

Engineering Report - Final

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1.0 INTRODUCTION

Rituximab is a monoclonal antibody that binds CD20 protein on the surface of leukemia and lymphoma cells.

The trade name for Rituximab is MabThera. The product is currently manufactured by F. Hoffmann-La Roche Ltd (hereafter referred to as Roche) and globally commercialized for the treatment of adults with the following blood cancers: previously untreated and relapsed/refractory follicular lymphoma, previously untreated diffuse large B-cell lymphoma, and previously untreated and relapsed/refractory chronic lymphocytic leukemia. MabThera is also approved for the treatment of adults in auto-immune diseases: severe active rheumatoid arthritis, pemphigus vulgaris and severe active granulomatosis with polyangiitis and microscopic polyangiitis (GPA/MPA).

Two (2) different dosages foreseen for MabThera drug product are:

1. MabThera 100 mg (100 mg / vial of concentrate for solution for infusion): the solution is filled in 10 mL glass vial type I and then sealed with 20 mm coated stoppers and flip-off aluminum seals
2. MabThera 500 mg (500 mg / vial of concentrate for solution for infusion): the solution is filled in 50 mL glass vial type I and then sealed with 20 mm coated stoppers and flip-off aluminum seals

MabThera manufacturing process of both dosage strengths (100mg/vial and 500 mg/vial) will be transferred from the Roche site at Mannheim, Germany (donor site) to Patheon (Thermo Fisher) Monza (hereafter referred to as Patheon), in Sterile Area 6 ("ST6") at the commercial scale of approx. 250 - 750 L bulk volume, as per change control n°191938 and technology transfer plan (TTP23701).

This report describes and analysis the activities executed during the engineering runs.

2.0 SCOPE AND PURPOSE

The scope of the present Engineering Final Report is to describe the activities performed to demonstrate that the procedures, equipment and processing parameters are adequate for the manufacturing of MabThera 500mg/vials in 50 mL Schott vials and 100 mg/vials in 10 mL Schott vials in Sterile Area 6 of the Patheon, Monza facility.

The Engineering trial covered one pooling/compounding of MabThera drug substance and buffer to reach ~ 250 L with the final concentration of 10mg/mL. The filling was then equally split into ~ 125L for MabThera 500 mg/vials and ~ 125L for MabThera 100 mg/vials.

These Engineering batches were not GMP and were not intended for human use. Nevertheless, cleanroom conditions were kept and physical parameters were monitored during the engineering runs, to closely simulate real compounding and filling conditions. Potential interactions of environment, equipment and procedures on process and product quality were investigated by these engineering runs. It must be noted that no formal deviations were opened in the QMS for the present study as this was a non-GMP Engineering batch and were managed within the Investigation form template (attached to this report) with the help of Quality operations.

The purposes of these batches were to:

- Performing the thawing of the Drug Substance and the manufacturing process of the Drug Product in preparation for future GMP manufacturing;
- Verify adequate machinability of the primary packaging components with the filling area equipment and dedicated format parts;
- Setup manufacturing line operational parameters (related to fill weight and crimping set up) which may have to be fine-tuned during manufacturing activities;
- Establish a suitable working range for potential critical process parameters (pCPP);
- Train personnel to operations required for future GMP activities, such as for manual handling, use of equipment, sampling and analytical testing required for manufacturing activities;
- Simulate Batch Record execution;
- Collect process monitoring data during the batch execution in order to provide useful information to demonstrate the performance of the manufacturing process and to enhance process understanding;
- Identify potential issues linked to the manufacturing process and take preventive measures to ensure PPQ readiness;
- Challenge of the process holding times and ensure PPQ readiness.

The collection of process monitoring data and the definition of the manufacturing process provided useful information to enhance process knowledge and understanding.

Each step of the whole manufacturing process were evaluated and the potential Critical Process Parameters (pCPP) were defined. The main objective was to increase the process knowledge, optimize the control strategy to ensure the stability of the potential CPPs over time, prior to the next PPQ batches manufacturing.

The applicable batch records and SOPs were followed during this manufacturing of the Engineering runs and the runs were documented according to the batch record.

In-process sampling and analytical testing were executed according to the sample plan specified in the relevant protocol (TT237B011) and Addendum (Addendum I to TT237B011).

Furthermore, the following process characterization activities were performed during the Engineering Batch and results were already reported and discussed in the Engineering interim report TT237C011:

- A filter flush study of sterilizing filters, to verify the volume of solution to be used to flush the residual water left in the filter after wetting as well as binding of active and/or excipients to the filter membrane;
- A stress study, to verify the proposed product quality attributes (CQAs) after mixing at the maximum speed at minimum batch size (250 L) in the compounding tank. The initial maximum speed to be challenged at the beginning of the trial based on results of surrogate trial was 250 rpm. This value, which could be adjusted during the engineering trial if necessary, was maintained at 250 rpm during all the trial.
- A filling performance to verify the proposed CQAs (homogeneity during filling) from the beginning, middle, and end of the filling process;
- Characterization of the filling process (e.g. check of filling accuracy including filling characteristic, simulation of process interruption)
- Characterization of stopper setting process
- Characterization of crimping process (crimping pressure and crimping height)
- VPHP study uptake to assess the rate of hydrogen peroxide pickup in open vials, and silicone tubing. This analysis has been performed at Roche side only during the run of MabThera 100 mg and on the water trial of MabThera 100 mg (executed for the particles/fiber investigation according to TT237Z051)
- Process Leachable studies during manufacturing. This analysis has been performed at Roche side on both MabThera 100mg and 500mg
- Elemental Impurity Analysis performed by Roche;
- Characterization of the product quality impact under relevant ambient light exposure during filling and inspection. The time the product was exposed to light during filling steps was evaluated, managing this activity through a dedicated tracking sheet;
- Tubing stress fatigue evaluation during filling through visible and subvisible particles (liquid samples as control);
- Challenge of process holding times
- Equilibration study before visual inspection operations

It must be highlighted that in the present Engineering Final Report is issued to add the results of the stability studies which were not available when interim report was issued because test were still ongoing. It must be highlighted that the stability studies, collected during engineering runs, for Mabthera 100 mg and 500 mg were development studies and for information only. For this reason, the data from the studies were not required for the initiation of the PPQ batches.

Process differences identified in GAP analysis (refer to TEC-0213431) were investigated during the engineering runs. The unexpected events occurred during the engineering run execution were managed with the support of Quality Operations and documented in the "Investigation form template" that are attached to this report. This has allowed Patheon to better understand the issue and promptly take preventive measures in the revision of future GMP batches manufacturing. Root causes of the events were defined, and corrective actions, if needed, will be implemented prior to proceeding with the next batches in manufacturing.

3.0 RESPONSIBILITIES

Process Engineering & Technology Transfer Patheon

- To ensure that the activities were conducted according to the protocol TT237B011 and Addendum I to protocol TT237B011
- To issue this relevant Report and archive approved version
- To share the results of the trial with the client
- To put in place identified corrective actions, if needed

Production Patheon

- To review and approve this Report
- To review manufacturing instructions
- To record observations, perform the required in-process controls and sampling, as stated in this Protocol and provide suggestions for improvement.

Quality Assurance (QA) Patheon

- To review / Approve the corresponding Report after execution
- To provide support just in case in case of critical events occur during the trial execution;
- To define the corrective actions to resolve investigations, if required

Quality Control (QC) Patheon

- To Review the Protocol before execution and this corresponding Report
- To Provide the resources necessary to collect and analyze samples during Protocol execution, as defined by this Protocol
- To Perform Analytical testing in accordance with methods

- To Provide analytical results

Quality Operations Thermo Fisher

- Support in case of the critical events that might occur on the line in order to understand possible root causes and carry out an investigation form if necessary.

Roche

- To approve the Report after execution of the trial
- To perform the requested analysis and provide results to be included in the final report

4.0 PRODUCT INFORMATION

MabThera 100 mg (100 mg/vial of concentrate for solution for infusion) is filled in 10 mL Schott Vials, with stopper 20mm D713 RTS and crimped with 20mm Flip-cap red seal.

MabThera 500 mg (500 mg/ vial of concentrate for solution for infusion) is filled in 50 mL Schott Vials fitted with stopper 20mm D713 RTS and crimped with 20mm Flip-cap grey seal.

The target vial fill volume for MabThera 100 mg is 10,525 mL (equal to 10,630 g considering a density equal to 1,01 g/mL) and for MabThera 500 mg is 51,109 mL (equal to 51,620 g considering a density equal to 1,01 g/mL).

MabThera products are “Unit Dose” products, meaning that the whole unit is administered to the patient, therefore the API active amount is guaranteed by the content in the entire dose volume.

Based on Patheon toxicity categorization (SOP 1622), MabThera drug products are classified as category 2. The categorization is carried out based on the therapeutical class, pharmaceutical potency and OEL. Category 2 substances are defined with reversible health effect, moderate pharmacological potency, no carcinogenic effects at industrially relevant doses, weak or rare sensitizers (skin or respiratory), lacks warning properties and a OEL range from 10 µg/m³ to 1 mg/m³.

The drug substance (BDS) was shipped by Roche at ≤ -20 °C in Freeze-Thaw tanks (F/T tanks). Particularly, two (2) different kinds of F/T tanks can be received for the manufacturing of MabThera 100 mg and 500 mg:

- 120 L US tank
- 300 L US tank

Different BDS manufacturers are foreseen for the MabThera transfer project. All the tanks (Table 1) will be shipped via the Basel Drug Substance unit in Switzerland, under Roche's responsibility.

In particular, for the manufacturing of these engineering batches, one (1) 300 L US F/T tank, manufactured by Samsung (South Korea), was received. Patheon code is 102706 (batch number T220766) and Roche code is 10174175 (batch number 13132583).

Table 1 Drug Substance for MabThera engineering manufacturing

Material	Description, supplier and manufacturing site	Supplier code	Patheon code	Supplier batch number	Patheon batch number	Storage condition
Rituximab DS in 300 L US	Supplier: DSTC (CH) Manufacturer: Samsung (South Korea)	10174175	102706	3132583	T220766	- 20°C max storage is 24 months including storage at site and shipment

MabThera BDS (bulk drug substance) is not expected to be light-sensitive product based on study provided at Roche side (RDR-1019102). However, light exposure during the process should be minimized. Considering that the manufacturing site is different, samples exposed to light were taken and analyzed during the run of MabThera 100 mg in order to evaluate the product exposure impact during the different manufacturing steps and a dedicated tracking sheets were used during the Engineering run.

Based on the duration of the data collected during these engineering runs and LUX value (Technical memo TT237Z011) a dedicated document (light technical assessment) has been issued (TT237F011) to assess the overall product light exposure during the manufacturing process steps in Patheon.

Roche provided CoC and CoA along with each BDS batch delivery at Patheon. No side samples were provided with the F/T tanks. The BDS release approval flow in Patheon was managed by - SOP-0000215286. The BDS identification for approval before manufacturing was paperwork-based (Patheon risk assessment QRSK-000249055). An analytical identification test was performed after thawing as IPC.

The composition of MabThera BDS for both 500mg/vial and 100 mg/vial dosage strength is reported in the table below (Table 2)

Table 2 Product composition MabThera - unit formula

Ingredient	Function	Concentration (mg/mL)	Unit formula (mg/vial)	Unit formula (mg/vial)
Rituximab	Active Ingredient	10	500	100
Sodium citrate dihydrate	Buffering Agent	7.35	367.5	73.5
Sodium chloride	Tonicity	9.0	450	90
Polysorbate 80	Surfactant	0.7	35.0	7.0
1N hydrochloride acid or 1N sodium hydroxyde	pH adjustment	q.s.ad pH 6.5	q.s.ad pH 6.5	q.s.ad pH 6.5
Water For Injection (WFI)	Solvent	q.s.	QS to 50 mL	QS to 10 mL
Density: 1.01 g/mL at room temperature (RT)				
Viscosity: 0.8 cP at 25°C and 1.4 cP at 5°C				

5.0 EQUIPMENT AND MATERIALS

In this paragraph, a summary of equipment and materials involved is reported, specifying if equipment and materials were required to manufacture MabThera 500mg/vial and MabThera 100mg/vial.

Major equipment involved in the engineering manufacturing process grouped by process step is reported in Table 3.

Table 3 Equipment used for MabThera engineering

Process Step	Equipment / Room
MabThera BDS storage (upon receipt)	For the engineering, the 300 L US F/T tank was stored in the cell (PTH ID: FRC086). This cell is at -20 °C. After the engineering campaign, all MabThera F/T tanks received at Patheon will be stored in Roche dedicated -20°C cells.
Thawing / re-freezing of MabThera BDS	300L US F/T tank placed in two thawing stations (TWU001 SN S452788 and TWU002 SN S453427) placed in Roche thawing room (PTH ID 2204). For these engineering runs, the thawing station used was the TWU002 SN S453427.

Process Step	Equipment / Room
MabThera BDS temporary storage, after thawing and after BDS pooling at 2-8°C (if necessary)	300 L US F/T tank stored in a 2-8°C cell (room 2207 and FRC114 as backup)
Buffer preparation	1x 1100L stainless steel tank (equipment ID: RTR342)
	J tube
	Funnel
	3 glass bottles
Pooling of MabThera BDS and buffer in compounding tank and mixing	1 x 300 L US F/T tank and 1 x 1100 L stainless steel tank (equipment ID: RTR343)
	32-14MP ECCENTRIC REDUCER
	J tube
BB filtration: solution transfer from compounding tank to storage tank through BB filter	Stainless steel trolley for the support of the disposable assembly that incorporates 1 (one) bioburden reduction filter
BDP storage in the storage tank	1 x 1100L stainless steel tank (equipment ID:RTR344)
Transport of storage tank in the filling room	Transpallet for tank transportation
Sterilizing filtration by two (2) 0.22µm sterilizing filters by nitrogen pressure	Stainless steel trolley for the support of the disposable assembly that incorporates 2 (two) in series sterilizing filters for sterile filtration
Vials Washing	Machine Vega8 (LFL013)
Vials Depyrogenation	Depyrogenation tunnel 1250FL DH (TST010)
Equipment sterilization	De Lama autoclave (ATC025) Or De Lama autoclave (ATC026)
Vials Filling	Machine Xtrema (INF021)
Vials Capping	ALU400 SA3122 (CPL012)
Finish DP storage at 2-8°C	Vials in 2-8°C cell

PRIMARY PACKAGING COMPONENTS

In the table below, the primary packaging components selected to manufacture MabThera 100mg/vial and 500mg/vial in Sterile area 6 during all the planned batches are listed.

Table 4 Primary packaging components

Material	Description	Supplier	Manufacturer	Patheon code
Vial (*)	Vial 22 mm 10 mL BB	Schott	Schott	241582
Vial (**)	Vial 42.5 mm 50 mL BB	Schott	Schott	241584
Stopper	Stopper 20 mm D713	Daikyo	Daikyo	273438
Seals (*)	Seals 20mm RED	Datwyler	Datwyler	273433
Seals (**)	Seals 20 mm GREY	Datwyler	Datwyler	273434

Vials, stoppers, and seals are materials that were used for both MabThera dosage strengths commercial manufacturing and technology transfer batches. The stopper was the same for the two dosage strengths, whereas the vial's size and the color of the seals are dedicated to the specific presentation: 10 mL vial and red seals (*) were dedicated to 100mg/vial dosage and the 50 mL vial and grey seals (**) were dedicated to 500mg/vial dosage.

According to procedure Patheon SOP 1602, as the batch was not intended for human use, some of the materials were used in a /TT status, therefore, no full incoming release testing was executed. Nevertheless, based on the available supplier certificates, materials were adequate for the indicated scope and no impact on the study is expected.

SECONDARY PACKAGING COMPONENTS

The secondary packaging components that were used to pack the samples for MabThera engineering trials are listed in Table 5 below.

Table 5 Secondary packaging components for samples MabThera

Description	Dosage	Description	Supplier	Patheon code
ALVEARE A-PET 358x268x30.2mm 130pz	MabThera 100 mg	To collect 10 mL finish product vials for incomplete boxes to prevent vials from slipping	Bachmann Fornig AG Hochdorf - Switzerland	261545
ALVEARE A-PET 358x268x38.5mm 35pz	MabThera 500 mg	To collect 50 mL finish product vials for incomplete boxes to prevent vials from slipping		261543
Space filler	MabThera 500 mg	To be used for 50mL Akylux boxes only	Comimbal SRL	261554

Description	Dosage	Description	Supplier	Patheon code
Akylux black	MabThera 100 and MabThera 500	To collect 10 mL and 50 mL vials for the shipping. The boxes are the same in which empty vials are supplied by Schott	Schott	NA (*)

(*) same boxes in which were supplied the empty vials by the supplier.

DISPOSABLE ASSEMBLIES

The disposable assemblies used for MabThera engineering Trials activities are detailed in the **Table 6** below. All assemblies in the table below were prototypes, as agreed with Roche.

Prototypes manufacturing process is the same of GMP assembly, the only difference is that the supplier did not provide the certificate (manufacturing and sterility) of the prototypes.

Table 6 Disposable assemblies used for the manufacturing of MabThera engineering batches

Process step	Material description	Patheon code	Supplier code
BDS recirculation during thawing	Two (2) disposable assemblies (connected in series) to connect F/T tanks inlet and outlet of the product throughLynx ST (*) (****)	273493	SGS04049
BDS pooling in compounding tank	Two (2) disposable assemblies to connect F/T tanks product outlet to the product inlet of the compounding 1100 L tank through tri-clamp connections	273493	SGS04049
Buffer transfer from the buffer tank to the compounding tank	Two (2) Y disposable tubes provided with steam-thru port to be connected to the tank and 2 (two) Aseptiquik ports for the connection to the extension tube disposable assembly	273499	SGS03744
	One (1) disposable extension tube with Aseptiquik ports for the connection of the SS buffer tank to the SS compounding tank through the Y assembly	273502	SGS04044
Bioburden reduction filtration and BDP transfer from compounding tank to storage tank	One (1) disposable system to transfer the solution from the compounding tank to the storage tank and for BB filtration. It is provided with one (1) Opticap XL10 capsule filter with 0,22 µm Durapore membrane for bioburden reduction filtration, provided with two Aseptiquik ports for the connection of the compounding tank to the storage tank through the Y assembly	273497	SGS04047

Process step	Material description	Patheon code	Supplier code
Transfer of BDP from SS tank to the filling line and sterilizing filtration for MabThera 100 mg	1 (One) Assembly for sterilizing filtration, provided with 2 (two) sterilizing filters (KVGLG10HH1 0.22 µm Opticap ® XL 10 capsule filters) already integrated for redundant filtration.	273504	SGS03875
	2 (two) disposable T tube provided with three (3) Aseptiquik ports for the connection to the Y line and sterilizing filtration assembly, for contingency (**)	273314	SGS03074
	1 (one) 8L filling bag provided with 10x SPT-60L surge tube 4.8mm x 8.0mm, 10x Accusil pump tube 6.8mm x3.2 mm, 10x SPT-60L dose tube 3.2mm x 6.8mm, 10x SS needles 3.0mm x 3.5mm	273508	SGS 03665
	1 (one) disposable extension tube for the connection of the SS tank to the assembly for sterile filtration	273502	SGS04044
Transfer of solution from SS tank to the filling line and sterilizing filtration for MabThera 500 mg	1 (One) Assembly for sterilizing filtration, provided with 2 (two) sterilizing filters (KVGLG10HH1 0.22 µm Opticap ® XL 10 capsule filters) already integrated for redundant filtration.	273504	SGS03875
	1 (One) 8L filling bag provided with 10x SPT-60L surge tube 8 mm x 11.2mm, 10x Accusil pump tube 6.8mmv x 10.2 mm , 10x SPT-60L dose tube 6 mm x 10.2 mm, 10x SS needles 6.0mm x 5.0 mm	273510	SGS 03667
Sampling assembly	Sampling assembly provided with 2 PETG Nalgene bottles of 500 mL and 2 PETG Nalgene bottles of 30 mL.	273220	SGS 04719
	Sampling assembly provided with 2 PETG Nalgene bottles of 500 mL and 2 PETG Nalgene bottles of 125 mL (***)	274061	B119646-I

(*) prototypes for engineering trials were provided by Saint Gobain with 1.5" tri-clamp connections instead of 1.5" ST lynx.

(**) Assembly 273314 were provided by Saint Gobain already gamma sterilized. During commercial manufacturing, the Y assembly provides the possibility to have a contingency line, but considering that during the engineering trials the filling was splitted between the two dosage, to maintain the possibility to have a contingency line, two additional 273314 assemblies were used only for the engineering trials. These assemblies were additional and foreseen only for the engineering runs.

(***) Assembly 274061 were provided by Thermo Fisher already gamma sterilized.

(****) Considering the additional F/T cycle executed, it was consumed in total four (4) recirculation assemblies, two (2) for every F/T cycle.

All assemblies were provided by the supplier, Saint Gobain, already sterilized by gamma irradiation and ready to use. Only exception is the 274061 sampling assembly, provided by Thermo Fisher, used to sample additional samples only for the engineering trials.

All the assemblies were introduced in the solution preparation room (room 823) and Xtrema filling room (room 843) of Sterile Area 6 through a dedicated airlock (room 824) and their packaging was removed just before performing their connections.

5.1 MABTHERA 500 AND MABTHERA 100 SET UP IN THAWING ROOM

In the thawing room (room 2204), the recirculation of the BDS (second step of the thawing. The first step of the thawing is without product recirculation) was performed from the bottom output valve of the F/T tank to the top valve of the F/T tank through two disposable assemblies "recirculation tubing" (code PTH:273493) connected in series. The assemblies for engineering trial were provided with two 1.5" tri-clamp connections. The recirculation was performed by peristaltic pump 620w N. After the thawing, the peristaltic pump was inverted and a manual squeezing of the tube with a dedicated tool was performed, to let the emptying of the tube during the challenging of the holding time.

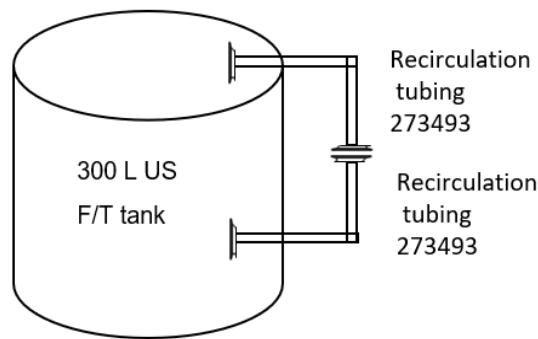


Figure 1 Set up in thawing room (both for MabThera 100 mg and MabThera 500 mg)

After the first thaw and the holding time challenge (room temperature holding time and 2-8°C holding time), the F/T tank was re-freezed.

Later, it was executed a second re-thaw, using the same set up listed in the present paragraph. For the second thaw, two (2) new recirculation tubing was used.

5.2 MABTHERA 500 AND MABTHERA 100 SET UP IN SOLUTION PREPARATION ROOM

After the second thaw, the F/T tank was then moved in the Xtrema solution preparation room (room 823) of the Sterile 06 department. In the solution preparation room the following 1100 L tanks were used:

- Buffer tank (RTR342) in position RP02
- Compounding tank (RTR343) in position RP03
- Storage tank (RTR344) in position RP04

First, the BDS was transferred (through peristaltic pump code PMP584) from the F/T tank to the compounding tank through two recirculation tubing in series and the eccentric adaptor. Then the compounding tank was connected to the buffer tank to allow the transfer (through nitrogen) of the buffer from the buffer to the compounding tank. Finally, the compounding tank was connected to the storage tank through the BB assembly (transfer of product by nitrogen).

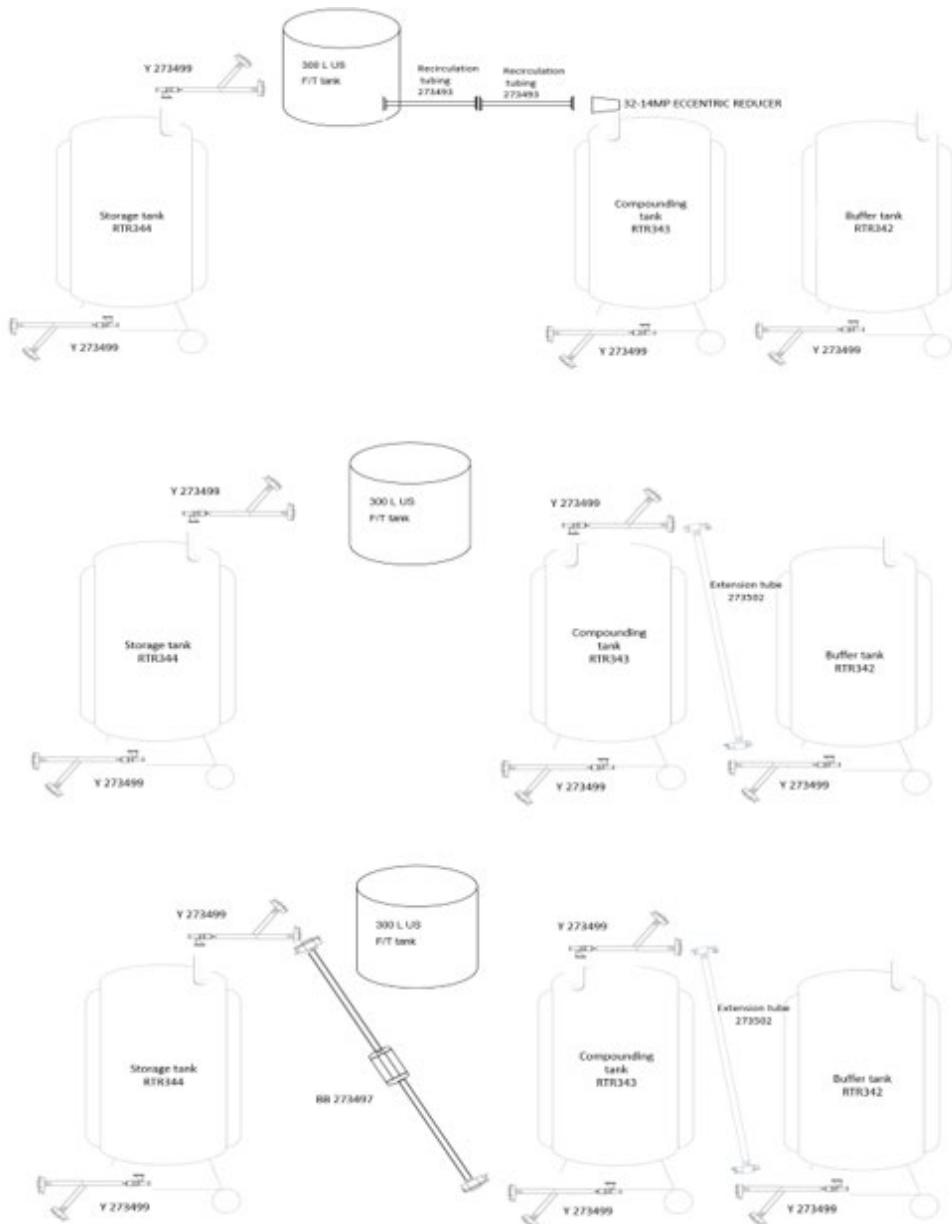


Figure 2 Set up in solution preparation room (both MabThera 100 mg and MabThera 500 mg)

5.3 MABTHERA 500 SET UP IN FILLING ROOM

In the following pictures is reported the set up in the filling room used for the filling of MabThera 500 mg in 50 mL vials.

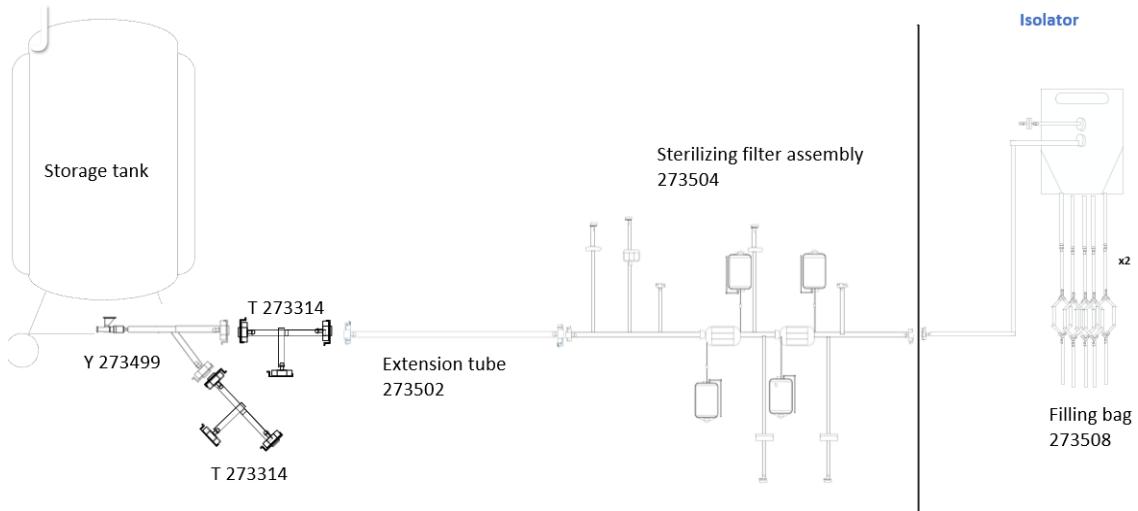


Figure 3 Set up in filling room for MabThera 500 mg

5.4 MABTHERA 100 SET UP IN FILLING ROOM

In the following pictures is reported the set up used in the filling room for the filling of MabThera 100 mg in 10 mL vials.

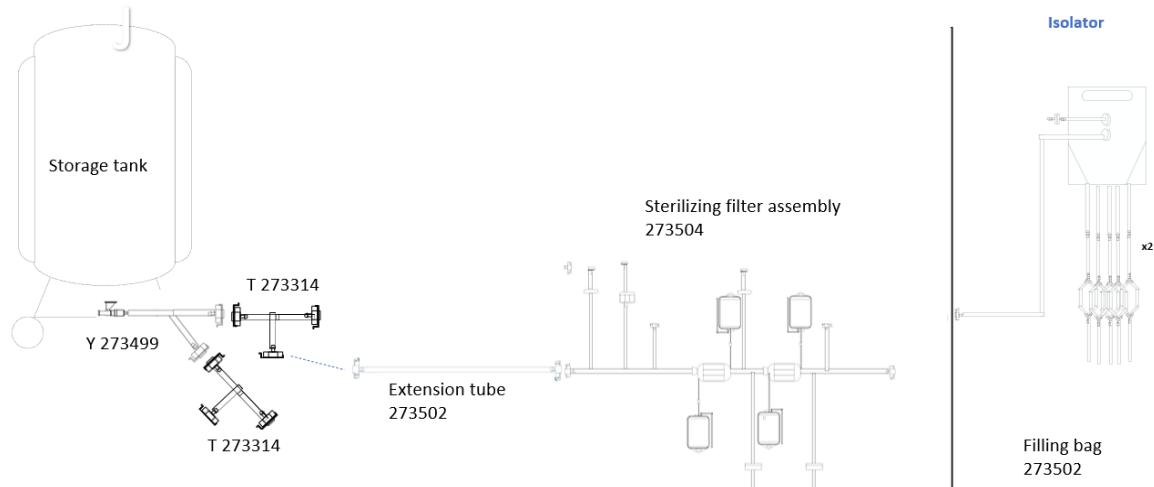


Figure 4 Set up in filling room for MabThera 100 mg

6.0 BATCH SIZE

The Engineering Batches were run at a minimum batch size (~ 250 L) as specifically requested by the client. The filling of the solution in the vials was split into two different batches:

- ~ 125 L of solution was used to fill the 50 mL vials (MabThera 500 mg/vials)
- ~ 125 L of solution was used to fill the 10 mL vials (MabThera 100 mg/vials)

Table below summarizes the proposed batch size considering a density of 1.01 g/mL

Table 7 MabThera 100 mg/vials and MabThera 500 mg/vials Batch Size

Batch Size assuming 100% yield				
Dosage	Batch size (L)	Batch size after crimping (pz)	Batch size after VI (pz)	Batch number
MabThera 500 mg	Approx. 125 L (*)	1482 (**)	1427 (**)	TT528
MabThera 100 mg	Approx. 125 L (*)	9248 (**)	9235 (**)	TT528/1

(*): the minimum batch size to be prepared in the compounding tank is 250 L. Only one preparation for MabThera DPS was done according to protocol TT237B011, the filling step was splitted in the two dosages (~ 125L to fill MabThera 100 mg and ~ 125L to fill MabThera 500 mg).

(**) Differences are due to:

- rejects in visual inspection;
- The number of vials after crimping steps are calculated by box, whereas the number of vials after the visual inspection are calculated one by one. A range is usually admitted but during engineering runs no limits are usually set.

The batch sizes shown in the table above were theoretical and do not consider line losses and sampling plan. The consideration about line losses and sampling plan are reported in the following paragraphs.

7.0 PROCESS DESCRIPTION

The manufacture of MabThera Engineering batches was performed following the Master Batch Record (MBR). The MBRs were approved by Patheon and Roche prior to the batch execution.

7.1 PROCESS OVERVIEW

The main process steps for the Engineering manufacturing process of MabThera 100 mg/vials and MabThera 500 mg/vial in Sterile Area 6 can be summarized as follows:

- Reception of F/T tanks containing formulated bulk drug substance and storage in Patheon Monza at ≤-20°C
- Thawing of MabThera BDS through dedicated thawing stations
- F/T tanks temporary storage at room temperature and at 2-8°C (storage at 2-8°C during commercial manufacturing is optional. During engineering run it was simulated as part of holding time challenge)
- After the holding time challenge on the F/T tank at room temperature (10 hours and 26 minutes) and at 2-8°C (10 days, 16 hours and 12 minutes), it was not possible to continue with the scheduled activities in solution preparation room. Thus, it was agreed with the client to proceed with a re-freezing, considering that for every F/T tank three (3) freeze/thaw cycle are possible as per validation.
- F/T tank was thawed for a second time through dedicated thawing station. Following this second thawing, after less than one hour, the F/T tank was put in the 2-8°C cell for approximately 2 days waiting for the readiness in the solution preparation room for the BDS pooling;
- Polysorbate stock solution and pH adjustment solution preparation in 2L, 5L, and 10L glass bottles.
- Preparation of buffer solution in SS buffer tank by pouring the number of excipients needed
- Pooling of the BDS contained in the F/T tanks into a stainless-steel compounding tank by a peristaltic pump.
- Second re-freezing of the BDS through dedicated thawing stations after holding time challenge considering that leftover BDS will be used also for the manufacturing of PPQs batches
- Buffer solution transfer to SS compounding tank by using nitrogen pressure through a disposable tubing
- Bioburden reduction filtration of the bulk drug product from the compounding tank into the stainless-steel storage tank by nitrogen pressure through a disposable line equipped with 1 (one) 0.22µm bioburden reduction filter.
- Post use integrity test on bioburden reduction filter
- Storage of the bulk solution into the storage tank at room temperature and then storage at 2-8°C (storage at 2-8°C was performed in Engineering, but during commercial manufacturing

will be not mandatory if filling activities can start within validated storage holding time at room temperature)

- Transferring of the storage tank from the solution preparation room to near the isolator by using an electric transpallet.
- Pre-use post sterilization integrity test of both the sterilizing filters to be performed through bubble point test and according to filters' supplier certificate (*)
- Sterilizing filtration of the BDP through 2 (two) 0.22µm sterilizing filters by nitrogen pressure (pressure monitoring in place).
- Vials filling driven by peristaltic pumps
- After filling, both sterilizing filters was tested for integrity (during commercial activities, only the filter closest to the filling bag will be tested and only in case of failure, the second sterilizing filter will also be integrity tested).
- Vials stoppering and crimping
- 100% Manual Visual Inspection
- Finished drug product storage at 2-8°C

(*) from this point, the engineering runs were splitted in two: the first for the manufacturing of MabThera 500 mg and the second for the manufacturing of MabThera 100 mg.

During commercial activities, storage of bulk solution in storage tank at 18-23°C will be performed when filling can start within 24 hours from the beginning of bioburden reduction filtration.

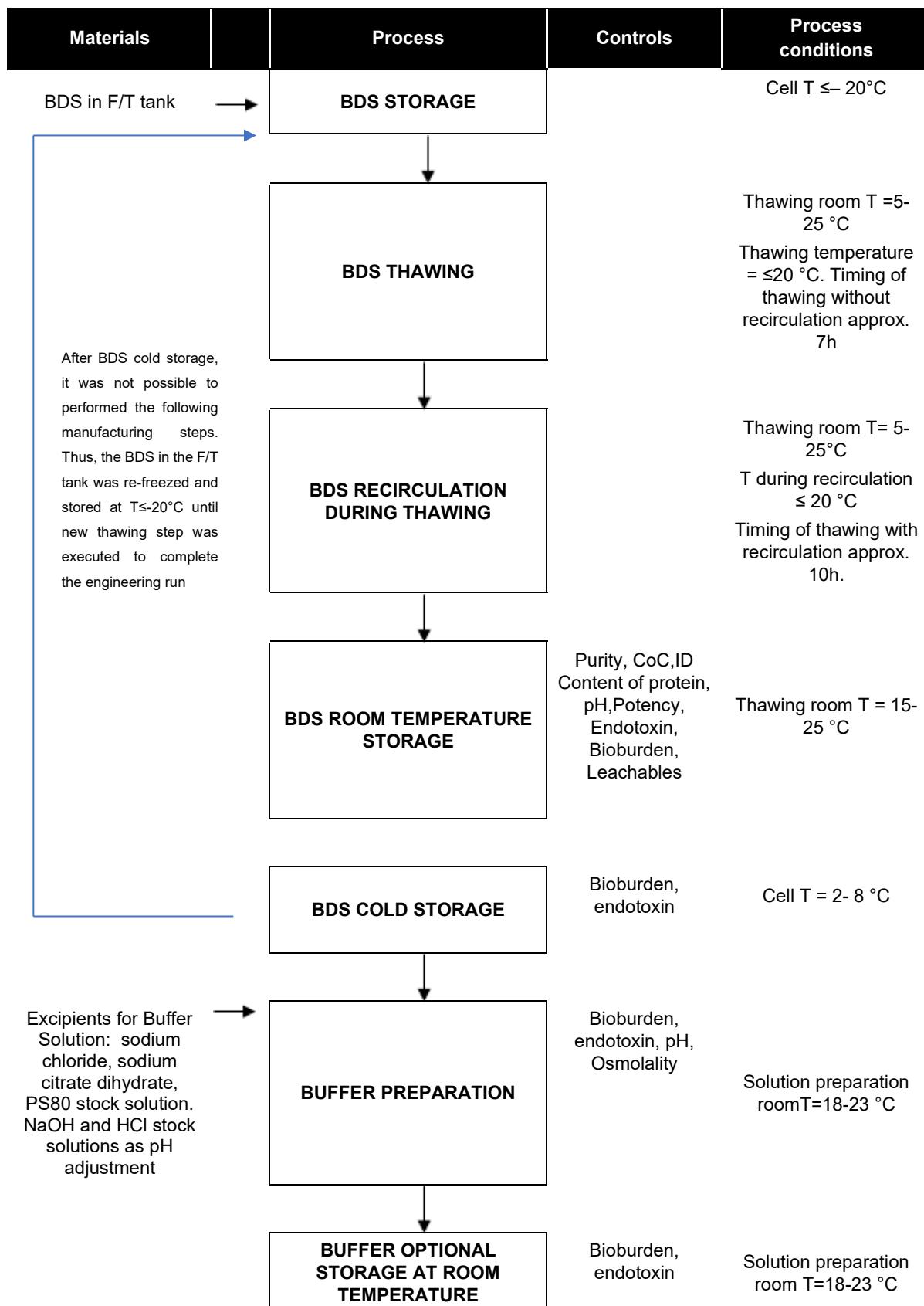
Otherwise, the solution will be stored at 2-8°C in the storage vessel for the needed amount of time and no re-equilibration to 18-23°C is required before starting the filling.

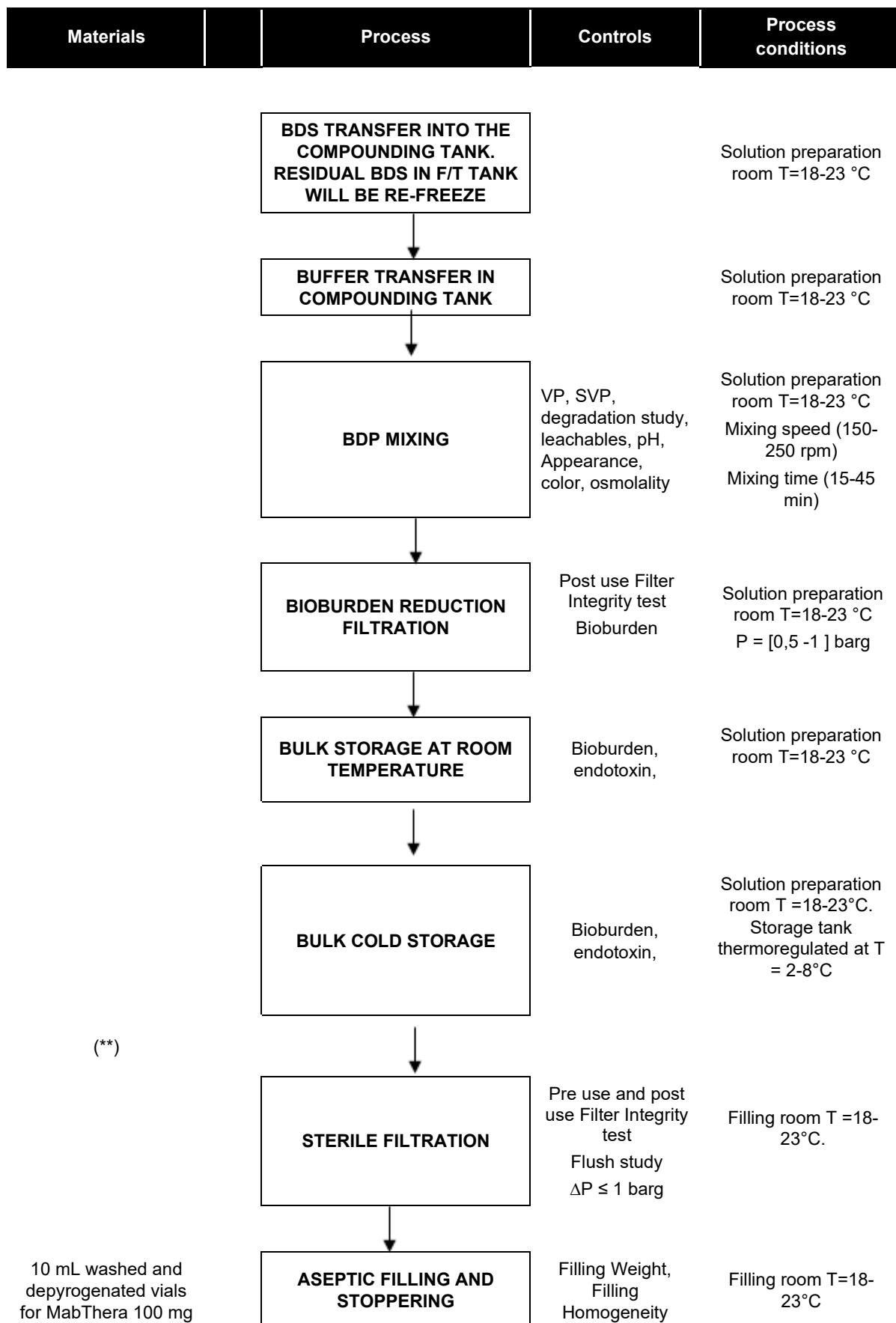
During these engineering trials, approximately 24 hours at room temperature was verified. After this time additionally approximately 24 hours at 2-8°C plus the actual time at 2-23°C needed for filling (this time was preliminary experimental evaluated with the data of execution of the trial) was challenged. This time will be than re-verified and validated during the PPQ batches.

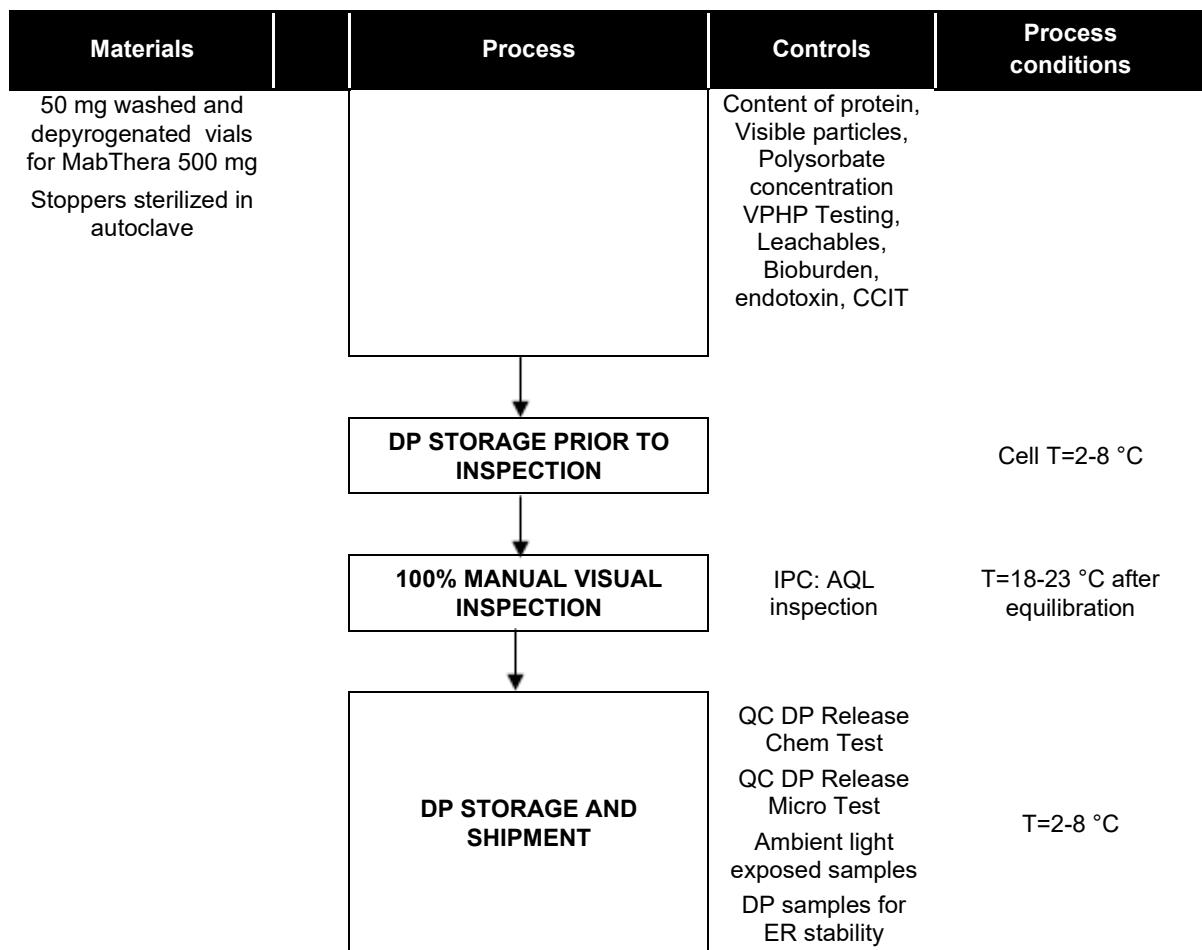
Details about the achieved holding time are reported in the dedicated paragraph (paragraph 8.0).

The maximum filling time allowed as per media fill is 48 hours.

The process flow for this Engineering run, including in process sampling and the tests designed with the accompanying product analytical sampling, are specified below.

Table 8 Schematic Process flow- MabThera Engineering trials





(*) After the BDS pooling step, the remaining solution was re-frozen considering that same F/T tank will be used also during PPQ batches. It must be noted that 3 maximum cycle of freeze/thawing for each F/T tank are allowed (either at the end of pooling (if residual BDS is present) or any time in between the end of thawing and the start of pooling if any technical issue arises that might prevent further BDS manufacturing. Considering the additional F/T cycle performed during this Engineering trial, for the involved F/T tank it is left one cycle of freeze/thawing.

After the BDS pooling and before the second re-freezing, during engineering run, additional holding time at 2-8°C for the F/T tank for 3 days was challenged (paragraph 8.0)

(**) from the sterile filtration step onwards, the manufacturing steps were doubled, one for MabThera 500 mg (first half of solution in the storage tank to fill 50 mL vials) and one for MabThera 100 mg (second half of solution in the storage tank to fill 10 mL vials). This approach was chosen considering that the only difference between the two strength is the fill volume. The steps in solution preparation room were the same for the two dosage strengths and so the evaluation is applicable for both MabThera 500 mg and MabThera 100 mg. Whereas the split was performed for the filling in order to evaluate the filling characteristics of both dosages. After the end of filling of the first format and before the start of filling of the second format the BDP in the storage tank was kept at 2-8°C.

8.0 RESULTS AND DISCUSSION

8.1 BDS RECEIPT AND STORAGE

The BDS solution needed to manufacture both MabThera dosages, for engineering trials, was shipped by Roche at $\leq -20^{\circ}\text{C}$ in one (1) 300L US F/T tank. For the Engineering batches the BDS were stored in Patheon -20°C walk-in cell (FRC086).

The set point of the temperature in the FRC086 is $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. It was checked the trend of the temperature in the timeframe 25/01/2022 -18/11/2022, when the involved F/T tank was stored in the FRC086. From the analysis on the raw data, it was identified that:

- during the involved timeframe, there was longer period in which the values fluctuated in the range between -20°C and -15°C ;
- there were some isolated events in which the temperature was outside -15°C . Particularly, the worst value reached was $-4,6^{\circ}\text{C}$ for 1 min, then the temperature increased up to -15°C in 14minutes. However, it has to be highlighted that no alarms occurred over this period considering that the room setting has a delay equal to 30minutes, so the temperature always was brought back to the range within this timeframe, without triggering any alarms.

The F/T tank was moved in Roche dedicated cell (FRC422), which is at $25 \pm 5^{\circ}\text{C}$, on 18/11/0222 and the transfer was completed in 12 minutes.

As per Roche study (VAL-0146645 and TEC-0186197), it can be assessed no impact on the quality of the BDS.

For commercial manufacturing, after the receipt of the tanks, Patheon will store the tanks in the warehouse at $-25 \pm 5^{\circ}\text{C}$ in Roche dedicated cells.

For the movement of the tanks among the different areas, it has to be highlighted that the 300L tank was placed on a dedicated trolley bought by Patheon.

8.2 DISPENSING: BDS THAWING STEP (FIRST THAWING STEP)

The BDS F/T tank was moved from the warehouse to the Roche dedicated thawing room according to the packing list provided by the client.

The thawing of the F/T tanks was performed at room temperature, using one of the two (2) dedicated thawing/freezing stations 620w NR (station 1: TWU001 SN S452788 and station 2: TWU002 SN S453427), supplied by Zeta. In particular, during engineering run, it was used the station 2.

The tank was connected to the silicone oil (heat exchange medium) supply through dedicated tubings. Moreover, the two-recirculation tubing (SGS04049) were connected, in series, before

starting in order to allow the recirculating of the product during the thawing step, by means of a peristaltic pump (630EnN/R).

During engineering run, considering that a 300 L F/T tank was used, the timing for the thawing step was approx. 17 hours (including 7 hours without product recirculation and 10 hours with product recirculation). At the end of the cycle the BDS temperature was kept at 2-8°C.

After the sampling activities at end of thawing (before the holding time challenge), to empty the recirculation tube from the BDS, first it was inverted the peristaltic pump and then it was also performed a manual squeezing of the tube (with the dedicated tool already used at the donor site).

Upon thawing completion, the F/T tank was held at 2-8°C. F/T tanks storage HT at 2-8°C is defined at Roche side as cumulative 60 days from first thawing till the transfer of the MabThera BDS to the compounding tank. For the Engineering manufacturing described in the protocol TT237B011, approximately 8 hours at room temperature following approximately 6 days at 2-8°C should have been challenged.

After the first thaw (started on 08/06/2022) it was challenged a holding time at room temperature of 8 hours and 34 minutes. Then, the F/T tank was put in the 2-8°C cell for the holding time challenge at 2-8°C.

Considering that it was not possible to perform manufacturing steps in sterile area, it was agreed with the client to re-freeze the F/T tank after having stored it at 2-8°C for 10 days, 16 hours and 12 minutes. At the end of the holding time at 2-8°C, microbiological samples were taken through the recirculation tubing, starting the peristaltic pump. After the sampling activities, the peristaltic pump was inverted and a manual squeezing of the tube with a dedicated tool was performed to let the emptying of the recirculation tube and the first freezing cycle started on 20/06/2022.

The main parameters documented during BDS first thawing step are listed in the table below

Table 9 Parameters documented during first thawing step (for 300 L tank)

Parameter	Requirements	Results	pCPP (Y/N)
Drug substance weight in Freeze/Thaw tanks	Weight \geq 51,5 Kg / Record	Weight \geq 51,5 Kg	Y
Number of freeze/thaw cycle	N° cycles \leq 3/Record	1° cycle	Y
Heat transfer fluid temperature set point	Target = 23°C Temperature \leq 26°C/ Record	Conforming (see graph)	Y
Recirculation Mixing peristaltic pump rpm	Record Target = 21 rpm [19-23 rpm]	Conforming (see graph)	Y
Recirculation mixing duration	Time \geq 10 hours / Record	Conforming	Y

Parameter	Requirements	Results	pCPP (Y/N)
Thaw operation duration	Time \geq 17 hours Record	Conforming	Y

The combination of all the pCPP indicated in table above have been selected because they potentially could have an impact on protein content, purity, CoC, particles generation, pH and osmolality of the released product.

Heat transfer fluid temperature

In the graph below it is reported the trend of the temperature of the silicon oil. In particular the red line is related to the temperature of the silicon oil when entering the F/T tank and the blue line is related to the temperature of the silicon oil when exiting the F/T tank.

The black line is instead the temperature of the product measured by the thermocouple inserted in the dedicate holding of the F/T tank. Nevertheless, the temperature of the product is not listed as potential pCPP, it is however displayed and described as outcome of the process.

The target of the temperature of the silicon oil during the thawing process was set as 23°C (recipe setpoint) both during the thawing without the product recirculation and during the thawing with the product recirculation. From the trend, it is possible to see that the temperature of the silicon oil stayed across the target (23°C) both at inlet and outlet of the F/T tank. After the end of the thawing with the product recirculation, it is present (in the recipe) a re-cooling of the product in which the temperature of the silicon oil goes down to approximately 5°C (recipe setpoint) in order to re-cool the product between 2-8°C.

From the graph, during the both phases of the thawing, the product temperature stayed below the maximum admissible product temperature which is 22 °C.

Finally, no alarms regarding heat transfer fluid temperature and product temperature were present during all the cycle. The cycle was conforming and the BDS was correctly thawed.

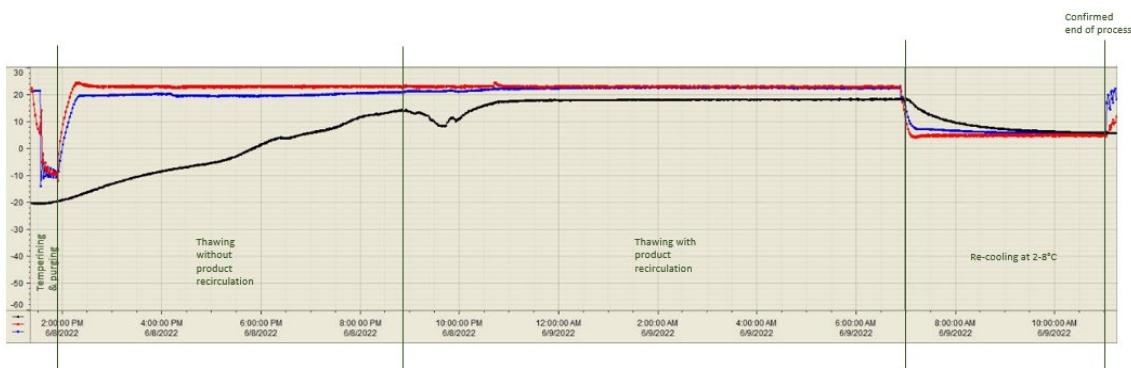


Figure 5 Temperature of Silicon oil and Product temperature during first thawing cycle

Product pump recirculation rpm

In the graph below it is reported the trend of rpm of the peristaltic pump during the product recirculation phase. The target of rpm, as per recipe, is 21 rpm and the range is [19-23] rpm. Along all product recirculation phase the rpm stayed in the range.

No alarm was present during the phase.

At the beginning of the recirculation phase, the connection between the two recirculation tubings showed a leakage. The operators promptly adjusted the connection without touching the gasket and without opening the connection. The event is detailed in the dedicated attachment "Investigation form template".

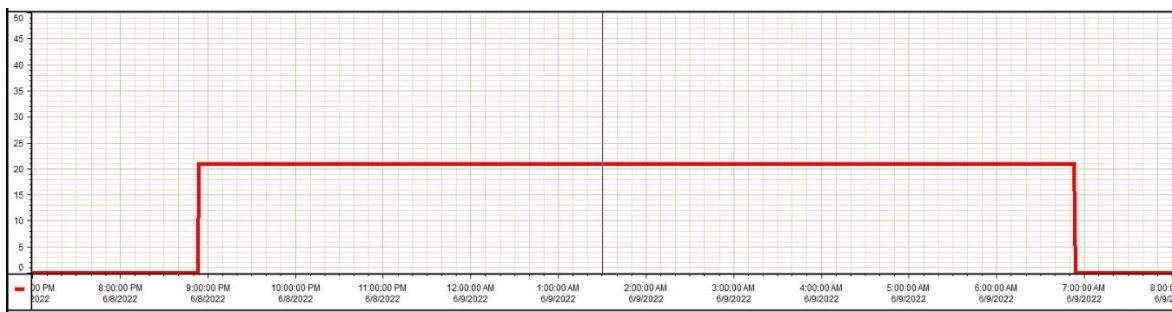


Figure 6 Peristaltic pump rpm during first thawing cycle thawing (product recirculation phase)

Sampling collection

At the end of the thawing, samples were taken to analyze purity (SE-HPLC and IE-HPLC), ID, CoC, Content of protein, pH, Osmolality, Potency by Bioassay, Leachable control sample, endotoxin, bioburden (in accordance with Attachment 1 of protocol TT237B011). Considering that the same F/T tank will be used also for manufacturing PPQs batches, retain sample was taken after the thawing and no needing to retake it during PPQs batches.

Results of analysis performed on samples are reported in the attachment 1 of this report TT237C011.

All the results met the acceptance criteria, confirming the good results of the thawing process.

After approximately 8 hours (8 hours and 34 minutes) at room temperature (from the disconnection of the F/T tank from the thawing station), samples were taken to analyze endotoxin and bioburden to challenge holding time at room temperature.

At the end of the previous one step, the F/T tank was foreseen to be placed for approximately 6 days at 2-8°C and at the end it was supposed to be taken samples to analyze endotoxin and bioburden to challenge holding time at 2-8°C, before pooling the BDS for compounding operations.

Instead, the F/T tank was hold 10 days, 16 hours and 12 minutes at 2-8°C before taking samples to analyze endotoxin and bioburden and re-freezing the tank.

All the results met the acceptance criteria (attachment 1 of this report TT237C011), confirming the good results of the holding time challenge.

Sampling were performed through sampling point (Nalgene bottles) provided with recirculation tubing (SGS04049) and sampling assembly (SGS04719).

Two additional sampling assemblies (274061) were added. 274061 assembly was provided with: two PETG Nalgene bottles (500 mL) and two PETG Nalgene bottles (125 mL) and two Aseptiquick connections. Assembly was manufactured by Thermo Fisher and provided already gamma irradiated.

All details about sampling are reported in attachment #1.

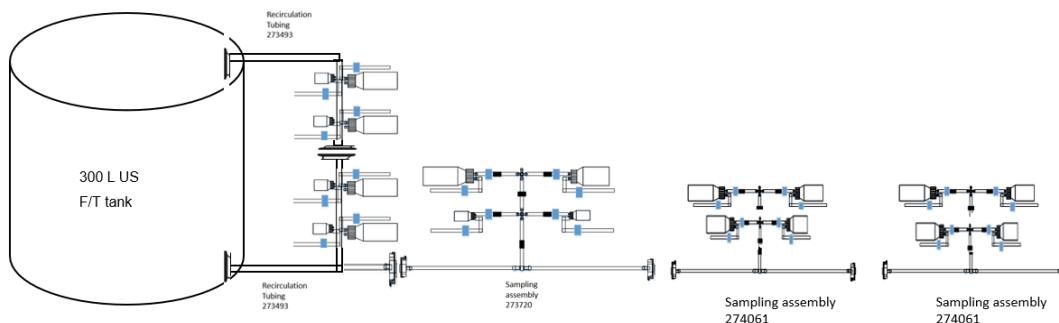


Figure 7 Sampling during BDS thawing step

8.3 DISPENSING: BDS RE-FREEZING STEP (FIRST)

The first re-freezing cycle started on 21/06/22.

Before the freezing cycle, samples for bioburden and endotoxin listed in previous one paragraph were taken. After having performed the sampling activities, the two recirculation tubings were detached and discharged.

During re-freezing phase, the following main parameters recorded are reported in the table below:

Table 10 Parameters documented during re-freezing step for 300 L tank

Parameter	Requirements	Results	pCPP (Y/N)
Drug substance weight in Freeze/Thaw tanks	Weight \geq 51,5 Kg / Record	Weight \geq 51,5 Kg	Y
Number of freeze/thaw cycle	N° cycles \leq 3/Record	2°cycle (*)	Y
Heat transfer fluid temperature set point	Record Target: -50°C Temperature \leq 26°C	Conforming (see graph)	Y
Freeze operation duration	Target =at least 19 hours/Record	Conforming	Y

(*) the freezing step is considered as second cycle because the first freezing cycle is the freezing cycle executed at the DS manufacturing site.

The combination of all the pCPP indicated in table above have been selected because they potentially could have an impact on product quality attributes like protein content, purity, CoC, particles generation, pH and osmolality

Heat transfer fluid temperature

In the graph below it is reported the trend of the temperature of the silicon oil. In particular the red line is related to the temperature of the silicon oil when entering the F/T tank and the blue line is related to the temperature of the silicon oil when exiting the F/T tank.

The black line is instead the temperature of the product measured by the thermocouple inserted in the dedicate holding of the F/T tank. Nevertheless, the temperature of the product is not listed as potential pCPP, it is however displayed and described as outcome of the process.

The target of the temperature of the silicon oil during the freezing process was set as -50°C (recipe setpoint) both during the first freezing step and during the second freezing step.

From the trend, it is possible to see that some peak in the temperature of the silicon oil is present both at inlet and outlet temperature graph of the F/T tank during:

- Purging step (preliminary step in which the air is evacuated from the pipeline of the silicon oil while silicon oil fills the circuit)
- First freezing step

The peaks were due to black out events in the facility during the cycle running. After the black out events, the power supply was guaranteed immediately but the machine put on hold the cycle and needed input from the operators to resume the cycle. It must be noted that however the signal coming from the thermocouples recorded also during the holding steps.

Here below the summary of the black out events: 1 happened during the purging phase, the other 6 during the first freezing step (of these, 4 events occurred one straight after the other and can be considered as a single event). No black out events happened during the secondary freezing.

Table 11 Black out events- details

Black out Time occurrence	Resume cycle Time occurrence	On HOLD duration	Step of the recipe black out happened	Recipe step duration	Each step Start/End time	Silicon oil temperature setpoint	Silicon oil temperature probe (inlet)	Silicon oil temperature probe (outlet)	Product Temperature probe
13:00	13:23	00:23	Purging step	At least 20 min	Start: 20.06.2022 12:55:56 End: 20.06.2022 13:39:39	NA	NA	NA	NA
13:41	15:45	02:04	Primary Freeze	At least 11h 30 min	Start: 20.06.2022 13:39:49 End: 21.06.2022 04:26:55	-50°C	Alarm High: 26°C Warning High: 30°C Warning Low: - 60°C	NA	Warning Low: - 55°C
18:49	18:51	00:01							
18:51	18:55	00:003							
18:55	18:55	00:00							
18:56	18:58	00:01							
19:53	21:03	01:10							

However, there was no alarm regarding the silicon oil product temperature during all cycle (temperature of the silicon oil was lower than 26°C which is the value for which the alarm is displayed and the cycle is interrupted).

Moreover, the product temperature never exceed 8°C during the holding.

Finally, considering that the recipe is time based (system has to actively work actually for the timing reported in the recipe) and if an unexpected holding happens during the cycle, the time of the holding is summed up to the overall time of the phase, it was also respected the pCPP of the duration of the cycle.

The cycle was conforming and the BDS was correctly thawed. Details about the un-expected occurrences are reported in the attached investigation form.

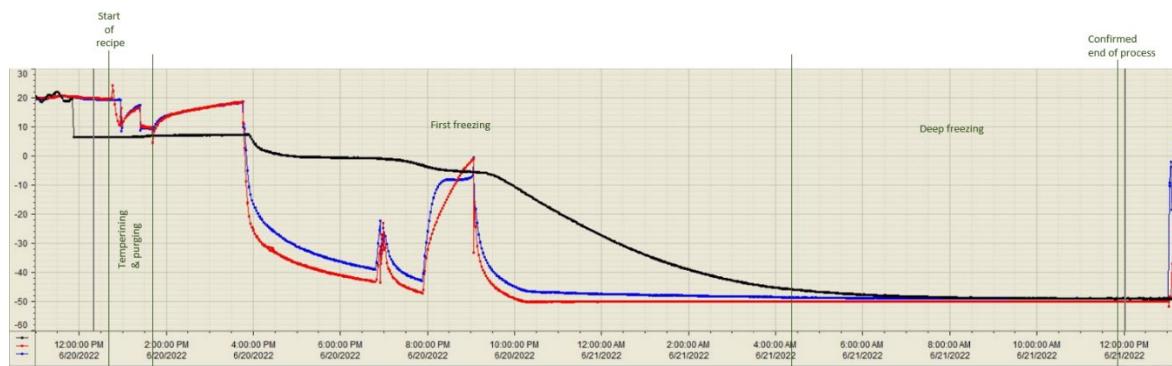


Figure 8 Silicon oil temperature and product temperature during first freezing cycle at Thermo Fisher

Sampling collection

Before the re-freeze, sampling for endotoxin and bioburden were taken using the bottles provided with the recirculation tubing (SGS04719). All the results met the acceptance criteria (Attachment 1 of this report TT237C011), confirming the good results of the holding time challenge.

8.4 BDS THAWING STEP (SECOND CYCLE)

After the first re-freezing step, the F/T tank was stored in the T≤ -20°C cell, until 01/07/2022 when the F/T tank was re-thawed according to certified copies of the MBR.

The sampling after the thawing station was managed according to Addendum I to Engineering protocol TT237B011.

Two new recirculation assemblies (273493) were supplied.

The main parameters documented during BDS second thawing step are listed in the table below

Table 12 Parameters documented during second thawing step for 300 L tank

Parameter	Requirements	Results	pCPP (Y/N)
Drug substance weight in Freeze/Thaw tanks	Weight \geq 51,5 Kg / Record	Weight \geq 51,5 Kg	Y
Number of freeze/thaw cycle	N° cycles \leq 3/Record	2 cycle	Y
Heat transfer fluid temperature set point	Target = 23°C / Record Temperature \leq 26°C/	Conforming (see graph)	Y
Recirculation Mixing peristaltic pump rpm	Record Target = 21 rpm [19-23 rpm]	Conforming (see graph)	Y
Recirculation mixing duration	Time \geq 10 hours / Record	Conforming	Y
Thaw operation duration	Time \geq 17 hours Record	Conforming	Y

The combination of all the pCPP indicated in table above have been selected because they potentially could have an impact on protein content, purity, CoC, particles generation, pH and osmolality of the released product.

Heat transfer fluid temperature

In the graph below it is reported the trend of the temperature of the silicon oil. In particular the red line is related to the temperature of the silicon oil when entering the F/T tank and the blue line is related to the temperature of the silicon oil when exiting the F/T tank.

The black line is instead the temperature of the product measured by the thermocouple inserted in the dedicate holding of the F/T tank. Nevertheless, the temperature of the product is not listed as potential pCPP, it is however displayed and described as outcome of the process.

The target of the temperature of the silicon oil during the thawing process was set as 23°C (recipe setpoint) both during the thawing without the product recirculation and during the thawing with the product recirculation. From the trend, it is possible to see that the temperature of the silicon oil stayed across the target (23°C) both at inlet and outlet of the F/T tank. After the end of the thawing with the product recirculation, it is present (in the recipe) a re-cooling of the product in which the temperature

of the silicon oil goes down to approximately 5°C (recipe setpoint) in order to re-cool the product between 2-8°C.

From the graph, during the both phases of the thawing, the product temperature stayed below the maximum admissible product temperature which is 22 °C.

No alarms regarding heat transfer fluid temperature and product temperature were present during all the cycle. The cycle was conforming and the BDS was correctly thawed.

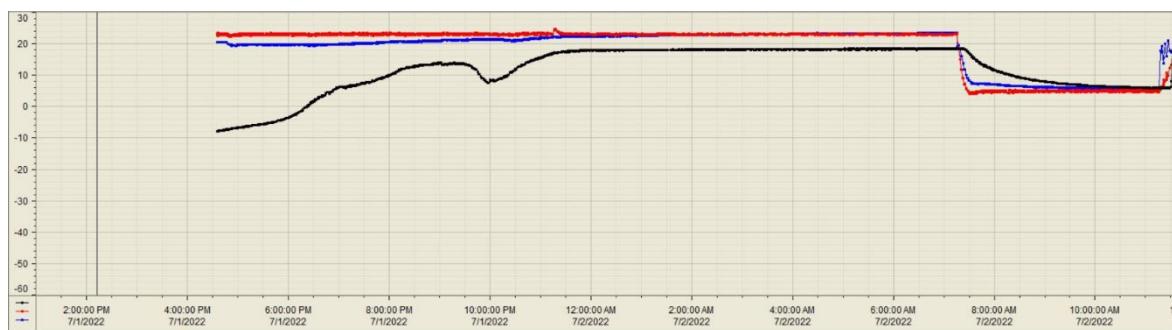


Figure 9 Temperature of Silicon oil and Product temperature during second thawing cycle

Product pump recirculation rpm

In the graph below it is reported the trend of rpm of the peristaltic pump during the product recirculation phase. The target rpm as per recipe is 21 rpm [19-23] rpm. Along all product recirculation phase the rpm stayed in the range.

During the product recirculation phase no alarm was present.

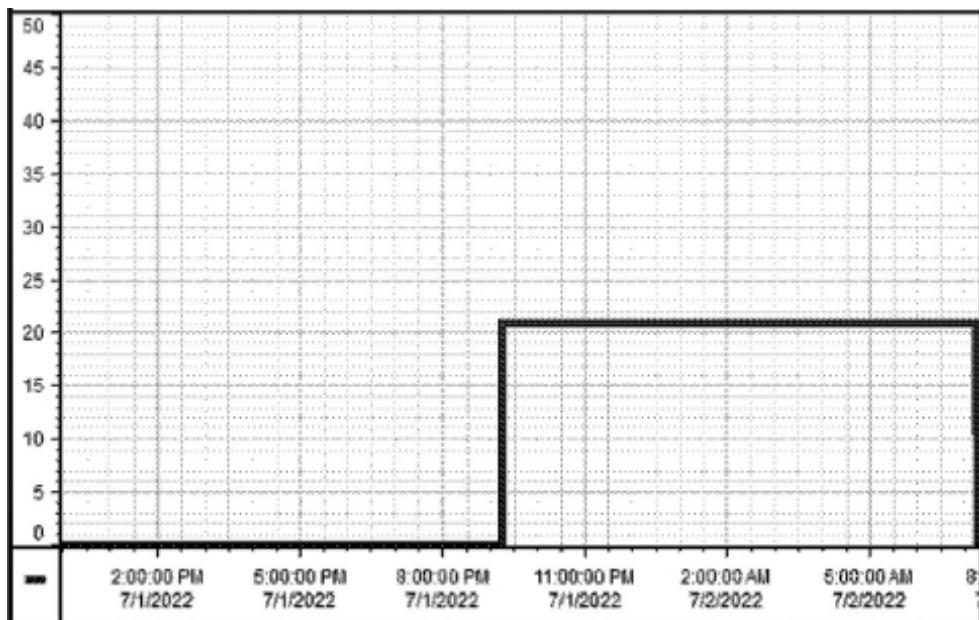


Figure 10 Peristaltic pump rpm during second thawing cycle (product recirculation phase)

Considering that F/T tank will be used also for the manufacturing of PPQ batches, the maximum number of manipulations of the interface between the recirculation tubing versus F/T tank product inlet/outlet (total of 10 manipulation for product inlet and 6 for product outlet) will be verified considering the cumulative engineering batches and PPQ batches. No simulation was performed. After the thawing, sampling for leachable, endotoxin and bioburden were taken. Bioburden and endotoxin met the acceptance criteria (attachment 1 of this report TT237C011), confirming the good results of the previous one cycle.

After the thawing the F/T tank was placed at 2-8°C cell. Before the starting of the manufacturing activities in solution preparation room, the F/T tank was moved from dispensing to Sterile Area in solution preparation room (823), wiping with IPA to Grade D and to Grade C.

8.5 DISPENSING: BDS RE-FREEZING STEP (SECOND)

After the BDS pooling, the F/T tank was hold 3 days and 34 minutes at 2-8°C before starting the re-freezing cycle as the remaining BDS will be used for PPQ activities.

The freezing cycle started on 07/07/2022 and before the freezing cycle, samples for bioburden and endotoxin were taken and the two recirculation tubings were detached and discharged.

During re-freezing phase, the following main parameters recorded are reported in the table below:

Table 13 Parameters documented during re-freezing step for 300 L tank

Parameter	Requirements	Results	pCPP (Y/N)
Drug substance weight in Freeze/Thaw tanks	Weight \geq 51,5 Kg / Record	Weight \geq 51,5 Kg	Y
Number of freeze/thaw cycle	N° cycles \leq 3/Record	3 cycle (*)	Y
Heat transfer fluid temperature set point	Target = -50°C Record Temperature \geq 26°C	Conforming (see graph)	Y
Freeze operation duration	Target = at least 19 hours/Record	Conforming	Y

(*) the freezing step is considered as second cycle because the first freezing cycle is the freezing cycle executed at the DS manufacturing site.

The combination of all the pCPP indicated in table above have been selected because they potentially could have an impact on product quality attributes like protein content, purity, CoC, particles generation, pH and osmolality

Heat transfer fluid temperature

In the graph below it is reported the trend of the temperature of the silicon oil. In particular the red line is related to the temperature of the silicon oil when entering the F/T tank and the blue line is related to the temperature of the silicon oil when exiting the F/T tank.

The black line is instead the temperature of the product measured by the thermocouple inserted in the dedicate holding of the F/T tank. Nevertheless, the temperature of the product is not listed as potential pCPP, it is however displayed and described as outcome of the process.

The target of the temperature of the silicon oil during the freeze process was set as -50°C (recipe setpoint) both during the first freezing step and during the second freezing step.

No anomalies in the graph is present and no alarm related to the silicon oil temperature was present during the cycle. Thus, the cycle was conforming and the BDS was correctly freezed and then stored at $T \leq -20^\circ C$.

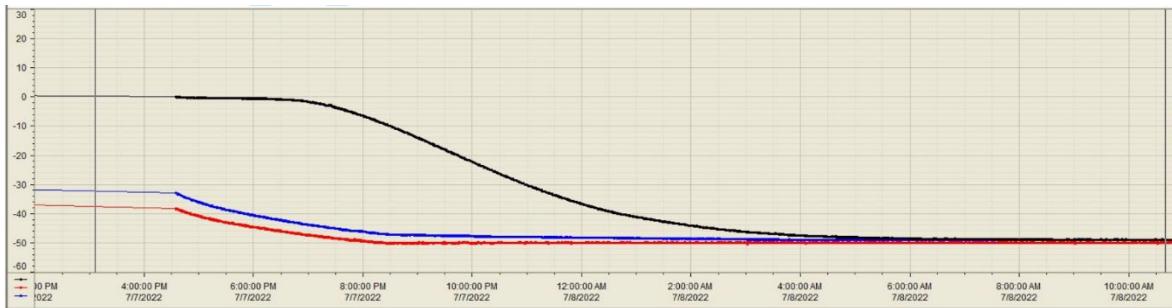


Figure 11 Temperature of Silicon oil and Product temperature during second freezing cycle

Before the freezing, samples for endotoxin and bioburden were taken. All the results met the acceptance criteria (attachment 1 of this report TT237C011), confirming the good results of the holding time challenge.

8.6 DISPENSING OF EXCIPIENTS

For the MabThera process, the excipients for the buffer were weighed in the Dispensing Department according to the Bill of Materials and delivered to Sterile Area 6. The water for injection (WFI) and nitrogen are supplied by Patheon.

The detailed Bill of Materials (BOM), the manufacturing instructions and the process parameters to be recorded, are captured in the relevant manufacturing batch record.

8.7 PREPARATION OF PRIMARY PACKAGING COMPONENTS

Glass vials

Schott vials (code PTH 241582 for MabThera 100mg and code PTH 242584 for MabThera 500mg) are used for both MabThera processes.

The vials were provided in filling room 843 from Dispensing Department to be washed. The vials were manually fed into the Washer Vega8 (LFL013). Vials were conveyed to a rotative carousel where they were washed with WFI and air-blown with compressed air filtered through a 0.22 µm filter.

After the washing process, the vials were conveyed into the depyrogenation tunnel 1250FL DH (TST010). Process parameters were identified and qualified and they are reported in document E878FE01 for the 50 ml format and in document E878BE01 for the 10 ml format.

After exiting the tunnel, the vials passed through an opening into the isolator and the filling machine.

Stoppers

The stoppers (code PTH 273438 both for MabThera 100mg and for MabThera 500mg) were provided by the vendor (West) and delivered to Patheon (4000 stoppers/bag) ready for sterilization. The packaging configuration foresees a double bag packaging, constituted of an internal bag with a rapid transfer port (RTP port GLC DPTE-BetaBag 190/25L) and an external polyethylene (PE) bag.

Stoppers were sterilized and dried in an autoclave (ATC-025 and ATC-026) in Patheon Sterile Area 6, using an appropriate validated cycle. It was used the recipe of the stopper code 273711 which holds also for the stopper code 273438 (sterilization equivalency evaluation according to TF document O927BI01)

After that, the autoclaved bags were connected to the isolator via the RTP port.

Seals (Cap)

Seals (code PTH 243433 for MabThera 100mg and code PTH 273433 for MabThera 500) were directly loaded to the capping machine and applied to the stoppered vials by Machine ALU400 SA5017 (CPL013) crimping Machine without being treated prior (no washing step is executed at Patheon considering that it is already executed by the supplier) to use by Patheon Monza.

8.8 BUFFER PREPARATION

As the first steps of the compounding operation, the buffer solution was prepared in a 1100 L stainless steel tank (RTR342) placed in position RP02 in a grade C area of Sterile area 6 (temperature = 18 °C - 23°C).

Each component concentration is reported in Table 14below.

Table 14 Buffer composition

Description	Starting concentration	Final concentration in the 1100L buffer tank
Sodium citrate dihydrate	NA	7.35 mg/mL
Sodium chloride	NA	9.0 mg/mL
Polysorbate 80 stock solution	NA	0.7 mg/mL

Description	Starting concentration	Final concentration in the 1100L buffer tank
Sodium hydroxide (pH adjuster)	1 N	q.s.ad pH 6.5
Hydrochloric acid (pH adjuster)	1 N	q.s.ad pH 6.5
WFI	N/A	N/A

The final buffer formulation procedure used is reported in Table below. Particularly, each excipient quantity is reported in order to manufacture 991 Kg of buffer (as per standard manufacturing process at the client). Sodium chloride and sodium citrate dihydrate were weighted by the dispensing department directly into a plastic bag and then delivered to the Sterile 6.

The amount of buffer to be added was adjusted based on the concentration of the received API. Details about buffer addition are reported in the following paragraph.

Table 15 Final buffer composition

Components addition	Quantity (grams) to be added to the tank
WFI	875,0 Kg [866,4-883,6] Kg
Sodium Chloride (weighted salt)	8840,0 g ± 1 % [8751,6-8928,4] g
Sodium Citrate Dihydrate (weighted salt)	7220,0 g ± 1 % [7147,8-7292,2] g
Polysorbate 80 stock solution	8000,0 g ± 1 % [7920,0-8080,0] g
WFI to rinse PS80 stock solution bottle	2000,0 g ± 1 % [1980,0-2020,0] g
Hydrochloric Acid stock solution bottle	pH adjustment approx 1700 g
Sodium Hydroxide stock solution bottle	pH adjustment (usually not necessary)
WFI to reach final weight	qs
Total buffer formulation	991,0 Kg ± 1 % [981,2-1000,8]Kg

1N HCl stock solution

4000.3 g of hydrochloric acid was prepared and stored in a 5 L portable glass container under laminar flow at a concentration of 1 N. The solution was prepared as pH adjuster and 1500mL were added in

order to get the final pH. Usually approx. 1,7 Kg is needed at the donor site in order to get the final desired pH value.

In particular, the dispensing department dispensed 391,9 g of HCl. In solution preparation room of sterile area 6 3001,6 g of WFI was added to 5L portable glass container where was then added the amount of HCl dispensed (391,9g). Then, after having added additional aliquot of WFI to reach the solution final weight (4000,3 g), the solution was mixed with magnetic stirrer under laminar flow at maximum speed (250 rpm) which will be the one that did not cause splashes during mixing, until visual checks confirmed the complete dissolution. It was also identified the minimum speed (150 rpm) that created a minimum movement on the surface.

Table 16 Recipe for HCl solution preparation

Component	Quantity (grams)
HCl 37 %	394,2 g [390.3 – 398.1]g
WFI	Approx. 3000 g
WFI to be added to get final weight	qs
Total HCl stock solution formulation	4000 g ± 1%

The main parameters recorded are listed in the table below:

Table 17 HCl solution preparation

Parameter	Results	pCPP (Y/N)
Start of HCl solution preparation (date dd.mm.yyyy, time hh:mm)	11:20 03/07/2022	N
HCl solution start of mixing (time hh:mm) and initial mixing speed	11:31 250 rpm	N
HCl solution end of mixing (time hh:mm)	11:46 03/07/2022	N
End of HCl solution preparation (date dd.mm.yyyy, time hh:mm)	11:46 03/07/2022	N
HCl solution mixing speed modification	150 rpm	N

1N NaOH stock solution

1000,2 g of Sodium Hydroxide was prepared and stored in a 2 L portable glass container under laminar flow at a concentration of 1 N. The solution was prepared as pH adjuster, but it was not

necessary the addition in the buffer tank RTR342 (usually also at Mannheim it is not needed NaOH addition). The solution was however prepared in order to identify the appropriate mixing speed range.

In particular, the dispensing department dispensed 40,1 g of NaOH. In solution preparation room of sterile area 6, 900,2 g of WFI was added to 2L portable glass container where was then added the amount of NaOH dispensed. Then, after having added additional aliquot of WFI to reach the solution final weight (1000,2 g), the solution was mixed with magnetic stirrer under laminar flow at the maximum speed which was the one that did not cause splashes during mixing. Visual checks confirmed the homogeneity of NaOH solution. It was also identified the minimum mixing speed that caused minimum movement of the solution.

Table 18 Recipe for NaOH stock solution preparation

Component	Quantity (grams)
NaOH	40g ± 0.5 %
WFI	Approximately 900 g
WFI to be added to get final weight	q.s
Total NaOH stock solution formulation	1000 g ± 1%

The main parameters recorded are listed in the table below

Table 19 NaOH solution preparation

Parameter	Results	pCPP (Y/N)
Start of NaOH solution preparation (date dd.mm.yyyy, time hh:mm)	13:28 03/07/2022	N
NaOH solution start of mixing (time hh:mm) and initial mixing speed	13:32 03/07/2022 330 rpm	N
NaOH solution end of mixing (time hh:mm)	13:46 03/07/2022	N
End of NaOH solution preparation (date dd.mm.yyyy, time hh:mm)	13:46 03/07/2022	N
NaOH solution mixing speed modification	220 rpm	N

Polysorbate 80 (PS80) stock solution

8000,6 g of polysorbate stock solution was prepared and stored in a 10 L portable glass container under laminar flow. The concentration of polysorbate in the final buffer tank was 0.7 mg/mL. The

starting amount of PS80 was added by the dispensing department directly in the autoclaved 10L bottle. It has to be highlighted that the PS80 container were used as “single use container”, meaning that, once opened, the leftover solution was delivered to the QC Laboratory in order to perform an identification test and then discarded.

The 10L bottle was then wrapped with foil in order to protect the PS80 from light and delivered to sterile 06. Then, WFI was added in sterile 06 in order to get PS80 final concentration.

In particular, the dispensing added 690 g in the glass bottle that was delivered to sterile area 6 where WFI will be added to obtain the final concentration. The PS80 solution was mixed using a magnetic stirring bar, applying a stirring speed between the value which let a minimum movement on the surface (minimum mixing speed) and the one which did not cause splashes or foam of the fluid (maximum mixing speed), until completely dissolved. Visual checks confirmed the homogeneity of PS80 solution.

Once prepared, the PS80 solution could be stored in solution preparation room, protected from the light, for up to 24 hours (starting from PS80 addition), but there was no needing.

Table 20 PS80 stock solution recipe

Component	Quantity (grams)
PS80	690g ± 0.5 %
WFI to be added to get the final weight	q.s
Total PS80 stock solution formulation	8000 g ± 1%

The main parameters recorded are listed in the table below

Table 21 PS80 solution preparation

Parameter	Results	pCPP (Y/N)
Start of PS80 solution preparation (date dd.mm.yyyy, time hh:mm)	03/07/2022 10:16	N
PS80 solution start of mixing (time hh:mm) and initial mixing speed	10:23 03/07/2022 450 rpm	N
PS80 solution mixing speed modification and start of mixing at the new mixing speed	10:27 03/07/2022 350 rpm	N
PS80 solution mixing speed modification and start of mixing at the new mixing speed	10:30 03/07/2022 400 rpm	N
PS80 solution mixing speed modification and start of mixing at the new mixing speed	10:38 03/07/2022 350 rpm	N

Parameter	Results	pCPP (Y/N)
PS80 solution end of mixing (time hh:mm)	12:21 03/07/2022	N
End of PS80 solution preparation (date dd.mm.yyyy, time hh:mm)	12:21 03/07/2022	N

Final buffer preparation

991,2 kg of buffer was prepared and stored in a 1100L stainless-steel vessel, positioned in position RP02. WFI from the loop was added to the tank first. Under mixing at minimum speed identified as 100 rpm during surrogate trial and confirmed during this engineering run. Then sodium chloride (8845,1 g) (weighted salt) and sodium citrate dihydrate (7224,9 g) (weighted salt) were added to the tank through the funnel at the level of the open port. Then, PS80 solution was added into the buffer tank through the funnel and J-tube placed on the open port, in order to minimize foaming. A WFI rinse of the PS80 container was performed. After this, the buffer was mixed for at least 15 minutes (mixing time equal to 56 minutes) when visual check confirmed complete dissolution of excipients and with a mixing speed identified before (100 rpm).

Before adding HCl, a pH measurement for information only was executed in sterile area 6.

HCl (1500 mL) was then added and then pH measured in sterile area 6 and confirmed at QC chemical: the blocking IPC was conforming and so after this point, the mixing was stopped and WFI was added to reach final weight.

After having reached the target weight, a final mixing at 200 rpm for 15 minutes was executed.

The above described excipients order addition was respected.

The parameters recorded during the preparation of the buffer are reported in the table below

Table 22 Parameters recorded during buffer step

Parameter	Results	pCPP (Y/N)
Initial weight of the tank	0 Kg	N
Start of buffer solution preparation (date dd.mm.yyyy, time hh:mm)	06:07 03/07/2022	N
Start of mixing in the tank (date dd.mm.yyyy, time hh:mm)	10:03 03/07/2022	N
Mixing rate (RPM)	100 rpm (100-200 rpm) (*)	Y
Quantity of sodium chloride to be compounded	8845,1 g (8751,6-8928,4) g	N
Quantity of sodium citrate dihydrate to be compounded	7224,9 g (7147,8-7292,2) g	N
Quantity of PS80 solution to be compounded	7996,3 g (7920,0 -8080,0) g	N
Quantity of HCl to be compounded (for pH adjustment)	1500 mL	N

Parameter	Results	pCPP (Y/N)
Excipients order addition	Respected	N
Mixing time	8 hours and 52 minutes (1hours and 53 min)**	Y
End of mixing in the tank before reaching the final weight (date dd.mm.yyyy, time hh:mm)	18:55 03/07/2022	N
Final weight of the tank	991,2 Kg [981,2-1000,8]Kg	Y
Start of mixing in the tank after having reach the final weight of the tank (date dd.mm.yyyy, time hh:mm)	19:11 03/07/2022	N
Mixing rate (RPM) after having reach the final weight of the tank	200 rpm [150-250]rpm (***)	Y
Mixing time after having reach the final weight of the tank	15 minutes	Y
Mixing temperature	20°C [15-25]°C	Y
End of mixing in the tank (date dd.mm.yyyy, time hh:mm)	19:27 03/07/2022	N

(*) range identified during surrogate trial part A (TT237D011)

(**) stirring time identified during surrogate trial part A (TT237D011) but no pH adjustment was executed during surrogate trial run.

(***) Mixing range identified during surrogate trial part A (TT237D011). During engineering we mixed at 200 rpm which can be considered the target value.

All the pCPP indicated in table above have been selected for the following reasons:

- Mixing rate (before and after having reached the final weight of the tank): mixing lower than the range identified during surrogate (TT237D011) can potentially have an impact on the homogeneity of the buffer and higher mixing rate could create excessive foam, polysorbate degradation and consequently affect the quality of the released product
- Final weight of the tank can potentially have an impact on the homogeneity of the buffer and consequently on the quality of the released product
- Mixing temperature can potentially have an impact on the quality of the buffer and consequently on the released product
- Mixing time (before and after having reached the final weight of the tank): lower time can potentially have an impact on the homogeneity of the buffer and consequently affect the quality of the released product

Sampling collection

At the end of buffer compounding, the samples to analyze pH, Osmolality, PS80 check, Endotoxin, and Bioburden were taken. Bioburden were analyzed for information only at this point.

Sampling were performed by the tank RTR342 through Novaseptum. Syringe and Novaseptum bag.

After almost 24 hours (from the start of WFI addition) additional samples was supposed to be taken (through Novaseptum) to analyze for bioburden and endotoxin to challenge buffer holding time in RTR342 tank. As agreed with the client, it was challenged an holding time of 33 hours and 45 minutes, taking samples for endotoxin and bioburden after this timing.

Also in this case, Bioburden was analyzed for information only at this point, because as agreed with the client, even if at donor side bioburden buffer reduction filtration was performed, in Patheon manufacturing process no bioburden reduction filtration is present on the buffer. The bioburden reduction filtration is performed on the BDP.

All samples analyzed met the acceptance criteria (details of the results of the sampling are reported in the attachment #1) confirming the good preparation of the buffer and good results and applicability of the holding time challenged.

8.9 BDS POOLING

The BDS solution was pooled into the cleaned and sterilized compounding tank (RTR343) placed in position RP03 using a peristaltic pump. The recirculation tubing (SGS04049), previously used to ensure product recirculation during the thawing step, was disconnected from the F/T tank and one end was connected to another recirculation tubing (SGS04049) which was connected on the top of the compounding tank through a reducer. All manipulation on the F/T tank were performed under LAF condition in position RP01, to minimize the risk of contamination.

For the pooling, the F/T tank was then moved near to the compounding tank in class C.

The target of rpm of the peristaltic pump was identified during this engineering trials. At the beginning the operators setted (and recorded) the minimum velocity (20 rpm) which let to transfer the solution. After that, the speed was increased (intermediate velocity tested were 30 rpm and 40 rpm) to reach the maximum velocity (50 rpm) which did not cause bubble or turbulence.

For the US F/T tank, the end of recirculation tubing, which remained connected to the tank, was at the F/T tank bottom outlet.

BDS was transferred into the compounding tank via J-tube and dedicated reducer for MabThera manufacturing. The total amount of BDS needed was calculated on the basis of the following formula provided by Roche. The calculation reported below is also reported in the master batch record.

Step 1: Calculate amount of protein needed for batch

$$(a) \text{ batch size [L]} \times \frac{10g}{L} = \text{amount of protein needed [g]}$$

where *batch size [L]* is the input from planning

For the engineering trials, considering that a 250 L batch size was foreseen, the amount of protein needed [g] were:

$$250 \text{ L} \times 10 \frac{\text{g}}{\text{L}} = 2500 \text{ g}$$

Step 2: Calculate amount of DS needed

$$(b) \text{ amount of DS [Kg]} = \frac{\text{amount of protein [g] from (a)}}{\text{protein concentration of DS} \frac{\text{g}}{\text{L}}} \times \text{density of DS} \frac{\text{Kg}}{\text{L}}$$

Where density of DS: 1,024 Kg/L

Protein concentration of DS [g/L] is the input form CoA for each DS batch

For the engineering trial, considering that the protein concentration of DS reported in the CoA was 55 mg/mL, the amount of DS [Kg] foreseen was:

$$\frac{2500 \times 10^3 \text{ mg}}{55 \text{ mg/mL}} \times \frac{1,024 \text{ Kg}}{10^3 \text{ mL}} = 46,55 \text{ Kg.}$$

After the pooling, the peristaltic pump was inverted and then the tubing was manually squeezed with the dedicated tool in order to reduce as much as possible the line loses.

The BDS leftover (which was equal to approximately 182,06 Kg which is a value inside the validated range for re-freezing) was delivered to dispensing.

For the engineering trials, the F/T tank was additionally stored at 2-8°C for 3 days and 34 minutes, before re-freezing.

At the end of this holding time challenge, F/T tank was transferred in the thawing room where samples for endotoxin and bioburden were taken as per Addendum I and the recirculation tubes were later discharged. Then, the F/T tank was re-frozen and then stored at -20°C . The delivery of the F/T tank to dispensing was performed as soon as the BDS pooling was completed.

For every new thawing cycle, new disposable recirculation tube will be used.

After having transferred the amount of needed BDS in the RTR343 1100L tank, operators weighted the RTR343 tank and calculated the real amount of protein transferred in the RTR343 tank.

$$(c) \text{ protein actually transferred [g]} = \frac{\text{amount DS actually transferred [Kg]} \times \text{protein concentration DS tank} \frac{\text{g}}{\text{L}}}{\text{density of DS} \frac{\text{Kg}}{\text{L}}}$$

$$\text{protein actually transferred [g]} = \frac{46,4 \text{ Kg} \times 55 \text{ mg/mL}}{1,024 \text{ Kg}/10^3 \text{ mL}} = 2492,2 \text{ g}$$

Where **Protein concentration of BDS [g/L]** is the input form CoA for each BDS batch

The parameters recorded during the pooling of the BDS are reported in the table below:

Table 23 Parameters recorded during BDS pooling

Parameter	Results	pCPP (Y/N)
Initial weight of the compounding tank	0 Kg	N
Start of transfer (date dd.mm.yyyy, time hh:mm)	12:27 04/07/2022	N
Peristaltic pump mixing rate (RPM)	20-50 rpm	N
End of transfer (date dd.mm.yyyy, time hh:mm)	12:52 04/07/2022	N
Final weight of the compounding tank	46,4 Kg (*)	Y

(*) see Table 23

The pCPP indicated in table above have been chosen for the following reasons:

- Final weight in the compounding tank can potentially have an impact on dilution formula and so on at least protein content and potency of the released product.

8.10 BUFFER DILUTION

For MabThera production, after pooling was completed, the buffer was added through SGS03744 (“Y” tank connector) and SGS04044 (“extension transfer tubing”) assemblies, in order to get the desired final composition. Buffer amount to be transferred was calculated based on BDS protein content stated in BDS CoA provided by Roche, the data obtain in **step 1** and **step 2** reported in paragraph 6.1.6 and following the below formula shared by Roche:

Step 3 Add buffer

Based on the real amount of protein transferred (c), it was calculated the batch size according to the following formula:

- Calculate batch size

$$(d) \text{final batch size [Kg]} = \frac{\text{totale protein transferred [g]from (c)}}{10 \frac{g}{L}} \times \text{density of DP} \left[\frac{Kg}{L} \right]$$

Where the **density of DP** is 1.012 Kg/L

$$\text{final batch size [Kg]} = \frac{2492,2 \text{ g}}{10 \frac{\text{g}}{\text{L}}} \times 1,012 \left[\frac{\text{Kg}}{\text{L}} \right] = 252,2 \text{ Kg}$$

After that, the following amount of buffer was calculated to be added, up to reach the final batch size calculated in (d)

- Buffer to be added = final batch size [Kg] – amount of BDS actually transferred [Kg]
= 252,2 Kg – 46,4 Kg = 205,8 Kg

The calculation for the buffer addition during the manufacturing of the batch was reported in the master batch record.

No buffer filtration was foreseen from the process before the transfer in the compounding tank. The transfer was performed through nitrogen at a pressure equal to 0,5 barg. No needing of adjustment because no foaming during transfer was present.

After buffer addition, a mixing step was performed, setting the mixing speed at maximum value identified during surrogate runs (250 rpm) in order to perform a stress study. For every time point (0, 15, 30 and 45 minutes) it was performed a visual check which confirmed the absence of splashes.

Sampling collection

At the start of mixing in compounding tank, samples to analyze visible and subvisible particles were taken (these samples are used to evaluate the contribution of worst case mixing to particles formation).

During the mixing step, a degradation study (purity IE-HPLC and SE-HPLC) was performed. The samples were taken in triplicate at the bottom of the tank from the minimum batch size condition (worst case) after having mixed at maximum speed. The maximum mixing speed identified during surrogate runs was 250 rpm. The mixing speed was set at this parameter. The timepoints to evaluate the stress study were: after 15 minutes, after 30 minutes and after 45 minutes. Degradation samples as purity were also collected.

At the end of the mixing, samples for leachable control (this sample were not analyzed because it was an extra control sample as per Roche document VAL-0212060 and the sample taken at the end of the F/T was representative as control sample), pH, appearance, Color, protein content, Osmolality, visible and subvisible particles were collected. All the samples were taken through Novaseptum.

All samples analyzed met the acceptance criteria (details of the results of the sampling are reported in the attachment #1) confirming the good preparation of the bulk drug product.

Moreover, all samples analyzed for purity met the acceptance criteria (details of the results of the sampling are reported in the attachment #1) and visible and subvisible particles at the end of mixing were comparable with the visible and subvisible particles before mixing, the bulk drug product can be mixed at maximum velocity (250 rpm) even for 45 minutes, without any impact on the quality of the released product.

The parameters recorded during the compounding of the BDS are reported in the table below:

Table 24 Parameters recorded during compounding

Parameter	Results	pCPP (Y/N)
Initial weight of compounding tank	47,2 Kg (*) (**)	N
Start of buffer transfer (date dd.mm.yyyy, time hh:mm)	15:24 04/07/2022	N
End of buffer transfer (date dd.mm.yyyy, time hh:mm)	15:39 04/07/2022	N
Start of mixing in compounding tank (date dd.mm.yyyy, time hh:mm)	04/07/2022 15:57 (first timepoint) 16:17 (second timepoint) 16:38 (third timepoint)	N
Mixing rate (RPM)	Maximum velocity =250 rpm (150-250)rpm (****)	Y
Final weight of compounding tank	253,0 Kg (***)	Y
Mixing temperature	18°C [2-25] °C (*****)	Y
Mixing time	Time ≤ 45 minutes/Record Minimum time challenged during surrogate trial A (TT237D011) was 15 minutes.	Y
End of mixing in compounding tank (date dd.mm.yyyy, time hh:mm)	04/07/2022 16:12 (first timepoint) 16:32 (second timepoint) 16:53 (third timepoint)	N

(*) (**) :between the weight of the compounding tank after BDS pooling (*) and before pooling of buffer there is a difference of 0,8 Kg which is due to the weight of assemblies for transfer the buffer. This second weight is taken only for operative purpose considering that assemblies is added and connector is removed with respect to set up present when tare was executed.

(***) final weight of compounding tank is 0,8 Kg higher than the batch size (252,2 Kg) because it is exactly the weight of the assemblies needed for the transfer of the buffer.

(****) range identified during MabThera surrogate trial A (TT237D011)

(*****) range is 2-25°C considering that the manufacturing process allow the possibility to pool the BDS straight after the 2-8°C cold storage without the needing of an equilibration step.

The pCPP indicated in table above have been selected for the following reason:

- weight of compounding tank together with mixing rate and mixing time potentially could impact product quality:
 - o Low volume with high mixing speed and high mixing time can potentially have an impact at least on purity, particle formation, CoC
 - o High volume with low mixing speed and low mixing time can potentially have an impact on at least the protein content and purity
- Mixing temperature potentially could impact on at least purity and protein content

8.11 BIOBURDEN REDUCTION FILTRATION AND STORAGE

The bulk drug product contained in the compounding vessel was transferred (after 19 hours and 15 minutes from the addition of the BDS in the compounding tank) , by nitrogen pressure, to the storage stainless-steel vessel placed in position RP04 through a Bioburden reduction line (SGS04047) which was equipped with an incorporated filter (FILTER-OPTICAP XL 10 CAPSULE 0.22 µm DURAPORE (KVGLG10HH1)) and through SGS03744 assembly ("Y" tank connector provided with steam thru and Aseptiquick connections).

Filtration pressure applied at the donor site during the bioburden reduction filtration is 0.5-1.0 bar. To minimize foam creation during the transfer, as a result of surrogate trial runs, the pressure applied in the RTR343 tank was 0,6 barg. During the bioburden filtration step, there was no need to control the temperature and no flush prior to filtration is requested.

According to Addendum I of protocol TT237B011, at the beginning at the middle and at the end of the bioburden filtration, samples from the bottom of the compounding tank (as representative samples of bottom, middle and top of the tank) were taken to gain information on the acceptance criteria to be applied for the bulk homogeneity study.

After the transfer in the storage tank, the solution was not needed to be mixed before the sterilizing filtration, according to the manufacturing process established at the donor site. Only during cooling down to 2-8°C a mixing at minimum mixing speed (50 rpm) was performed to have a homogeneity in temperature.

The mixing was activated on 06/07/2022 at 7:49 and it was switched off on 06/07/2022at 9:21 when the probe inside the tank measured 8°C. The temperature than went down to 5°C at approximately 10:20 on 06/07/2022. The Temperature trend is reported in attachment #9.

During these engineering runs, approximately 24 hours at RT and approximately 24 hours at 2-8°C was challenged. These timing will be validated during the PPQ. Exactly timing challenged during this engineering runs will be reported in the dedicated paragraph 8.0.

At the end of bioburden reduction filtration, a nitrogen overlay (approximately 0.5 barg) in the storage tank was applied to the tank.

After intermediate storage at 2-8°C, there was no need to re-equilibrate the solution at room temperature before starting the filling considering that the density and viscosity features of the product will not be impacted due to the cool storage period, as per Roche indication (ref TEC-0213431).

The parameters recorded during the bioburden reduction filtration of the BDS and the following storage are reported in the table below:

Table 25 Parameters recorded during BB filtration and storage steps

Parameter	Requirement	Results	pCPP (Y/N)
Filtration pressure	[0,5-1]bar	Ptarget=0,6 Barg range [0,5-1barg]	Y
Volume to surface area ratio (batch size / filter area)	Record	342,19 Kg/m ²	Y
Filtration time	Record	44 minutes	N
Filter integrity test post use	Conform if pressure ≥ 3450 mbar	3500 mbar	Y
Initial weight of storage tank	Record	0 Kg	N
Start of formulated bulk transfer (date dd.mm.yyyy, time hh:mm)	Record	07:42 05/07/2022	N
End of formulated bulk transfer (date dd.mm.yyyy, time hh:mm)	Record	08:26 05/07/2022	N
Final weight of storage tank	Record	249,8 Kg	N
Temperature in the compounding tank at end of bioburden reduction filtration	[15-25°C]	19°C [15-25°C]	N
Temperature in the storage tank	[15-25°C]	19°C [15-25°C]	N
Mixing rpm during cooling down (*)	Target = 50 rpm	50 rpm	N

Parameter	Requirement	Results	pCPP (Y/N)
Mixing time during cooling down (*)	Record	1 hours and 32 minutes	N

(*)These parameters were evaluated during engineering trial because storage at 2-8°C was performed. During commercial, considering that this step is optional, they will be applied only in case optional storage at 2-8°C will be performed.

The pCPP indicated in table above have been selected as they might impact product quality (filtration pressure together with volume to surface area ratio can potentially have an impact on attributes like purity, protein content, CoC particulates potency).

Finally, filters integrity test post use can potentially impact on bioburden

Sample collection

Bioburden samples were taken before the bioburden filter at the end of compounding (from RTR343 tank) after 19 hours and 44 minutes (at the end of the bioburden reduction filtration step). Samples were taken for information only and the results was <1CFU/100ml.

Sample to analyze endotoxin, bioburden, were taken after 23 hours and 38 minutes in the storage tank to challenge holding time at room temperature. The same samples were taken after additionally 24 hours at 2-8°C to challenge holding time at 2-8°C. Same results for bioburden was obtained at the two time points, confirming the good results of the holding time challenge.

In addition VPHP control samples were taken as a control for the VPHP study performed during filling.

These samples were taken from the storage tank (RTR344) through Novaseptum.

Finally, samples before the first sterilizing filter were taken at the end of sterilizing filtration step for the first filling (MabThera 500 mg) to analyze endotoxin and bioburden to challenge the holding time. Same results for bioburden was obtained with respect to samples taken at time zero in the storage tank, confirming the good results of the holding time challenge.

Even if the holding time challenge was not performed during the second filling (MabThera 100 mg), the same sampling (bioburden and endotoxin) was performed also at the end of the second filling (MabThera 100 mg), as routine IPC sampling during commercial manufacturing. Moreover, for engineering trials purpose, these sampling will be used as information considering that the solution was additionally stored in the storage tank, before the second filling (100 mg), while performing Xtrema machine format part changing and VPHP cycle in preparation of the filling of the second vials format (100 mg).

It must be noted that this additional storage is not process related, but it was performed only during engineering trials to be able to split the filling in the two formats.

Indeed, same results for bioburden was obtained with respect to samples taken at end of the first filling confirming that no adverse occurrences happened due to the holding time for splitting the formats.

8.12 STERILE FILTRATION

After the storage and before starting with the filling operations, the RTR344 1100L tank was moved by electrical transpallet from the solution preparation room to the filling room. No mixing step was required before starting with the filling.

Before filling, the bulk solution was sterilized through two (2) 0.22 μ m sterilizing grade membrane filters OPTICAP XL 10 CAPSULE 0.22 μ m DURAPORE PVDF (KVGLG10HH1) incorporated in series in the SGS03875 assembly. Particularly the assembly with the incorporated filters is sterilized by gamma irradiation by the supplier.

Particularly, the assembly was connected through a sterile-to-sterile connection to the T assembly connected to the Y assembly placed at the bottom of the storage tank at one end and to the filling bag (filling bag placed inside isolator) at the other end. The sterile filtration was performed in grade C through nitrogen (the details about pressure during sterilizing filtration are reported in the following paragraph).

The filtration and the filling temperature was equal to 2-25°C, considering the possibility to keep the product at 2-8°C and no equilibration before filling.

During engineering run, a filter flush study was performed, prior to starting with the sterile filtration and the related amount of solution to be discarded was evaluated (see attachment 1).

After the sterile filtration, the solution was conveyed into an 8 L filling bag (during engineering trials the minimum working range set was 50% of the net weight (set as 5 kg) and maximum working range was 85% of the net weight (set as 5 Kg)).

The parameters monitored during sterilizing filtration are reported in the table below.

8.12.1 MabThera 500 mg

Table 26 Parameters documented during sterilizing filtration

Parameter	Results	pCPP
Filter flush with product at the beginning of the sterilizing filtration	2000 mL	Y

Parameter	Results	pCPP
Filtration ΔP on filters	$\Delta P \leq 1$ bar (max value recorded 0,59 bar)	Y
Volume to surface area ratio (*)	342,19 Kg/m ²	Y
Filtration time	2 hours and 36 minutes	Y
Temperature during filtration	Temperature between range [2-25°C]	Y

(*) for this point we can rely on the calculation already performed at bioburden reduction filtration step and storage, considering that:

- no weight load is present in filling room, the weight was considered (as worst case) the weight in the storage tank at the end of the bioburden reduction step;
- bioburden filter and sterilizing filter are identical

As per microbial retention study, the maximum filter contact time validated is 72 hours (R-21-04210-BRTA).

The pCPP indicated in table above have been selected for the following reason:

- volume to surface ratio combined to temperature and flush amount with product could potentially have an impact on adsorption and therefore on attributes like protein content and polysorbate content
- volume to surface area ratio in combination with pressure, temperature could potentially have an impact on the compatibility (attributes like Protein content, CoC, Particulate, purity, potency)
- pressure in combination to Volume to surface area ratio, temperature could potentially have an impact on filter capacity and so on attributes like protein content, CoC Particulate analysis and purity

Sample collection

The filter flush aliquot was taken from the sampling assembly connected to the sterilizing filter assembly.

As per Millipore's recommendation, the volume of flush for one filter is approximately 1000 mL. Considering two filters in series but considering that the product that passes through the first filter and second filter is the same, circa 2000 mL was flushed. Aliquoted every 500 mL should have been taken. Instead, it was taken 5 aliquots: the first aliquot was 250 mL, the second aliquot was 500 mL, the third aliquot was 250 mL, the fourth aliquot was 500 mL and it was taken an additional fifth aliquot of 500 mL to reach the 2000 mL.

The aliquots were analyzed for protein content only for MabThera 500 mg (attachment 1). Even from the third aliquot (after having flushed 1000 mL) the protein content met the acceptance criteria and after 2000 mL the protein content was at the target of the acceptance criteria.

The set up of the sampling assembly connected to the sterilizing filter assembly is reported in the figure below.

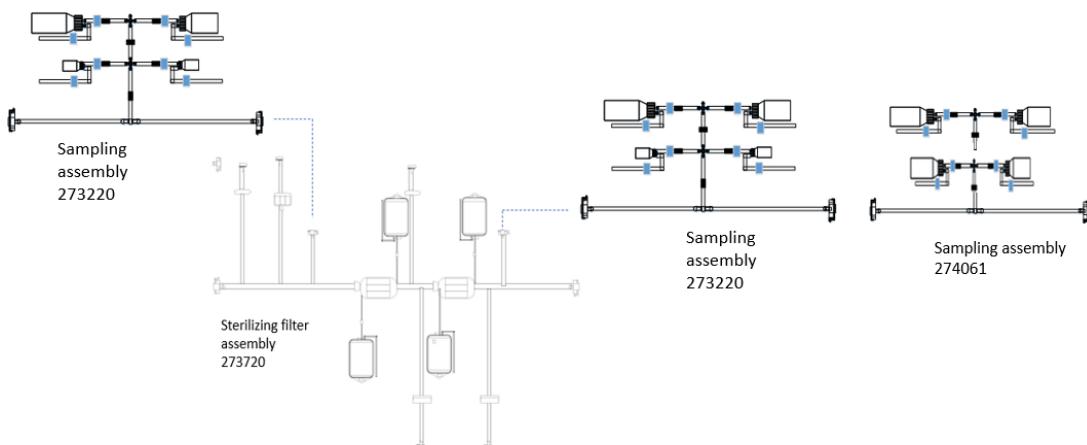


Figure 12 set up for sampling

7.2.1.1 Execution of Pre-use Filter Integrity test

Before performing all the connections, a pre-use filter integrity test on both sterilizing filters was executed.

The following steps were performed:

- The filter was wetted with WFI for ten minutes
- Pre-use test was performed using the Bubble point method

The recipe to be used for the filter integrity test and related parameters was reported in the master batch record. The bubble point value is ≥ 3450 mbar at 15-25 °C for KVGLG10HH1 filter, as per Millipore recommendation.

7.2.1.2 Execution of Post-use Filter Integrity test

After the filtration, the filter integrity test (FIT) of filters included in the disposable assembly 273504 was performed offline.

The following steps were executed for each filter:

- Disconnection of the filter from the disposable assembly 273504
- Flush of the filter with WFI
- Filter integrity test with bubble point method in accordance to filter's supplier data

The filters were the following:

- 2 (two) nitrogen filters KEGBG050HH00 (≥ 1170 mbar nitrogen with 100% IPA)

- 1 (one) WFI filter KA3EKVP6G (≥ 3320 mbar nitrogen with WFI)
- 2 (two) barrier filters MSP010012 (≥ 1280 mbar nitrogen with 70/30 % IPA/water).
- 2 (two) sterilizing filters KVGLG10HH1 (≥ 3450 mbar nitrogen with WFI)

During commercial manufacturing, only the filter closer to the filling line will be integrity tested and, in case of failure, the other filter will also be tested. In the present trial, both filters were tested in order to assess the entire filtration assembly.

The parameters documented are listed in **table** below.

Table 27 Parameters recorded during integrity tests

Parameter	Criteria	Bubble point results	pCPP
Pre-Use Sterilizing Filter I Integrity test	Conform If bubble point ≥ 3450 mbar	3800 mbar Conforming	Y
Pre-Use Sterilizing Filter II Integrity test	Conform If bubble point ≥ 3450 mbar	3900 mbar Conforming	Y
Post-use Sterilizing Filters I Integrity test	Conform If bubble point ≥ 3450 mbar	3450 mbar Conforming	Y
Post-use Sterilizing Filters II Integrity test	Conform If bubble point ≥ 3450 mbar	3700 mbar Conforming	Y
Barrier I Filters Integrity test	Conform If bubble point ≥ 1280 mbar	1400 mbar Conforming	N
Barrier II Filters Integrity test	Conform If bubble point ≥ 1280 mbar	1400 mbar Conforming	N
WFI filter Integrity test	Conform If bubble point ≥ 3320 mbar	3400 mbar Conforming	N
Nitrogen I filters Integrity test	Conform If bubble point ≥ 1170 mbar	1400 mbar Conforming	N
Nitrogen II filters Integrity test	Conform If bubble point ≥ 1170 mbar	1400 mbar Conforming	N

The pCPP indicated in table above have been selected for the following reason:

- Pre-Use Sterilizing Filter Integrity test can have an impact on the sterility of the released product
- Post-use Sterilizing Filters Integrity test can have an impact on the sterility of the released product

For these engineering trials, the approx. first 125 L of solution was filtered with the setup reported in paragraph 4.3 for manufacturing MabThera 500 mg.

After this, the extension tube, the filter assembly and the filling bag were changed, to have the configuration reported in paragraph 4.4 for the manufacturing of MabThera 100 mg with the second 125 L of solution.

8.12.2 MabThera 100 mg

Table 28 Parameters documented during sterilizing filtration

Parameter	Results	pCPP
Filter flush with product at the beginning of the sterilizing filtration	2000 mL	Y
Filtration ΔP on filters	$\Delta P \leq 1$ barg (max value recorded 0,38 bar)	Y
Volume to surface area ratio (*)	342,19 Kg/m ² (*)	Y
Filtration time	3 hours and 54 minutes	Y
Temperature during filtration	Temperature between range [2-25°C]	Y

(*) for this point we can rely on the calculation already performed at bioburden reduction filtration step and storage, considering that:

- no weight load is present in filling room, the weight was considered (as worst case) the weight in the storage tank at the end of the bioburden reduction step;
- bioburden filter and sterilizing filter are identical

The pCPP indicated in table above have been selected for the following reason:

- volume to surface ratio combined to temperature and flush amount with product could potentially have an impact on adsorption and therefore on attributes like protein content and polysorbate content
- volume to surface area ratio in combination with pressure, temperature could potentially have an impact on the compatibility (attributes like Protein content, CoC, Particulate, purity, potency)
- pressure in combination to Volume to surface area ratio, temperature could potentially have an impact on filter capacity and so on attributes like protein content, CoC Particulate analysis and purity

Sample collection

The filter flush aliquot (reported in Table 28) was taken from the sampling assembly connected to the sterilizing filter assembly.

For MabThera 100 mg, considering the same BDP and same filtration assembly as of MabThera 500 mg, no analysis on the aliquot was performed. However, the same amount of solution was discharged in order to have similar condition between the two filling.

The set up of the sampling assembly connected to the sterilizing filter assembly is reported in the figure below.

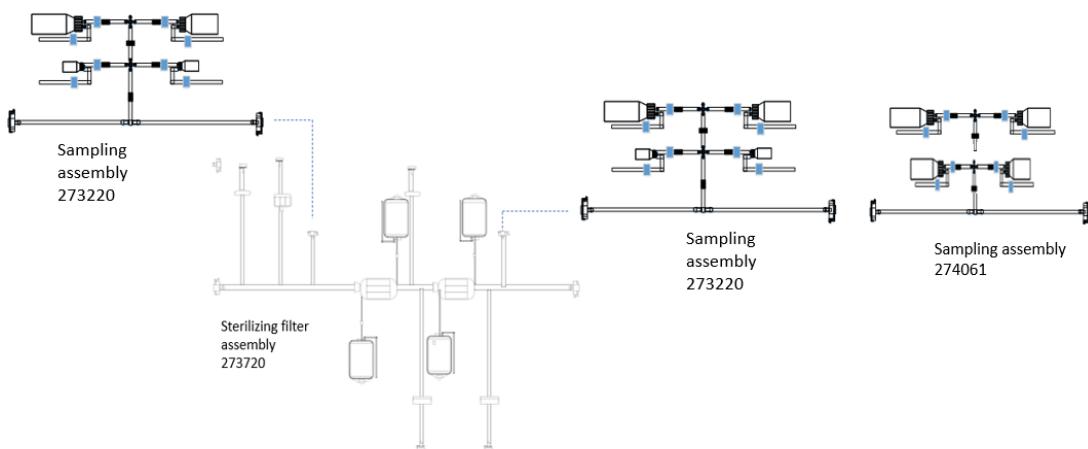


Figure 13 set up for sampling

7.2.1.3 Execution of Pre-use Filter Integrity test

Before performing all the connections, a pre-use filter integrity test on both sterilizing filters was executed.

The following steps were executed:

- The filter was wetted with WFI for ten minutes
- Pre-use test was performed using the Bubble point method

The recipe to be used for the filter integrity test and related parameters was reported in the master batch record. The bubble point value is ≥ 3450 mbar at 15-25 °C for KVGLG10HH1 filter, as per Millipore recommendation.

7.2.1.4 Execution of Post-use Filter Integrity test

After the filtration, the filter integrity test (FIT) of filters included in the disposable assembly 273504 was performed offline.

The following steps were executed for each filter:

- Disconnection of the filter from the disposable assembly 273504
- Flush of the filter with WFI

- Filter integrity test with bubble point method in accordance to filter's supplier data

The filters are the following:

- 2 (two) nitrogen filters KEGBG050HH00 (≥ 1170 mbar nitrogen with 100% IPA)
- 1 (one) WFI filter KA3EKVP6G (≥ 3320 mbar nitrogen with WFI)
- 2 (two) barrier filters MSP010012 (≥ 1280 mbar nitrogen with 70/30 % IPA/water).
- 2 (two) sterilizing filters KVGLG10HH1 (≥ 3450 mbar nitrogen with WFI)

During commercial manufacturing, only the filter closer to the filling line will be integrity tested and, in case of failure, the other filter will also be tested. In the present trial, both filters will be tested in order to assess the entire filtration assembly.

The parameters documented are listed in **table** below.

Table 29 Parameters recorded during integrity tests

Parameter	Requirements	Bubble point -value	pCPP
Pre-Use Sterilizing Filter I Integrity test	Conform if bubble point ≥ 3450 mbar	3850 mbar Conforming	Y
Pre-Use Sterilizing Filter II Integrity test	Conform if bubble point ≥ 3450 mbar	4000 mbar Conforming	Y
Post-use Sterilizing Filters I Integrity test	Conform if bubble point ≥ 3450 mbar	3550 mbar Conforming	Y
Post-use Sterilizing Filters II Integrity test	Conform if bubble point ≥ 3450 mbar	3550 mbar Conforming	Y
Barrier I Filters Integrity test	Conform if bubble point ≥ 1280 mbar	1400 mbar Conforming	N
Barrier II Filters Integrity test	Conform if bubble point ≥ 1280 mbar	1400 mbar Conforming	N
WFI filter Integrity test	Conform Conform if bubble point ≥ 3320 mbar	3600 mbar Conforming	N
Nitrogen I filters Integrity test	Conform Conform if bubble point ≥ 1170 mbar	1700 mbar Conforming	N
Nitrogen II filters Integrity test	Conform Conform if bubble point ≥ 1170 mbar	1700 mbar Conforming	N

The pCPP indicated in table above have been selected for the following reason:

- Pre-Use Sterilizing Filter Integrity test can have an impact on the sterility of the released product
- Post-use Sterilizing Filters Integrity test can have an impact on the sterility of the released product

8.13 VIALS FILLING AND STOPPERING

The filling operation was carried on using the X TREMA filling machine, which is installed into an isolator (Grade A), surrounded by grade C. The aseptic filling process in line is an automated system with eight dosing peristaltic pumps and needles. The machine automatically performs the following steps:

- Vial transfer at machine in-feed
- Weighing of the tare of all the vials (IPC 100%)
- Liquid solution dosing into vials with 8 filling needles
- Weighing of the gross weight of all the vials (IPC 100%)
- Rejection of non-conforming vials/transferring conforming vials to the machine outfeed

Also, the filling step was divided into two: approx. 125 L of the solution to manufacture MabThera 500 mg in vials 50 mL, stoppers, and grey seals. After this, a change of format in the filling machine was performed and approx. 125 L of the solution to manufacture MabThera 100 mg in vials 10 mL, stoppers and red seals. Between the filling of the two formats, during the mechanical activities which was performed to change the format in the filling machine and the VPHP cycle, the BDP in the storage tank was kept at 2-8°C.

8.13.1 MabThera 500 mg

During the filling of MabThera 500 mg, after having taken samples for VPHP and leachable, an optimization (eg: needle set up and distance from level of liquid) was performed to minimize foam creation.

In particular, the distance between the tip of the needle and the level of the liquid during the filling was reduced by 20 mm (Y cam rise nozzle head changed from 30 mm to 50 mm). After the optimization, a little improvement in the foaming inside the vials was noticed.

Below in table, it is reported the fill weight parameters for MabThera 500 mg presentation. Moreover, alert and action limits reported in the tables below were defined based on data provided by dosing tests performed at the supplier (IMA). The maximum filling speed was identified during surrogate batches (100 vials/min for MabThera 500 mg) and it was set at the beginning of the trial and it was confirmed during this engineering trial.

The maximum filling speed is considered as worst case speed in terms of filling accuracy.

During the filling steps, the process interruption events, and relative time were recorded in the machine record according to SOP 000136665.

Table 30 MabThera 500 mg filling weight

Dosage	Limits	Fill volume (ml)	Fill weight (g)
500 mg/vial	Upper Action Limit	52,099	52,620
	Upper Alert Limit	51,366	51,880
	Target	51,109	51,620
	Lower Alert Limit	50,851	51,360
	Lower Action Limit	50,396	50,900
	Target filling speed: 100 vials/min		
	Density of BDP= 1,01 g/mL		

The parameter documented during the sterilizing filtration and filling operations are listed in Table below.

Table 31 Parameters documented during sterilizing filtration and filling Operations

Parameter	Specification	Results	pCPP
Filling machine speed	Target =100 vials/min [80 vials/min – 100 vials/min] (*)	100 vials/min	Y
Start of filling (date dd.mm.yyyy, hh:mm)	Record	10:16 07/07/2022	N
End of filling (date dd.mm.yyyy, hh:mm)	Record	12:40 07/07/2022	N
Pressure value in storage tank during filling	Target = 0,4 barg, Working range $\pm 0,1$ bar	$0,43 \leq P \leq 0,63$ bar (see graph below)	N
IPC vials weight	Record	Record -see data below	Y

(*) minimum filling speed is the one validated per media fill.

The lowest limit for the minimum machine filling speed was set at 80 vials/min, as per media fill. After the additional water trial (executed after the engineering runs in accordance with Technical Memo TT237Z051) the minimum filling speed was optimized to 90 vials/min in order to avoid any risk of wetting the needles during the filling.

The pCPP indicated in table above have been selected for the following reason:

- Filling machine speed: higher filling speed can impact on product quality (due to shear stress), uniformity of dosage, extractable volumes and excessive foam creation; lower machine speed could bring the process outside the media filled validated range and so it could have potentially an impact on sterility of the released product;
- IPC vials weight could affect at least the uniformity of dosage and extractable volumes of the released product;

Before the start of the solution transfer, the storage tank was stored at nitrogen overpressure at 0,5 bar ± 0,1 bar.

It was checked the pressure trend of the storage tank during the filling, after the storage. According to Engineering protocol TT237B011 and relative MBR, the setpoint requested was 0,4 bar. Solution transfer from the storage tank to the filling bag was done by pressuring the storage tank with nitrogen, setting a set point and a working range. In particular, the setpoint requested was 0,4 barg and the working range was ± 0,1 bar. If the pressure is > 0,4 + 0,1 bar the system opens automatically the vent, if the pressure is < 0,4 – 0,1 bar, the system gives automatically nitrogen.

From the audit trail it was checked that at 10:03:30 on 07/07/2022 the operators set on HMI 0,8 bar as set point. Immediately after (10:04:04), the pressure was set at 0,4 bar as request by the MBR. As a consequence, at 10:16 the start of the filling (which is after the filer flush and needle purging) was performed at pressure >0,5 bar and no foam creation in the filling bag was observed.

In the graphs below it is reported:

- The trend of the pressure in the storage tank in blue
- The trend of the rpm in the storage tank (0 rpm because no mixing) in green
- The trend of the temperature in the storage tank in red

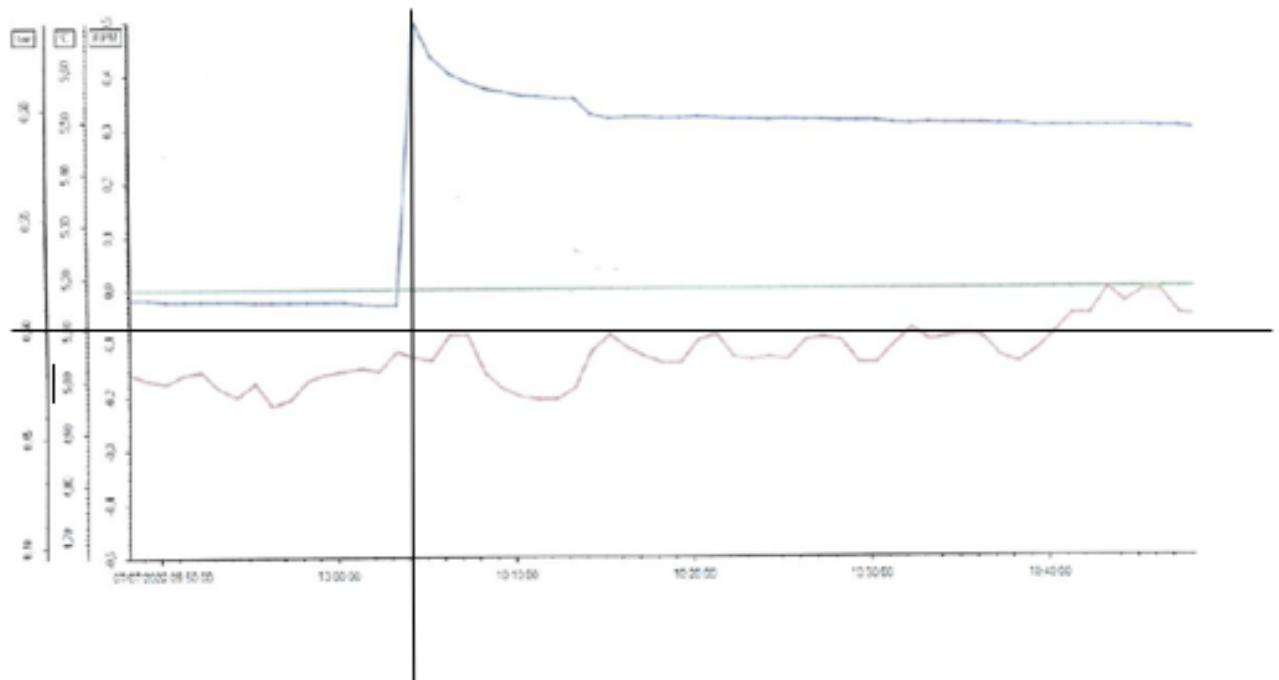


Figure 14 Pressure trend in the storage tank - part I

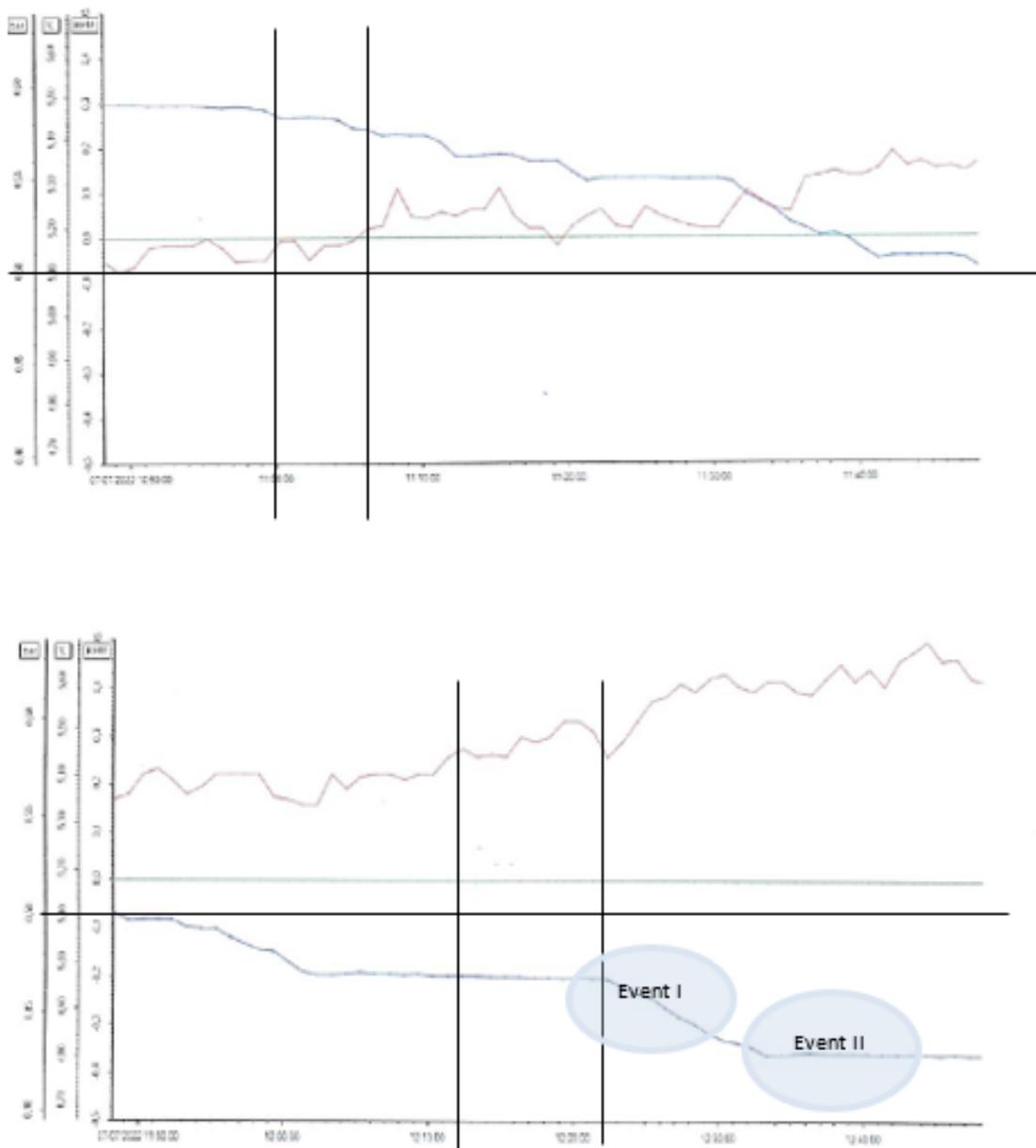


Figure 15 Pressure trend in the storage tank - part II and III

During the last part of the filling two events were identified and were mapped on the graph:

- Event I: is the closure and reopening of the bottom valve;
- Event II: it is the emptiness of the filling bag which is worsening by the low pressure during transfer

The details about the two events will be reported after the rejects analysis.

For MabThera 500 mg filling, the analysis between goods and machine rejects (both during set up (purge and calibration) and from the machine during production) which were obtained, dividing them between mode of working of the machine, are reported in the table below.

Table 32 Scarp analysis

Mode	Good	Machine rejects
Purge	0	54
Calibration	132	48
Production	2109	77
End of production	67	20

In particular, during production mode about 3% of rejects were done considering only the filled vials

Table 33 Rejects rate during production mode

Rejects rate during production mode – filled vials	3 %
Rejects – filled vials [qty]	67
Total = goods + rejects filled vials [qty]	2176

Considering the results listed above, a deeper analysis was performed on the type of rejects obtained during production mode and end of production mode.

- Production mode

It was checked the type of rejects obtained. First it was checked if the vial was weighted or not and secondly if specific reoccurrence between scales and needles was present.

Table 34 Production mode: deeper analysis on cause of rejects

Production mode		
Cause of rejects	# rejects	Note
stopper not selected	1	vial weighted
filling interrupted	9	vial not weighted

Production mode		
Cause of rejects	# rejects	Note
net OOS scale 1	27	vial weighted: 26 from needle 1, 1 form needle 4 (*)
net OOS scale 2	28	vial weighted: 26 from needle 5, 2 from needle 2 (*)
net OOS scale 3	12	vial weighted: 8 from needle 3, 4 from needle 6 (*)

(*) For MabThera 500 mg 6 peristaltic pumps are used

- End of production mode

It was checked the type of rejects obtained. First it was checked if the vial was weighted or not and secondly if specific reoccurrence between scales and needles was present.

Table 35 End of production mode: deeper analysis on cause of rejects

End of production mode		
Cause of rejects	# scarp	Note
error scale 1 tare	1	vial not weighted
error scale 2 tare	1	vial not weighted
error scale 3 tare	1	vial not weighted
error scale 1 gross	1	vial not weighted
error scale 2 gross	1	vial not weighted
error scale 3 gross	1	vial not weighted
net OOS scale 1	5	vial weighted (4 from needles 1 and 1 from needle 4)
net OOS scale 2	4	vial weighted (3 from needle 5 and 1 from needle 2)
net OOS scale 3	5	vial weighted (4 from needle 3 and 1 from needle 6)

Then, it was plotted the trend of the filling weight dived also per needles in order to check if there was a specific reoccurrence in the needles, after having first checked that no specific occurrence between the scale was present.

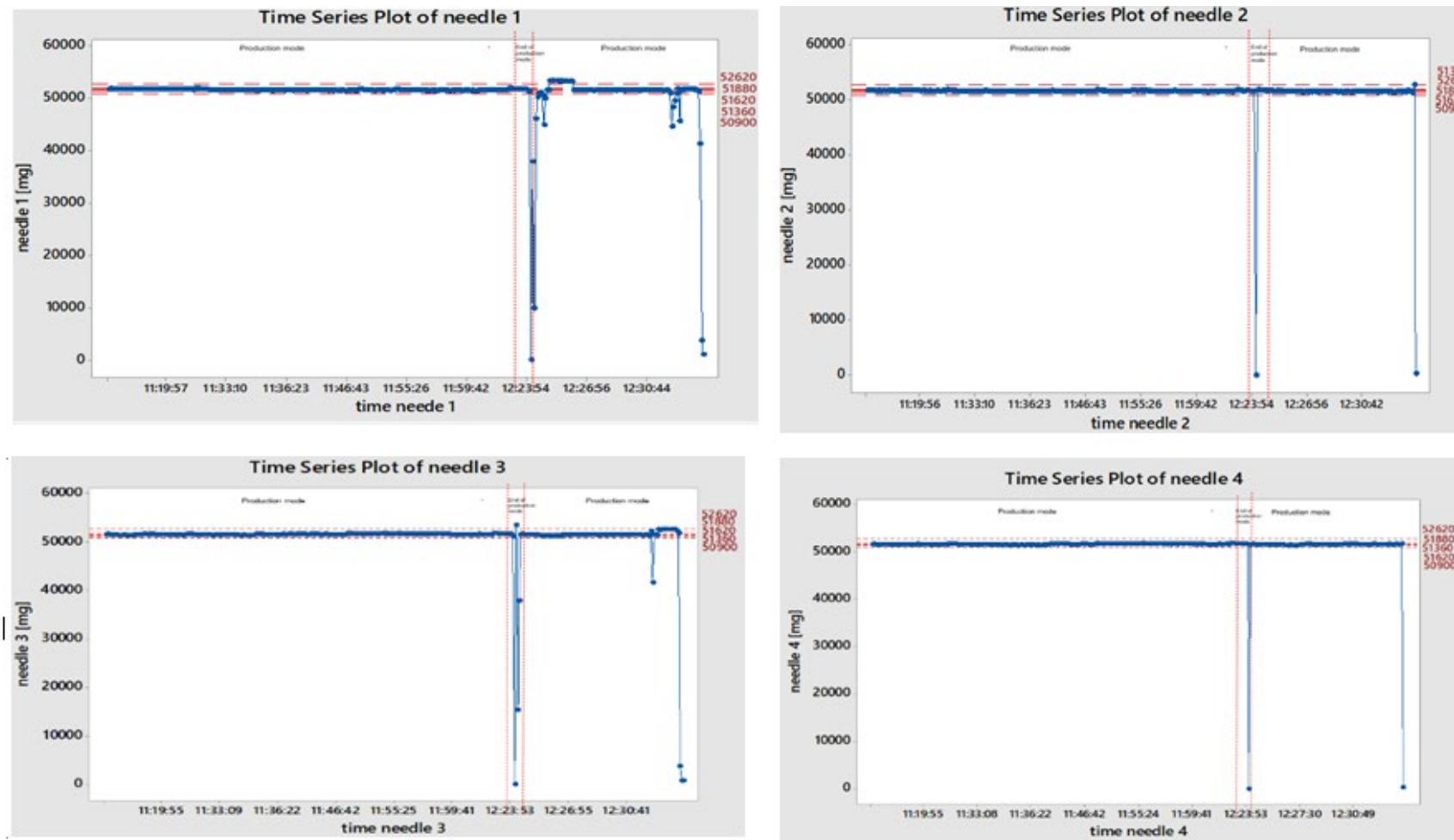


Figure 16 100% IPC fill weight per needle – part I

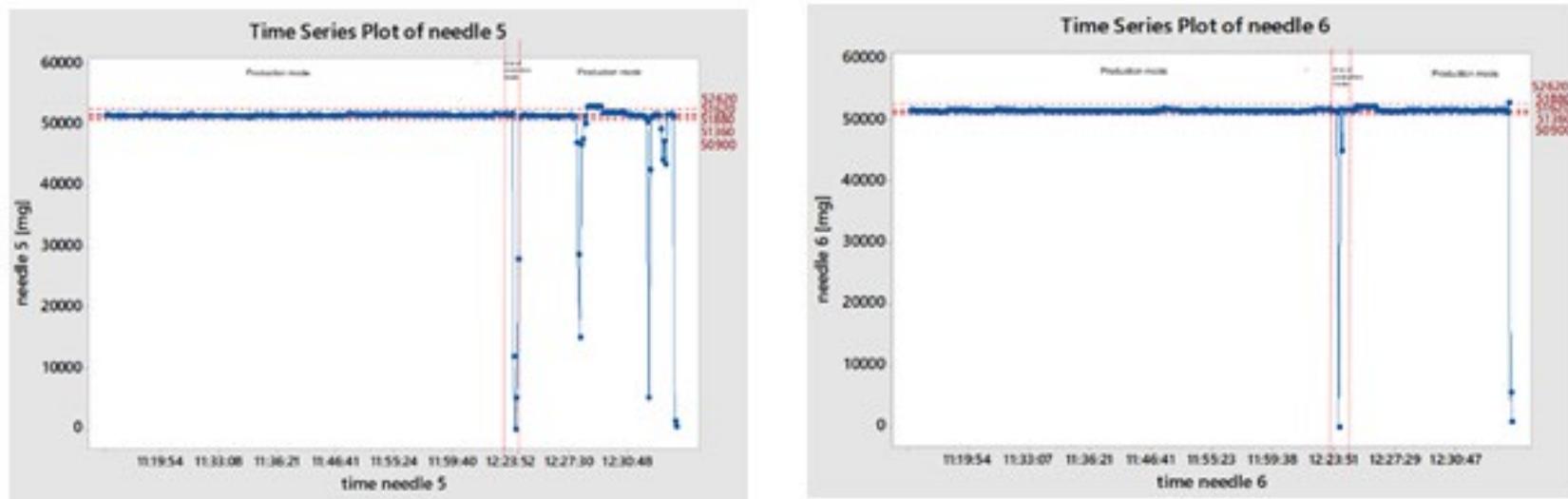


Figure 17 100% IPC fill weight per needle – part II

Even if from the rejects rate it seems that some needles (eg: needle 1 and needle 5) had more rejects, there is not a specific trend in needles. This is typical for the filling bag used in this trial that has not a single outlet but rather a separate tubes for every needles that goes out from the bottom of the bag. Moreover, it was not identified either a specific link with the peristaltic pump, considering that:

- rejects related to different needles are also related to different pump (eg: pump of needle 1 is different from pump of needle 5)
- at the beginning of every run the pumps are calibrated,

Then, from the graph above and from the raw data (see graph below) of the 100% fill weight IPC performed by the machine, it was observed two clusters of rejects.

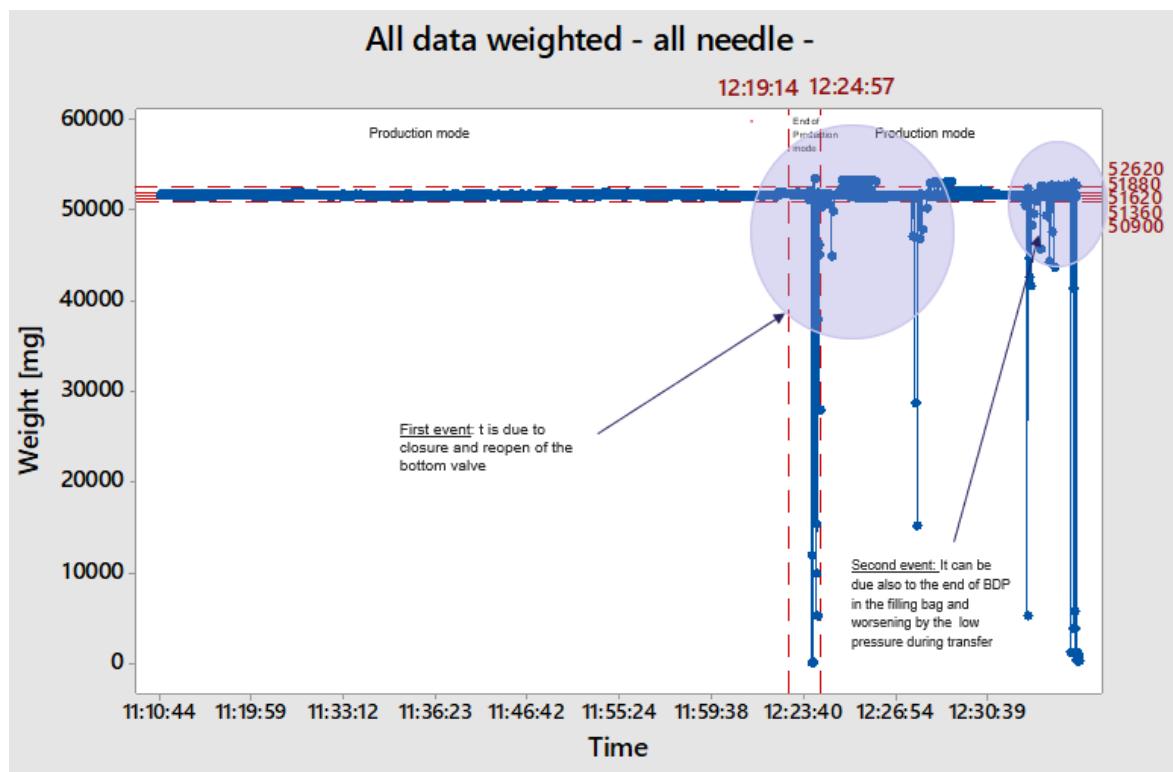


Figure 18 Filling IPC - all needles

For the first cluster (first event in the graph of figure 16), it can be observed that it was present when the machine was in the final part of “end of production mode” and at beginning of “production mode”.

In particular, the storage tank contained around 250 L of BDP. The first half of BDP was intended to fill the 50 mL formats, whereas the second half to fill the 10 mL formats. For this reason, the end of first filling (50 mL format) was calculated based on an estimation of number of vials theoretically expected.

At 12:12:15 the bottom valve of the storage tank was closed assuming that the BDP volume remained in the tubing and in the filling bag would be enough to reach the estimated number of vials. During this time the machine filled vials in "end of production mode".

When it was realized that the BDP remained in the tubing and filling bag was not enough (filling bag emptied) to complete filling of 50ml format, rejects vials beginning to appear and so the bottom valve was reopened at 12:22:43 to allow the refilling of solution. The machine was reset in production mode. At the resetting and after the machine rejects vials for weight out of limit, some minutes (approximately 4 minutes) was needed for the machine to restore the regime. No manual operations was performed by operators.

However, this operation will be not needed during PPQs and commercial manufacturing because splitting of a batch across multiple formats is note foreseen and so this event is not representative of the regime filling performance of the machine.

The second cluster of rejects (second event in the graph of figure 16) instead are present at the end of the filling. It was than checked the pressure in the storage tank during this part of the filling and it was possible to see that during that part of the filling the pressure in the storage tank was approximately 0,43 bar. In the first part of the filling instead the pressure was never below 0,45 bar and reach approximately also 0,6 bar during the calibration phase in which it was processed 180 vials (48 rejects and 132 goods). Moreover approximately 340 vials were processed with a pressure higher than 0,57 bar and approximately 1270 vials (50% of processed vials) were processed with a pressure higher than 0,5 bar without any rejects. This is a consequence of having initially set the pressure in the storage tank at 0,8 bar. The value was, immediately after, setted at 0,4 bar, as request by the MBR, but time was needed to decrease the pressure in the storage tank (the pressure in the storage tank is regulated by the nitrogen filter which is vent if pressure is higher than the maximum working range setted on HMI).

It was theoretically calculated the minimum pressure needed in the storage tank to allow a flow rate entering in the filling bag equal to the flow rate exiting the filling bag (maximum value of vial weight (52,10 mL/vial) x line velocity (100 vial/min) = 5209,90 ml/min) without considering the localized and distributed pressure drop across the line, applying the following Bernoulli equation:

$$P_1 + \frac{1}{2}\rho v_1^2 + \rho gh_1 = P_2 + \frac{1}{2}\rho v_2^2 + \rho gh_2 + \Delta P$$

Where:

P1 is the pressure in the pipeline at the bottom of the storage tank

P2 is the pressure in the filling bag which can be considered atmospheric pressure considering that the filling bag is vented

V1 = is the flux velocity in the pipeline which is equal to the flow rate divided by the cross section of the pipeline

V₂ = is the velocity in the filling bag which can be approximated equal to 0

H₁ = is the height of the pipeline at the bottom of the storage tank which can be approximated equal to 0

H₂ = is the height of the filling bag

ΔP = pressure drop (sum of localized ΔP_l and distributed pressure drops ΔP_d) which was in first approximation considered equal to 0

Without considering the pressure drops (either localized and distributed) the pressure in the storage tank (P₁) minimum required is approximately 0,2 bar.

Than to evaluate the localized pressure drop on the filters it was checked the trend of the pressure sensor placed on the sterilizing filter assemblies during the filling: for every sterilizing filter approximately 0,1 bar of localized pressure drop was present. So in total the localized pressure drops (ΔP_l) can be estimated as 0,2 bar.

Without considering the distributed pressure drop, the minimum theoretical pressure to be applied in the storage tank to guarantee a flow rate equal to 5209,90 ml/min entering the filling bag is 0,4 bar, applying the following formula:

$$P_1 = 0,2 \text{ bar} + \Delta P_l = 0,2 \text{ bar} + 0,2 \text{ bar} = 0,4 \text{ bar}$$

This pressure however can be considered risky taking into account:

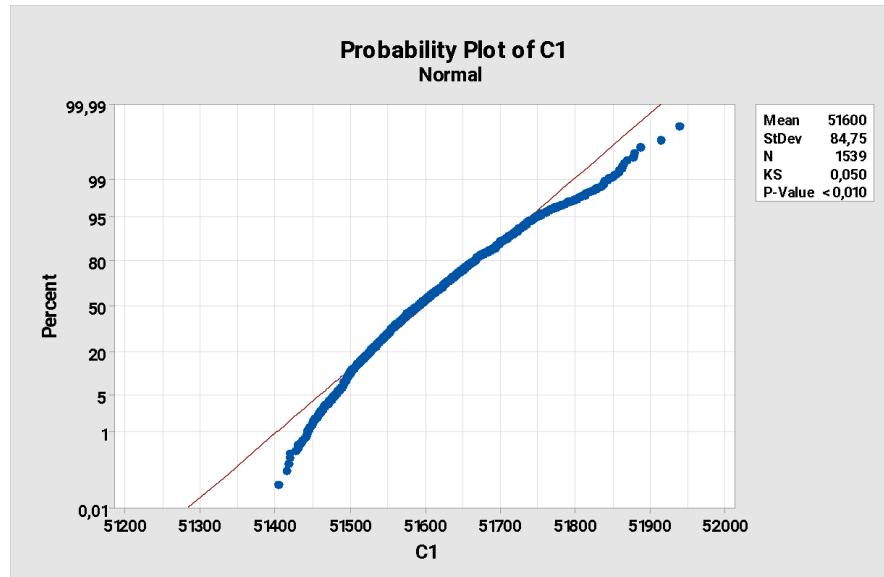
- the localized pressure drop across the circuit
- sampling from the assembly
- pressure trend during time can decrease
- pressure drop due the filter can increase due to possible clogging

Thus, the second cluster of filling rejects can be explained due to the emptiness of the filling bag due to not enough product supply for lower pressure in the storage tank.

For this reason, data from the event I and the event II were not considered in the filling capability analysis of the IMA filling line.

The filling performance capability of the machine in terms of filling weight was evaluated below using MiniTab 18.

Since the sample size is large, the Kolmogorov-Smirnov normality test was executed to assess the normality of the probability distribution of the available data. The results are briefly summarized in figure below.

**Figure 19 Normality test**

As shown in figure above, the p-value found to be <0,05 indicates that the data do not follow the normal distribution, but for sample size larger than 100-200 normality tests tend to be too sensitive and should be interpreted alongside histograms with the fitted normal curve. This hypothesis is supported by the figure that shows the histogram of the values which approximate a gaussian curve.

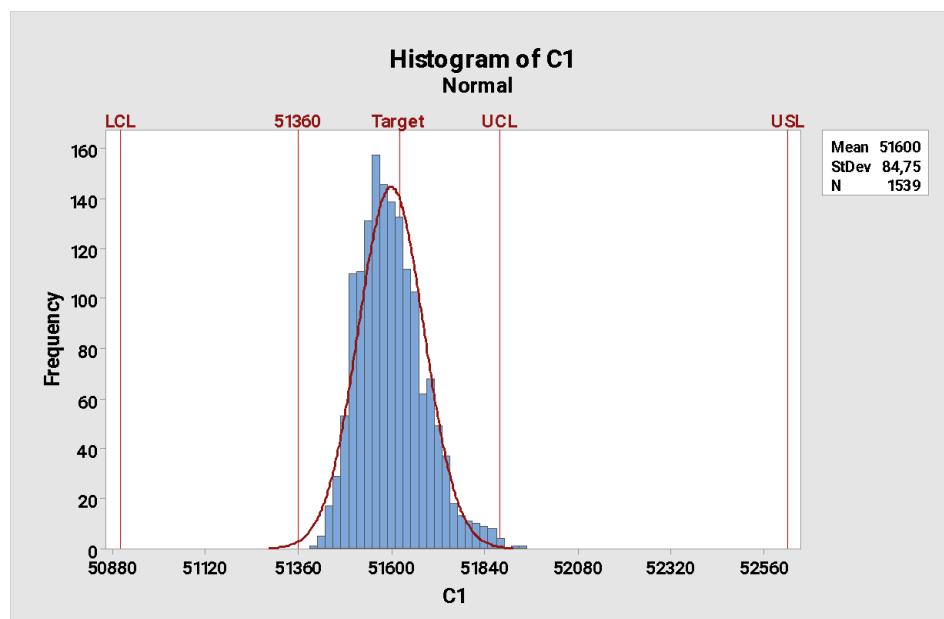
**Figure 20 Histogram of IPC filling weight**

Figure below shows the filling process capability. The data is not collected in subgroups because the sampling was 100% IPC (subgroup size = 1).

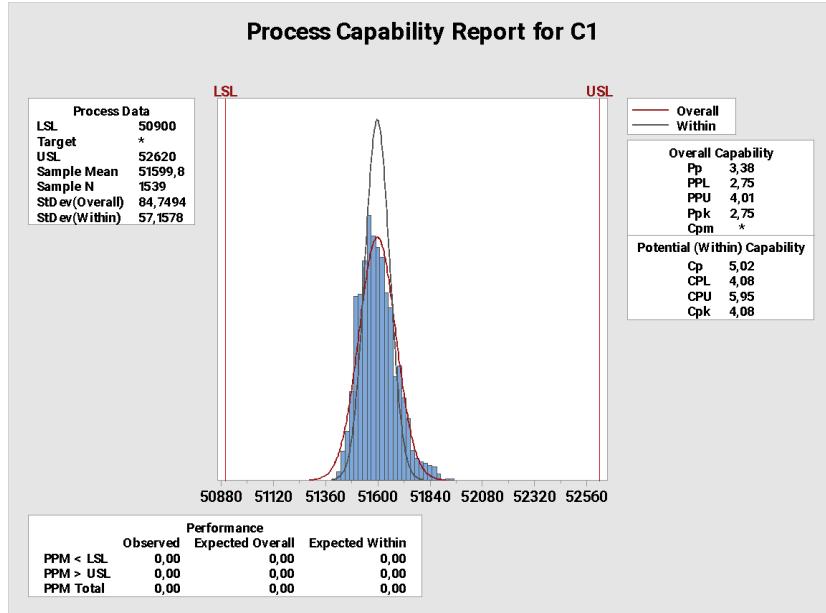


Figure 21 Filling capability

As shown in figure above the fill weight IPCs during the filling operation showed the process in controlled status and capable considering a P_{pk} = 2,75 and C_{pk} = 4,08.

As information, C_{pk} and P_{pk} value higher than 1.33 gives the assurance that the 99.99% (4 σ) of data will be able to meet the specifications.

Finally, the overall performance of the filling process can be considered robust, as the mean value recorded is close to the target filling value and the filling weight checks obtained show a tight variability around the mean value, confirming good accuracy and precision of the filling phase.

Finally, during the filling, the following times were identified (reported in table below) taking into account the VPHP study.

Table 36 Parameters documented related to VPHP

Parameter	Report	pCPP
End of aeration (*)	06/07/2022 06:33:13	N
start of bag/tubing installation	06/07/2022 21:14	N
end of bag/tubing installation	06/07/2022 22:40	N

Parameter	Report	pCPP
Start of sterile filtration	07/07/2022 10:04	N
Starting of the filling	07/07/2022 10:16	N
End of pump calibration/beginning of production	07/07/2022 11:06	N

(*) This information is reported in the machine report

Sample collection during MabThera 500 mg

3 vials every 40 locations (equally distributed along all the batch) were sampled to be tested for filling homogeneity (UV content). No significant events happened during MabThera 500 mg so no additional 3 samples after significant events were needed to be collected.

Considering that MabThera is a products single dose, which have content uniformity requirement as part of release specification, the filling homogeneity was assessed by following ASTM E2709, detailed in the Standard Corporate Guideline "Content Uniformity" QS05-G10-01.

In detail the sampling plan, the testing and acceptance criteria are summarized below:

Table 37 Sampling plan, testing and acceptance criteria for filling homogeneity for product single dose which have content uniformity as release test

Sampling	Testing	Acceptance Criteria
Sample 3 units from 40 locations throughout the batch. Sample 3 units from each significant event	Test 3 samples from 20 locations (including beginning, end and all significant events)	PVT Stage 1 Individual values 75.0% - 125.0% Meets 90% confidence 95% coverage for n=60
	Test 3 samples from 20 of the remaining locations	PVT Stage 2 All individual values 75.0% - 125.0% Meets 90% confidence 95% coverage for n=120

¹ Any result outside 75.0% - 125.0% in PVT Stage 1 will be a failure of the test and will not proceed to PVT Stage 2;

² Based on the overall mean being within the range on the look-up table.

The PVT Stage 1 foresaw the following acceptance criteria:

- individual values between 75.0% - 125.0%: this requirement was fulfilled
- statistical evaluation applying the Bargum approach: from the corporate guideline the lower limit (LL) and upper limit (UL) calculated for the results obtained for MabThera 500mg are 84,8% and

115,2%. The mean was 101,4 % and it was inside the LL and UL, and this provide that with 90% of confidence at least 95% has fulfilled the compendial Uniformity of Dosage Unit requirements.

Considering that the requirements for the PVT stage 1 were fulfilled, there was no need to do the PVT stage 2.

Samples to evaluate leachable was also taken at the following steps of filling:

- First filled vials after needle purge and calibration (approximate #1- #2) ;
- After first tubing hold up volume sample. Considering that the refilling is automatic and there is not an emptiness of the bag before the filling, it was agreed with the client to take vials approximately between #19-#20 as representative for this step;
- After approximately having filled 2 L of solution, which can be estimated in vials approximately between #41 and #42.

Finally, at the beginning of filling, vials were taken also to evaluate visible particles.

These samples are representative of filling steps but they were collected after crimping step, considering that crimping is in line with the filling machine. On the same samples it was also executed elemental impurities analysis by Roche. The results were complaint (RPT-0304717) confirming no impact on the drug product during the manufacturing process.

All details are reported in attachment #1

8.13.2 MabThera 100 mg

During the filling of MabThera 100 mg, after having taken samples for VPHP and leachable, an optimization (eg: needle set up and distance from level of liquid) was performed to minimize foam creation.

In particular, the following activities were performed:

- Y cam rise nozzle head modification. This parameter is the distance between the tip of the needle and the level of the liquid during the filling. It was reduced of 5 mm (Y cam rise nozzle head changed from 25 mm to 30 mm).
- Dosing start (on doser encoder): this parameter can be set in radiant degrees, within a range preset by the control system (it is not possible to set a dosing outside the vial). A such, the start of the expulsion of the liquid can be delayed or brought forward in relation to the position of the nozzles inside the vial. It was changed from 154° to 160°C
- Peristaltic pump dosing acceleration: this parameter set the acceleration of the peristaltic pump. It was changed form 5000 rpm/s to 1500 rpm/s
- Peristaltic pump dosing speed: this parameter set the speed of the dosing (product movement speed). This imposes the speed to the dosing units with which product is transferred into the vials, regardless of machine speed. Consequently, this allows keeping

constant all the run settings and micro-adjustments to optimize the dosing operation and maintain its efficiency and repeatability within preset precision ranges. It was changed from 305.0 rpm to 315.0 rpm.

Following this adjustment, an improvement in the foam creation during filling was observed.

During MabThera 100 mg a filling interruption (30 minutes) in the filling was simulated. After the stop, all vials that during stoppage were filled but not stoppered plus another 40 vials filled after the stoppage were taken to evaluate VPHP. The interruption was performed after having taken previous one sampling for VPHP and leachable, therefore after having filled approximately 220 vials.

At the end of the filling, another 30 minutes of stoppage was performed. Samples (vials) which were filled and already stoppered during the stoppage was collected and they underwent four visual inspections (paragraph 6.1.12).

In the Table below are reported the fill weight parameters for MabThera 100 mg presentations.

Moreover, alert and action limits reported in the tables below were defined based on data provided by dosing tests performed at the supplier (IMA). The maximum filling speed has been verified during surrogate batches (180 vials/min for MabThera 100 mg) and it was set at the beginning of the trial and it was confirmed during this engineering trial.

During the filling steps, the process interruption events, and relative time were recorded in the machine record according to SOP 000136665.

Table 38 MabThera 100 mg filling weight limits

Dosage	Limits	Fill volume (ml)	Fill weight (g)
100 mg/vial	Upper Action Limit	10,921	11,030
	Upper Alert Limit	10,634	10,740
	Target	10,525	10,630
	Lower Alert Limit	10,416	10,520
	Lower Action Limit	10,297	10,400
	Theoretical filling speed: 180 vials/min		

The parameter documented during the sterilizing filtration and filling operations are listed in table below.

Table 39 Parameters documented during sterilizing filtration and filling Operations

Parameter	Specification	Results	pCPP
Filling machine speed	(*) 80- 180 vials / min	180 vials/min	Y
Start of filling (date dd.mm.yyyy, hh:mm)	Record	09/07/2022 11:13	N
End of filling (date dd.mm.yyyy, hh:mm)	Record	09/07/2022 14:37	N
Pressure value in storage tank during filling	Target = 0,3 bar, target range [0,2-0,4] bar	Pressure between [0,2-0,4] bar	N
IPC vials weight	Record	see graph below	Y

(*) the minimum filling speed is the one identified as per media fill

The pCPP indicated in table above have been selected for the following reason:

- Filling machine speed: higher filling speed can impact on product quality (due to shear stress), uniformity of dosage, extractable volumes and excessive foam creation; lower machine speed could bring the process outside the media filled validated range and so it could have potentially an impact on sterility of the released product;
- IPC vials weight could affect at least the uniformity of dosage and extractable volumes of the released product;

First, it was checked the pressure trend of the storage tank during the filling. According to Engineering protocol TT237B011 and relative MBR, the setpoint requested was 0,3 bar. Solution transfer from the storage tank to the filling bag was done by pressuring the storage tank with nitrogen, setting a set point and a working range. In particular, the setpoint requested was 0,3 barg and the working range was $\pm 0,1$ bar. If the pressure is $> 0,3 + 0,1$ bar the system opens automatically the vent, if the pressure is $< 0,3 - 0,1$ bar, the system gives automatically nitrogen.

The pressure trend stayed across the range identified.

In the graphs below it is reported:

- The trend of the pressure in the storage tank in blue
- The trend of the rpm in the storage tank (0 rpm because no mixing) in green
- The trend of the temperature in the storage tank in red

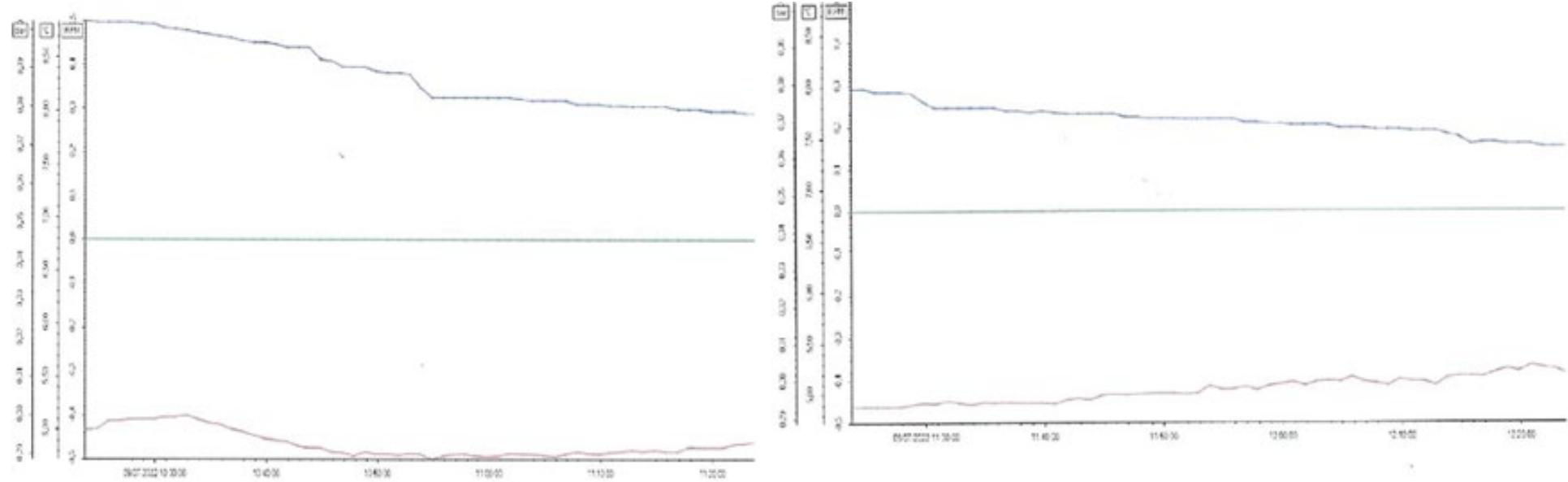


Figure 22 Pressure value in the storage tank – part I

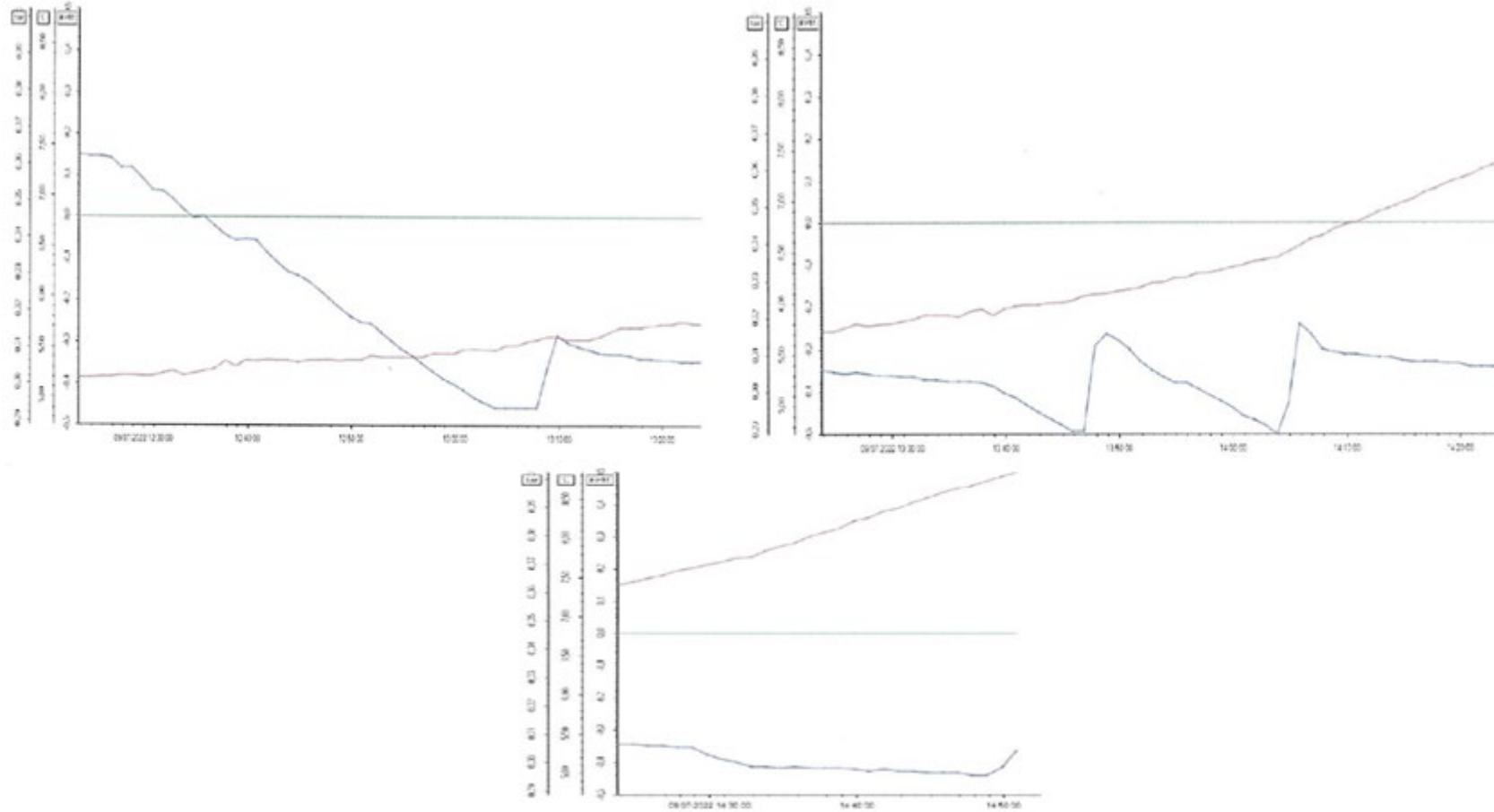


Figure 23 Pressure value in the storage tank – part II

For MabThera 100 mg filling, the analysis between goods and rejects machine which were obtained, dividing them between mode, are reported in the table below.

Table 40 Rejects analysis

Mode	Good	Rejects
Purge	0	113
Calibration	29	51
Production	10356	732
End of production	79	89

In particular, during production mode of MabThera 100 mg filling, about 0,3% of rejects were done considering only the filled vials.

Table 41 Rejects rate during production mode

Rejects rate during production mode – filled vials		0,3 %
Rejects – filled vials [qty]		32
Total = goods + reject filled vials [qty]		10388

Considering the results listed above, a deeper analysis was performed on the type of rejects obtained during production mode and end of production mode.

- Production mode

It was checked the type of rejects obtained. First it was checked if the vial was weighted or not and secondly if specific reoccurrence between scales and needles was present.

Table 42 Production mode: deeper analysis on cause of rejects

Cause of rejects	Production mode		Note
	# rejects		
stopper not selected	11		vial weighted
missed stop	3		vial weighted
filling interrupted	9		vial not weighted
net OOS scale 2	3		vial weighted: all from needle 8
net OOS scale 4	2		vial weighted: all from needle 8
net OOS scale 3	4		vial weighted: all from needle 8

- End of production mode

It was checked the type of rejects obtained. First it was checked if the vial was weighted or not and secondly if specific reoccurrence between scales and needles was present

Table 43 End of production mode: deeper analysis on cause of rejects

Cause of rejects	# sca rp	End of production mode	
			Note
net OOS scale 1	10	vial weighted: 1 from needle 1, 2 from needle 3, 2 from needle 5, 5 from needle 7	
net OOS scale 2	19	vial weighted: 5 from needle 2, 4 from needle 4, 4 from needle 6, 6 from needle 8	
net OOS scale 3	11	vial weighted: 2 from needle 1, 2 from needle 3, 2 from needle 5, 5 from needle 7	
net OOS scale 4	20	vial weighted: 5 from needle 2, 5 from needle 4, 4 from needle 6, 6 from needle 8	
net OOS scale 5	10	vial weighted: 2 from needle 1, 2 from needle 3, 2 from needle 5, 4 from needle 7	
net OOS scale 6	19	vial weighted: 4 from needle 2, 4 from needle 4, 5 from needle 6, 6 from needle 8	

The data of end of production were considered in the rejects analysis but they were not plotted in the graphs below because the multiple rejects present at the very end of the "end of production mode" can not be considered as representative of the filling step. Indeed, no load cell is present in the filling room and these rejects were the trigger to stop the production.

Then, it was plotted the trend of the filling weight (during the production mode) dived also per needles in order to check if there was a specific reoccurrence in the needles, after having first checked that no specific occurrence between the scale was present (as per Table 42 and Table 43 above).

After having checked that no specific reoccurrence between scales was present, it was checked if specific reoccurrence between needles was present. Even if from the rejects rate it seems that needle 8 had more rejects than other needles, there is not a specific trend in needles because this is typical for a filling bag that has not a single outlet as the one used which has a tubes for every needles that goes out form the bottom of the bag.

No specific issue in the peristaltic pump was identified considering that out of specification were not constant along all the filling.

Then, from the raw data (see graph below) of the 100% IPC performed by the machine, it was observed that no specific clusters of rejects were present.

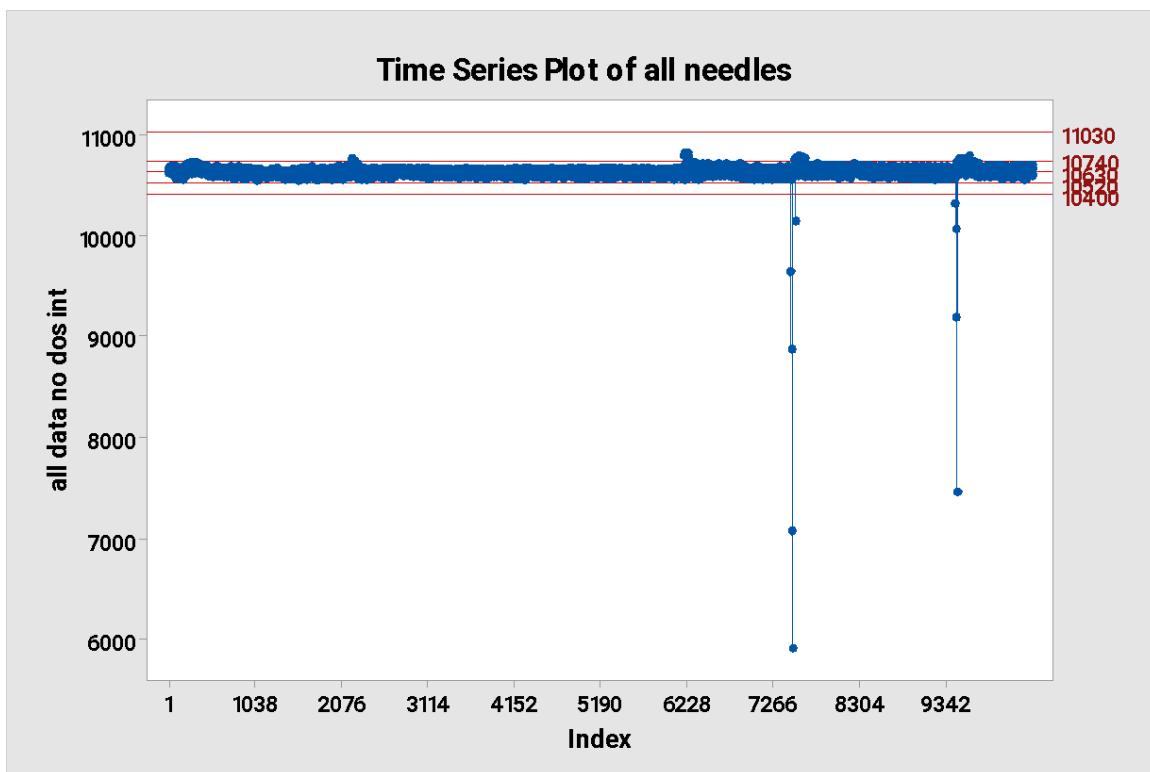


Figure 24 Filling IPC - all needles

The filling performance capability of the machine in terms of filling weight was evaluated, using MiniTab 18. It was not considered the 9 rejects of the needle 8 because they can be considered narrowed occurrences happened at the very end of the filling (also sampling from the sterilizing filter assembly for endotoxin and bioburden was executed at that moment which could explain those rejects).

Since the sample size is large, the Kolmogorov-Smirnov normality test was executed to assess the normality of the probability distribution of the available data. The results are briefly summarized in figure below.

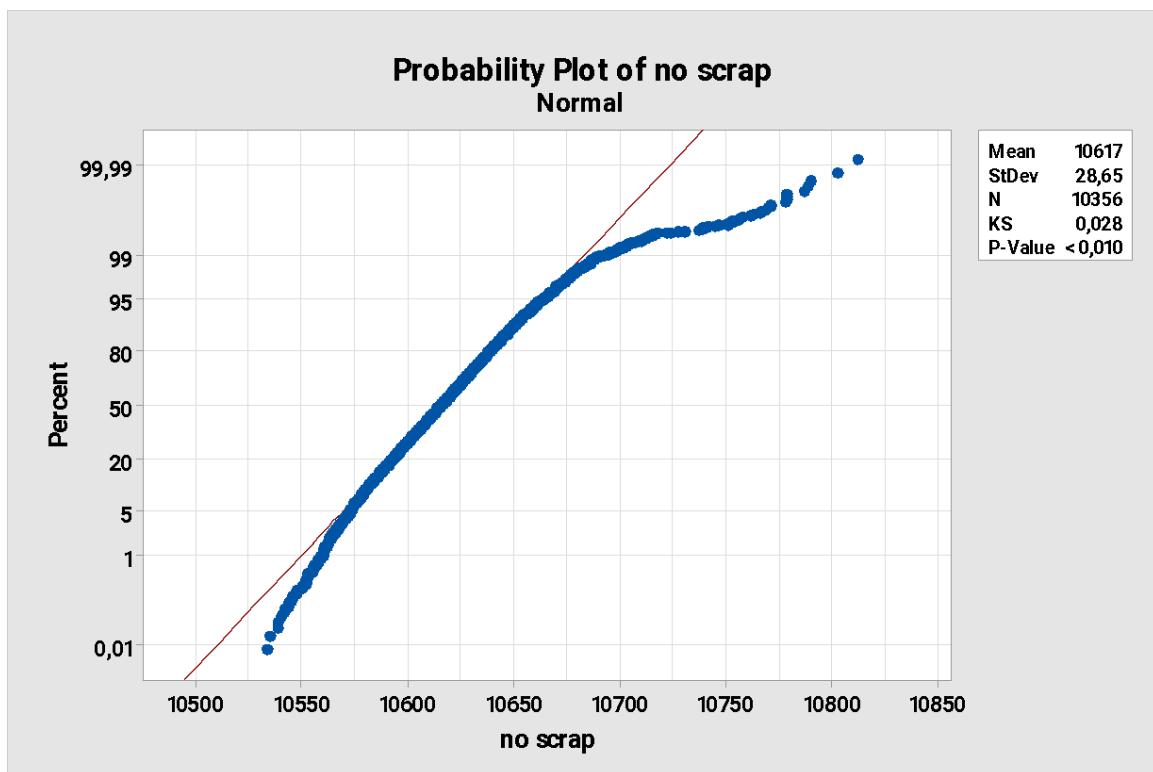


Figure 25 Normality test

As shown in figure above, the p-value found to be <0,05 indicates that the data do not follow the normal distribution, but for sample size larger than 100-200 normality tests tend to be too sensitive and should be interpreted alongside histograms with the fitted normal curve. This hypothesis is supported by the figure that shows the histogram of the values which approximate a gaussian curve.

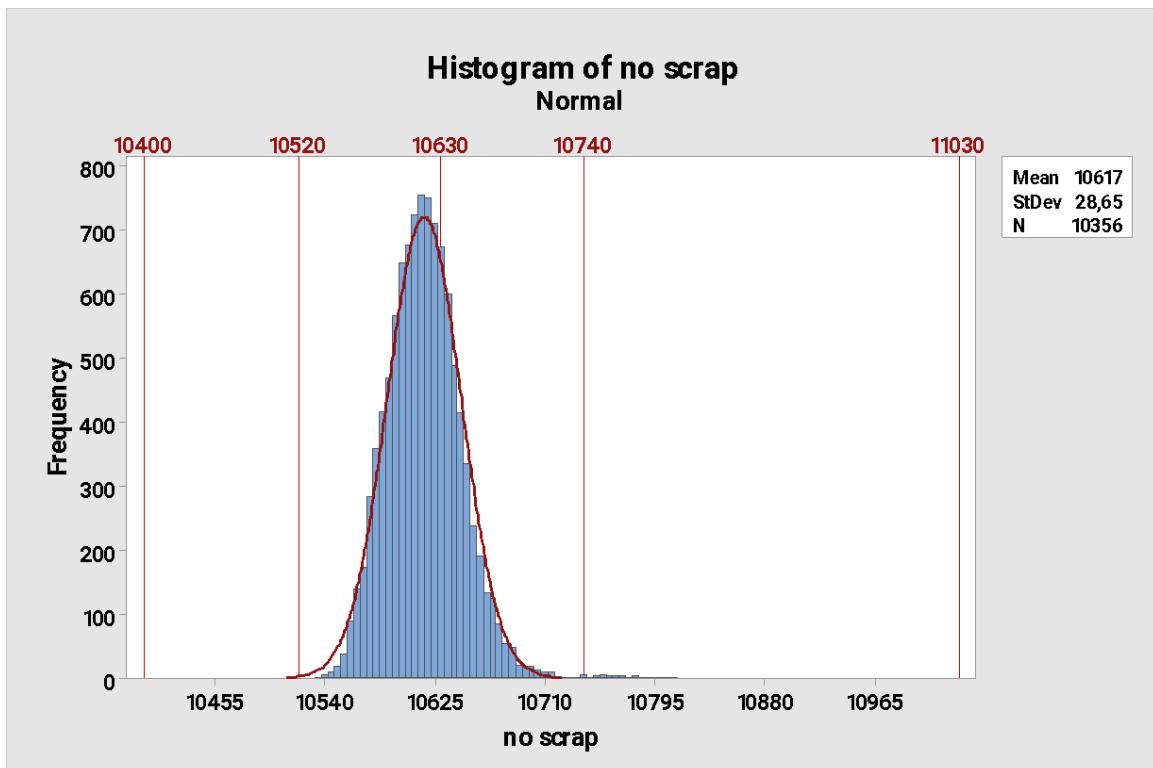
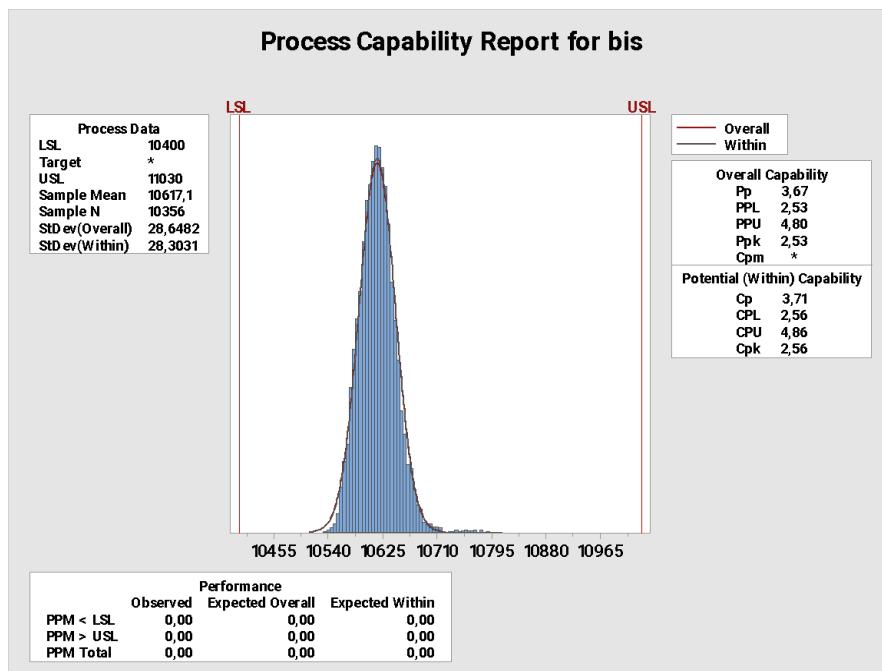
**Figure 26 Histogram of IPC filling weight**

Figure below shows the filling process capability. The data is not collected in subgroups because the sampling was 100% IPC (subgroup size = 1).

**Figure 27 Filling - process capability**

As shown in figure above, the fill weight IPCs during the filling operation showed the process in controlled status and capable considering a $P_{pk} = 2.53$ and $C_{pk} = 2.56$.

As information, a C_{pk} and P_{pk} value higher than 1.33 gives the assurance that the 99.99% (4σ) of data will be able to meet the filling weight specifications.

Finally, the overall performance of the filling process can be considered robust, as the mean value recorded is close to the target filling value and the filling weight checks obtained show a tight variability around the mean value, confirming good accuracy and precision of the filling phase.

Finally, during the filling, the following time were identified (reported in table below) taking into account the VPHP study.

Table 44 Parameters documented related to VPHP

Parameter	Recorded times	pCPP
End of aeration (*)	09/07/2022 06:14:29	N
start of bag/tubing installation	09/07/2022 08:07	N
end of bag/tubing installation	09/07/2022 09:38	N
Start of sterile filtration	09/07/2022 10:43	N
Starting of the filling	09/07/2022 11:13	N
End of pump calibration/beginning of production	09/07/2022 11:23	N

(*) This information is reported in the machine report therefore information can be retrieved.

Sample collection during MabThera 100 mg

3 vials every 40 locations (equally distributed along all the batch) plus three vials after every significant events were sampled to be tested for filling homogeneity (UV content).

Considering that MabThera is a products single dose, which have content uniformity requirement as part of release specification, the filling homogeneity was assessed by following ASTM E2709, detailed in the Standard Corporate Guideline "Content Uniformity" QS05-G10-01.

In detail the sampling plan, the testing and acceptance criteria are summarized below:

Table 45 Sampling plan, testing and acceptance criteria for filling homogeneity for product single dose which have content uniformity as release test

Sampling	Testing	Acceptance Criteria
Sample 3 units from 40 locations throughout the batch. Sample 3 units from each significant event	Test 3 samples from 20 locations (including beginning, end and all significant events)	PVT Stage 1 Individual values 75.0% - 125.0% Meets 90% confidence 95% coverage for n=60
	Test 3 samples from 20 of the remaining locations	PVT Stage 2 All individual values 75.0% - 125.0% Meets 90% confidence 95% coverage for n=120

¹ Any result outside 75.0% - 125.0% in PVT Stage 1 will be a failure of the test and will not proceed to PVT Stage 2;

² Based on the overall mean being within the range on the look-up table.

The PVT Stage 1 foresaw the following acceptance criteria:

- individual values between 75.0% - 125.0%: this requirement was fulfilled
- statistical evaluation applying the Bargum approach: from the corporate guideline the lower limit (LL) and upper limit (UL) calculated for the results obtained for MabThera 100mg are 86,3% and 113,4%. The mean was 101,5 % and it was inside the LL and UL, and this provide that with 90% of confidence at least 95% has fulfilled the compendial Uniformity of Dosage Unit requirements.

Considering that the requirements for the PVT stage 1 were fulfilled, there was no need to do the PVT stage 2.

Samples to evaluate leachable were also taken at the following steps of filling:

- First filled vials after needle purge and calibration (10 vials were taken approximately from vial #1 - #40);
- After first tubing hold up volume sample . Considering that the refilling is automatic and there is not an emptiness of the bag before the filling, it was agreed with the client to take vials approximately between vials #41- #50 as representative for this step (for details see attachment #1);
- After approximately having filled 2 L of solution, which approximately can be estimated in vials approximate between vials #201-#210.

On the same samples it was also executed elemental impurities analysis by Roche.

In particular, on the first vials sampled after calibration, the client has performed also the following tests: content of protein (by UV) polysorbate concentration and VPHP studies.

Finally, at the beginning of filling, vials were taken also to evaluate visible particles and after having performed all the sampling for leachable, the filling was interrupted for 30 minutes and the first vials after the interruption were samples for VPHP by the client. For the visible particles, results were compliant confirming that no impact on the product quality of the filling steps.

At the end of the filling, another 30 minutes filling interruption were performed and 10 vials (details in attachment #1) were than subjected to light exposure (attachment #10) for 30 minutes in AVI room (room 1116) and finally they underwent MVI (under BAO light source) for four times in order to evaluate light impact on product.

During filling steps additional 10 vials were collected and later exposed to light for 48 hours in filling room (attachment #11) and then exposed to light (attachment #11) for 30 minutes in AVI room (room 1116) and finally they underwent MVI (under BAO light source) for four times in order to evaluate, also in this case, the light impact on product.

These samples were representative of filling steps but they were collected after crimping step.

The results of the study executed by Roche are summarized below:

- content of protein and polysorbate: concentration was conforming (RPT-0308922)
- VPHP studies: were conforming (RPT-0308922)
- leachables and elemental impurities analysis: were conforming (RPT-0304717)

confirming no impact on the drug product during the manufacturing process.

Moreover, results of samples exposed to light (RPT-0309669) were conforming and identified the suitability of Xtrema filling line for the manufacturing of MabThera.

All details are reported in attachment 1.

8.13.3 Additional studies for VPHP Uptake

During the water trial, executed after the engineering trials, in accordance with Technical memo TT237Z051, additional samples were taken for VPHP uptake studies in order to test samples also during calibration phase.

Considering that the format 100 mg represents the worst case in terms of VPHP uptake, during water trial additional samples for 100 mg format were taken and the results can be considered applicable also for the format 500 mg.

In particular, during the water trial, the following vials were pulled:

- vials from 1 to 242 (good from calibration phase) were taken and labeled sequentially. In order to reach the final number of desired 250 vials, 8 vials from the first filled in production mode were taken and labeled (from 243 to 250).

- discarded vials from calibration step (73 vials) have been taken and kept separately.

Samples have been stored at 2-8°C, as indicated by Roche.

The results on the vials collected on both engineering runs and water trial (Roche report RPT-0309731 for VPHP study) were conforming.

In table below it is also reported the relevant information about purging cycle and calibration cycle obtained during both engineering runs and water trial.

Table 46 Purging and calibration cycle comparison between water trial and engineering runs - MabThera 500 mg and MabThera 100 mg

	MabThera 10 mL		MabThera 50 mL	
	Engineering trial	Water trial	Engineering trial	Water trial
Purging: n° cycles	14 (*)	14 (*)	9 (**)	10 (**)
Calibration: n° cycles	10	40	30	20

(*) purging volume/needle at every cycle in the recipe = 52 mL

(**) purging volume/needle at every cycle in the recipe = 12 mL

Finally, during the water trial of MabThera 500 mg, it has been noticed that during the purging phase the needles were very near to the product surface (during purging phase the filling machine speed is lower with respect to standard production to allow gently the evacuation of air from tubing and needles without causing splashing) considering also the optimization executed during engineering trial in terms of distance between needle and product surface for foam optimization (paragraph 8.13.1). For this reason, in order to avoid any risk of wetting the needles during purging phase, but still to fulfill the requirement of minimum flushing volume per needle (equal to purging volume/needle at every cycle per number of purging cycle), it is suggested to halve the purging volume (used during engineering and water trial) but at the same time to double the minimum purging cycle which were identified as per outcome of engineering and water trial.

In particular, as per outcome of engineering and water trial (data supported by the conforming results on samples (RPT-0309731)), the indication for the 50 mL was:

- at least 10 cycle with a purging volume per needle at every cycle equal to 52 mL (total flushing volume equal to 520 mL),

which is recommended to be updated to:

- at least 20 cycle with purging volume per needle at every cycle equal to 26 mL(total flushing volume still equal to 520 mL)

Finally, for the 100 mg the indication is:

- at least 14 cycle wit purging volume per needle at every cycle equal to 12 mL, for a minimum 168 mL of flushing volume/needle.

8.14 CRIMPING

The vials were capped and crimped under RABS (LAF surrounded by grade C) equipped with a dedicated HVAC system and terminal HEPA filters for particle protection.

Capping parameters were first challenged in surrogate batches to challenge the limit of capping pressure and capping height.

During engineering, the capping parameter for MabThera 50 mL applied were at the target value between the following range:

- Capping pressure: 90KPa-120 KPa (capping pressure setted at target, equal to 100 KPa)
- Capping height: 67,5 mm – 68,5 mm (capping height setted at target, equal to 68 mm).

Capping parameters for MabThera 10 mL were at the target value between the following range:

- Capping pressure: 80KPa-100 KPa (capping pressure setted at target, equal to 90 KPa);
- Capping height: 46 mm- 47 mm (capping height setted at target, equal to 46.5 mm)

The lower and higher limit for the capping pressure and capping height were identified during the surrogate trial for both formats (TT237D011).

The maximum capping speed (100 vials/min for MabThera 500 mg and 175 vials/min for MabThera 100 mg) were also challenged during surrogate batches and re-verified during these engineering trials. During engineering trials no adjustment was needed.

An IMA ALU 400 rotary capping machine with a continuous motion was used for applying and flanging aluminum caps on stoppered vials. The machine is equipped with a SEA VISION system in order to check the stopper position and presence.

The machine is particularly suitable for capping aseptic products since it can operate in a classified environment under laminar flow in a conventional sterile chamber or an isolator.

The capping process flow was carried out through the following operations:

- Automatically conveying the vials to machine infeed
- Pre-feeding the caps
- Feeding and distributing the caps
- Capping the vials with eight capping heads
- Rejecting the non-conforming vials
- Conveying the conforming vials to machine outfeed

Upon completion of capping, the vials were collected into polypropylene black trays (the same trays used for the receipt of the empty vials were used). The trays were placed on pallets and then moved and stored in the warehouse at 2-8°C.

The crimping step was also divided into two phases. Approx. 125 L of the solution to manufacture MabThera 500 mg in vials 50 mL, stoppers and grey seals. After this, approx. 125 L of the solution to manufacturing MabThera 100 mg in vials 10 mL, stoppers and red seals.

8.13.1 MabThera 500 mg

The parameters documented during the crimping phase are listed in table below.

Table 47 Parameters documented during Crimping Operations

Parameter	Specification	Results	pCPP
Crimping machine speed	Record	100 vials/min	N
Crimping Pressure	Target = 100 KPa During surrogate TT237D011 tested [90-120]KPa	100 KPa	Y
Crimping Height	Target = 68 mm During surrogate TT237D011 tested [67,5-68,5]mm	68 mm	Y
Start of crimping (time hh:mm)	Record	07/07/2022 11:07	N
End of crimping (time hh:mm)	Record	07/07/2022 12:45	N

The pCPP indicated in table above have been selected for the following reasons:

- Crimping height can have an impact on the container closure integrity of the released product
- Crimping pressure can have an impact on the container closure integrity of the released product

During the manufacturing of the batch, it was approximately checked the time needed to complete a tray at the goods exiting. Indeed, during this time the vials remained exposed to light of the filling

room. The checking was performed four times randomly: the maximum time recorded was 9 minutes and the minimum one was 1 minute.

Sample collection during MabThera 500 mg

Vials for CCIT were collected through the crimping activities and the sampling size was identified on the base of the batch size for the two DP and according to ISO 2859-1.

All samples (attachment 1) were compliant confirming the good results of the process parameter applied during the crimping step.

8.13.2 MabThera 100 mg

The parameters documented during the crimping phase are listed in table below.

Table 48 Parameters documented during Crimping Operations

Parameter	Specification	Results	pCPP
Crimping machine speed	Record	175 vials/min	N
Crimping Pressure	Target = 90 KPa [80-100]KPa During surrogate TT237D011 tested [80-100]KPa	90 KPa	Y
Crimping Height	Target = 46.5 mm [46-47]mm During surrogate TT237D011 tested [46-47] mm	46.5 mm	Y
Start of crimping (time hh:mm)	Record	09/07/2022 11:28	N
End of crimping (time hh:mm)	Record	09/07/2022 14:50	N

The pCPP indicated in table above have been selected for the following reasons:

- Crimping height can have an impact on the container closure integrity of the released product
- Crimping pressure can have an impact on the container closure integrity of the released product

During the manufacturing of the batch, it was approximately checked the time needed to complete a tray at the goods exiting. Indeed, during this time the vials remained exposed to light of the filling room. The checking was performed three times randomly: the maximum time recorded was 13 minutes and the minimum one was 3 minutes.

Sample collection during MabThera 100 mg

Vials for CCIT were collected through the crimping activities and the sampling size was identified on the base of the batch size for the two DP and ISO 2859-1.

All samples (attachment 1) were compliant confirming the good results of the process parameter applied during the crimping step.

8.15 VISUAL INSPECTION

After being removed from cold storage, the vials were transported to the inspection room.

During these engineering trials, the time for the equilibration of vials before the visual inspection was studied. Particularly, operators took a box from the middle of the pallet and from it, 10 vials in the middle of the box were checked for the absence of condensate and a temperature check ($T = 15^{\circ}\text{C}$) was performed every 30 minutes. The first check should have been performed after 60 minutes.

For MabThera 500 mg considering that the vials showed condense, the first check was instead performed after 08 hours and 39 minutes. The vials equilibrated after 14 hours and 39 minutes from the exiting of the $2\text{-}8^{\circ}\text{C}$ cell.

For MabThera 100 mg, the first check was instead performed after 07 hours and 55 minutes. The vials equilibrated after 13 hours and 25 minutes from the exiting of the $2\text{-}8^{\circ}\text{C}$ cell.

At the same timepoint a check for absence of bubble, prior to perform the visual inspection, was performed both for 50 mL and 10 mL formats and no bubble was ever detected.

8.15.1 Mabthera 500 mg

For MabThera 500 mg engineering batch (TT528), vials were visually inspected through manual visual inspection by qualified operators.

The defect list of the product for the manual visual inspection has been agreed with Roche and it is reported below, along with the results of the MVI divided between the prioritized samples and rest of the batch.

Table 49 Defect List – MVI

Defect	Description	Classification	Prioritized samples [pcs]	Batch TT528 [pcs]
VIAL	Vial with defected glass (chipped – body, bottom)	M	0/483	1/1427
	Cracked vial (body, bottom)	C	11/483	0/1427
	Dirty external glass	m	0/483	0/1427
	Surface scratch on vial body (length > 1 cm, width > 2 mm)	m	12/483	9/1427
PRODUCT	Empty vial	C	0/483	0/1427
	Vial with glass fragment, particles, foreign bodies in the solution	C	25/483	39/1427
CLOSURE	Vial without stopper	C	0/483	0/1427
	Vial with wrong stopper (mixup – lyo vs liquid)	C	0/483	0/1427
	Vial without seal / flip-off	C	0/483	0/1427
	Foreign seal (mixup)	C	0/483	0/1427
	Vial with defected flip-off/seal (scratched, dented, dirty, damaged)	m	0/483	1/1427
	Seal with wrongly positioned/non-sealing seal – not crimped	C	0/483	0/1427
	Seal with wrongly positioned/non-sealing seal – Partially crimped	M	0/483	0/1427
	Partially detached flip-off (evident)	m	0/483	0/1427

In the table below are reported the defective percentage divided per critical, major and minor defects.

Table 50 Defective % critical, major and minor defects

Defect Classification	Prioritized samples [%]	Batch TT528 [%]
M	0	0,1
C	7,5	2,6
m	2,5	0,7

8.15.1.1 Cracked vials (body, bottoms)

On the prioritized samples, some cracked vials were present, whereas there was not any cracked vials in the rest of the batch TT528.

Indeed, the prioritized samples were taken manually by the operators from the complete boxes at the exiting goods of the Xtrema line. Considering that the 50 mL is a big format, some friction could be present between adjacent vials, in extracting some vials manually from the rest of the box. The friction could result in having cracks on the body of the vials.

This hypothesis was also confirmed by the fact that no vial with the same defects was found during the visual inspection of the rest of the batch, for which instead no manually sampling was performed. It must be noted that this type of intensive sampling will be not performed during routine batches.

8.15.1.2 Surface scratch on vial body (length > 1 cm, width > 2 mm)

On some vials were present rounded scratches on the bottom and on the upper part of the body. According to the defect list, if the scratch is lower than 1 cm and has less than 2 mm width, the vials are conforming. In case the scratch is higher than 1 cm and has a width higher than 2 mm the vial is rejected.

The rounded scratches are caused by a guide in the vials washing machine. Indeed, the guide used during the engineering trials were made of two beams in stainless steel. The position of the two beams is exactly comparable with the position of the rounded scratches and moreover for other production on the same Xtrema line the defects were eliminated by changing the beams from stainless steel to PEEK. This implementation has been done also for MabThera formats, before the manufacturing of PPQ batches.

8.15.1.3 Vial with glass fragment, particles, and foreign bodies in the solution

Some vials (43 pcs) were found with particles. Considering that same defects (12 vials) were found also in MabThera 100 mg, the details are reported in the MabThera 100 mg section

8.15.1.4 AQL check

Vials from the inspected batch were randomly sampled as per ISO 2859-1, applying a general inspection level II (plan for normal inspection), as per SOP-000118671, to perform a statistical control (AQL) during the visual inspection activities as per Patheon Monza SOP-000028049 (SOP-1804/F). QC Packaging operators performed the statistical check (AQL).

The sampling plan was defined according to the batch size and the critical defects acceptance = 0 criteria, as per SOP-000028049 (SOP-1804/F) current revision. The check performed by QC packaging was conforming, finding 0 non-conforming vials.

The defects are classified as explained in table below.

Table 51 Classification of defects

Defect category	Description
m	Minor defects do not impact product performance or compliance; they are often cosmetic in nature, affecting only product appearance or pharmaceutical elegance.
M	Major defects carry the risk of a temporary impairment or medically reversible reaction or involve a remote probability of a serious adverse reaction. This classification is also assigned to any defect which causes impairment to the use of the product. These may result in a malfunction that makes the product unusable
C	Critical defects are those that may cause serious adverse reaction or death of the patient if the product is used. This classification includes any nonconformity that compromises the integrity of the container and thereby risks microbiological contamination of the sterile product.

Table below show the AQL that was applied during the statistical control and the relative results.

Table 52 AQL Table

	AQL for Critical defects	AQL for Major defects	AQL for minor defects
Acceptance criteria	None Allowed	0.65	2.5
Results	Compliant	Compliant	Compliant

Visible particles test was executed according to USP <790> and Ph.Eur.2.9.20 using an AQL of 0.65. Table 2 A (Single sampling plans for normal inspection) of ISO2859-1 defines the acceptance criteria for each classification of defects (Critical, Major or minor) as per SOP-000028049 (SOP-1804/F). The results were compliant.

The attachment #2 and attachment #3 of the protocol TT237B011 were used by QC Packaging analysts to record the statistical check (AQL) activities.

8.15.1.5 Sample collection

During the manual visual inspection, it was executed the sampling as per AQL check (paragraph 8.15.1.4)

No other sampling collection was executed because all the samples (Container/Appearance, Clarity, Color, pH, Osmolality, Extractable volume (min), Uniformity of dosage unit, Content of protein (by UV), Identity of rituximab (CZE), Purity by SE-HPLC, Purity by IE-HPLC, Visible particles, Subvisible particles, Sterility, Endotoxin) were taken during crimping steps and manual visual inspected as prioritized as compare to the rest of the batch, as per protocol TT237B011.

Finally, during crimping steps were collected and then prioritized in the visual inspection also samples for potency and stability challenge was collected and these vials were analyzed by Roche.

The result for potency were compliant, as per Roche results (attachment #10).

The analytical results from MabThera 500 mg stability studies showed that the results of the side-by-side stressed stability study demonstrate that the MabThera DP (500 mg) manufactured at Patheon Monza changes in a similar way to GNE Rituxan DP Controls with regards to degradation mode, chromatograms, and degradation rates.

8.15.2 MabThera 100 mg

For these engineering batches, MabThera 100 mg was visually inspected through manual visual inspection by qualified operators.

The defect list of the product for the manual visual inspection has been agreed with Roche and it is reported below, along with the results of the MVI divided between the prioritized samples and rets of the batch.

Table 53 Defect List – MVI

Defect	Description	Classification	Prioritized samples	Batch TT528/1
VIAL	Vial with defected glass (chipped – body, bottom)	M	0/443	0/9235
	Cracked vial (body, bottom)	C	0/443	0/9235
	Dirty external glass	m	0/443	0/9235
	Surface scratch on vial body (length > 1 cm, width > 2 mm)	m	2/443	8/9235
PRODUCT	Empty vial	C	0/443	0/9235
	Vial with glass fragment, particles, foreign bodies in the solution	C	0/443	12/9235
CLOSURE	Vial without stopper	C	0/443	0/9235
	Vial with wrong stopper (mixup – lyo vs liquid)	C	0/443	0/9235
	Vial without seal / flip-off	C	0/443	0/9235
	Foreign seal (mixup)	C	0/443	0/9235
	Vial with defected flip-off/seal (scratched, dented, dirty, damaged)	m	0/443	0/9235
	Seal with wrongly positioned/non-sealing seal – not crimped	C	0/443	0/9235
	Seal with wrongly positioned/non-sealing seal – Partially crimped	M	1/443	0/9235
	Partially detached flip-off (evident)	m	0/443	0/9235

In the table below are reported the defective percentage divided per critical, major and minor defects.

Table 54 Defective % critical, major and minor defects

Defect Classification	Prioritized samples [%]	Batch TT528/1 [%]
M	0,2	0,1
C	0	2,6
m	0,5	0,7

8.15.2.1 Vial with glass fragment, particles, and foreign bodies in the solution

During the manual visual inspection of MabThera 100 mg, out of 9678 inspected vials, 12 vials were found with particles/fibers (all during the visual inspection of the batch TT528/1 and no vials with particles were found in prioritized samples). The % defective rate is 0,1 %.

After the visual inspection, the statistical check was performed by the QC packaging laboratory, and it met the foreseen Acceptance Quality Level (AQL) (0 defective units found, including 0 particles found out of 200 vials inspected). The criteria applied for the check are briefly reported below:

- critical defects (acceptance criteria = none defects allowed);
- major defects (AQL =0,65);
- minor defects (AQL=2,5);
- visible particles according to USP <790> and Ph.Eur.2.9.20 using an AQL of 0.65,

Also during the manual visual inspection of MabThera 500 mg, some vials with particles were found. In particular, out of 1910 inspected vials, 64 vials were found with particles/fibers (25 vials were found in prioritized samples and 39 vials in batch TT528). The % defective rate is 3%,

After the visual inspection, the statistical check was performed by the QC packaging laboratory, with conforming results (0 defective units found, including 0 particles found, out of 200 vials inspected). The criteria applied for the check are briefly reported below:

- critical defects (acceptance criteria = none defects allowed);
- major defects (AQL =0,65);
- minor defects (AQL=2,5);
- visible particles according to USP <790> and Ph.Eur.2.9.20 using an AQL of 0.65,

Characterization of particles

In order to assess the origin of particles found in the defective units reported in the paragraph above, three representative samples (as the most reoccurring defects) from MabThera 500 mg and three representative samples form MabThera 100 mg (evaluated by Patheon between reflecting particles, not reflecting particles, white fibers, black fibers) were sent to an external laboratory to perform a characterization.

The fragments were analyzed after filtering through FTIR whose results were compared to a library.

According to results provided by the external lab (Redox report 479/2022 (Ed. 01), for MabThera 500 mg it was found silicone rubber in all three samples. Moreover, in one sample also cellulose and polyamide/nylon and inorganics material were found (for inorganics it was also conducted a SEM analysis whose outcome is reported in table below).

All the intrinsic particles have a dimension bigger than 0,22 µm (pore size of the two sterilizing filters, see table below), so it is likely that particles have been introduced into the vials after the two sterilizing filters, excluding any contribution due to the solution preparation and storage.

Table 55 Characterization MabThera 500mg

Sample	FTIR attribuition	Match	SEM	Dimension	Shape
Sample 1 – P1	Polyamide and inorganics	NA	Silicon, Magnesium, Iron, Aluminium, Potassium, Titanium. Carbon, Oxygen	300 x 500 µm	Particle
Sample 1 – P2	Cellulose	81%	NA	200 x 100 µm	Particle
Sample 1 – P3	Silicon rubber	95%	NA	50 x 100 µm	Particle
Sample 2 – P1	Silicon rubber	93%	NA	600 x 100 µm	Particle
Sample 3 – P1	Silicon rubber	97%	NA	500 x 100 µm	Particle

For MabThera 100 mg (Redox report 578/2022 (Ed. 01), it was found cellulose in all three samples. Moreover, in two samples it was also found polyamide/nylon.

All the intrinsic particles have a dimension bigger than 0,22 µm (pore size of the two sterilizing filters, see table below), so also in this case it is likely that particles have been introduced after the two sterilizing filters.

Table 56 Characterization MabThera 100 mg

Sample	FTIR attribution	Match	Dimension *	Shape
Vial 1 – P1	Cellulose	76%	700 µm	Fiber
Vial 1 – P2	Polyamide/nylon	66%	500 x 100 µm	Particle
Vial 2 – P1	Polyamide/nylon	56%	150 x 150 µm	Particle
Vial 2 – P2	Cellulose	76%	>1mm	Fiber
Vial 3 - P1	Cellulose	78%	>1mm	Fiber

In order to identify the possible source of the particles found all the production steps, equipment and materials were mapped, focusing on: the primary packing, the disposable assemblies, the stainless-steel tanks, the vial washer, the depyrogenation tunnel, the filling and stoppering machine and the sterile area 6 operators gowning procedure.

The aim of the present section is to identify the root- cause related to the occurred event.

Potential root- causes have been analyzed starting from the major root cause categories summarized below (“6Ms”):

- 1- MATERIAL: raw material/ components used during manufacturing, with particular focus on stoppers flow and material/ equipment preparation
- 2- MACHINE: facility, systems, tools and equipment used during manufacturing
- 3- MEASUREMENT: physical measurements/ process parameters to be measured during manufacturing
- 4- MILIEU/ MOTHER NATURE: uncontrollable/ unpredictable external environmental factors occurred during material preparation and manufacturing
- 5- METHOD: (process, procedures) operative instructions regarding the material/ equipment preparation and how to behave in terms of gowning and during manufacturing
- 6- MANPOWER: operations executed by operators during manufacturing.

- **Material: Stoppers – Most likely root- cause**

As the vials are investigated in the dedicated paragraph along with the vials washing machine, in this section there is a specific focus on the stoppers.

Materials direct in contact with the product are the stopper product code 273438 and batch number M220112, used for the manufacturing of both TT528 and TT528/1.

The stoppers are manufactured by West Daikyo according to Roche dedicated specification and are made of Butyl Rubber (D 713) coated with silicone RB2-40 and a lamination of ETFE (ethylene tetra fluoro ethylene).

The stoppers are provided by the supplier to Patheon in a beta bag (GCL 190 25 L Port-bag, made of a film of Tyvek) and are ready to be sterilized. In each beta bag 4000 pcs are present. They are sterilized as per SOP-0001100043 (121°C for 40 minutes). During the preparation of the materials (e.g.: sterilization) and during the manufacturing process steps, the beta bags containing the stoppers were not opened.

As per Daikyo specification (22-Y-141), the stoppers are supplied already washed by Daikyo with WFI.

The washing step and drying step are performed at Daikyo manufacturing site after the molding step and coating step, but before the final 100% visual inspection. The visual inspection is performed by automatic machine in an ISO 7 clean room applying the following criteria: foreign particles not less than 0,05 mm² and fibers not less than 0,5 mm in length.

Daikyo stopper specification classifies the defects for foreign matter (embedded, adhered) and fiber (embedded, adhered) in major, moderate and minor depending on the size. Here below is reported the classification along with the respective AQL level.

Table 57 Daikyo AQL for particles/fibres

	Major AQL =0,025%	Moderate AQL =0,25 %	Minor AQL =1,0%
Foreign matter (embedded, ashered)	Greater than or equal to 1.00 mm ²	0,20 mm ² or more and less than 1.0 mm ²	0,05 mm ² or more and less than 0,20 mm ²
Fiber (embedded, adhered)	Greater than or equal to 10,0 mm	2.0 mm or more and less than 10.0 mm	0.5 mm or more and less than 2.0 mm

The statistical check (destructive tests executed on a sample size statistically significant) of the involved stopper (batch number M220112 and product code 273438) performed by Thermo Fisher QC packaging during incoming inspection was conforming to the foreseen AQL (0 defective samples founded on a total of 500 stoppers analyzed).

During AQL check is applied the same defect list of the manual visual inspection.

In order to support the investigation on the stoppers as potential root cause, the following steps has been performed:

- Historical data collected from complaints opened by Patheon on similar RSV stoppers at Daikyo have been evaluated
- Daikyo complaint opened specifically for supporting the investigation on MabThera stoppers
- Rinsing study executed at Patheon to assess the stoppers/stoppers bag as potential source of contamination
- Water trial execution at maximum batch size (approximately 71000 vials for MabThera 100 mg and approximately 15000 vials for MabThera 500 mg)

Historical data (complaints EN3041221 and EN3046214)

Based on the visual inspection, outcome previously complaints were checked in order to understand if similar events were already investigated. Historical data review showed that two complaints raised to Daikyo (complaint EN3041221, West QNS 200024332 on July 2022 and complaint EN3046214; West QNS 200007669 on July 2021) have been opened by Patheon, due to similar findings during different products' production.

Referring to the first one (complaint EN3041221, West QNS 200024332 on pre-washedr stoppers from Daikyo used in Patheon), the supplier production flow was mapped, confirming the presence of polyamide/nylon and cellulose, same materials also identified by FTIR analysis for the MabThera defective drug products units. Particularly polyamide/nylon and cellulose are used in Daikyo manufacturing process, respectively in the recording paper and in the hairnet worn under the operator's cap.

The EN3041221 complaint is linked to an anomaly found for a product manufactured on a different line than the one used for MabThera.

The affected stoppers which also in this particular case are RSV stoppers require the following manufacturing steps at Daikyo.

The supplier provided with a detailed description of the manufacturing process at their side, the environment where the process is performed is ISO8 class for steps 2 and 3 while points 4 to 10 in ISO7 class.

1. Compounding and Mixing: each raw material is compounded by the operators and mixed by the mixing machine to obtain mixed rubber.
2. Pre-forming: mixed rubber sheet is pre-formed into flat rubber sheets (pre-formed sheets) by the pre-forming machine.
3. Molding: pre-formed sheets are set in the molds to be formed into the rubber stopper configuration by the molding machine.
4. RB2-coating: RB2-coating solution is sprayed in a fine mist on the top surface of molded rubber sheets by the RB2-coating solution machine.

5. Sheet inspection: as an in-process inspection, molded rubber sheets are 100% manually inspected by the inspectors.
6. Trimming: molded rubber sheets are trimmed into individual stoppers.
7. Washing and Drying: pre-washing: In order to remove adherent foreign particles, shower washing using RO water is performed.
Main washing: in order to obtain a 3-log reduction of endotoxin, washing is performed with alkaline treatment under high-temperature and high-pressure conditions, and with acid neutralization using hot water. In addition, the final rinse is performed using WFI.
Drying: drying treatment is carried out by far-infrared and microwave.
8. Visual inspection: the 100% inspection is performed by the visual inspection machine to remove products affected by defects as per Daikyo'sr defect detection specification
(foreign particles : not less than 0.05mm² in size, fibres : not less than 0.5mm in length).
9. Sampling appearance: inspection Conforming products are sampled and visually inspected by the inspectors using a magnifying glass (3x).The check is performed on samples size of 500 pieces sampled across one lot in chronological order. The AQL is 0,025% for major defects, 0,25% for moderate defects and 1,00% for minor defects (Table 57 Daikyo AQL for particles/fibres).
10. Packaging -> Packaging bags with products are heat-sealed by the operators.
11. Packing -> Packaged products are packed into cardboard boxes by the operators.

Considering the outcome of the investigation performed by the supplier and considering the number of defects found in the final product, despite it was not possible to exclude the supplier responsibility, no mitigation action was implemented by the supplier since the defects found were within the total number of possible defects retrievable based on the quality level of the stoppers currently bought (Patheon code 273438).

Moreover, the defects size complained of the particles described were considered within the specification, for Daikyo fibers greater than or equal to 0.5mm in length and foreign particles greater than or equal to 0.05mm² in size are deemed defects.

Another complaint (complaint EN3046214; West QNS 200007669) has been raised by Patheon for the presence of cellulose, silica, silicon rubber, polyamide and polystyrene in the vials of a product manufactured in Patheon Monza on a different line with respect to the MabThera's one and also in this case using RSV quality stoppers. In order to complete the investigation, a rinsing study was carried out by Patheon: stoppers coming from one bag were washed and the rinsing water was filtered. The filter was then analyzed, and particles/fibers trapped on the filter were categorized by the external laboratory which confirmed the presence of polyamide and cellulose coming from the stoppers bag. As mitigation, in this case, the Dsigma quality stoppers were introduced for this particular product, in order to improve the stoppers quality.

In the manufacturing of Dsigma quality stopper, the camera (used by Daikyo during the 100% automatic visual inspection step) are set to be able to detect particles with dimension $\geq 0.01 \text{ mm}^2$, instead for standard RSV quality stopper the camera can detect particles with dimension $\geq 0.05 \text{ mm}^2$. Moreover, the final manual visual inspection, conducted by Daikyo on statistical sample size, has a tighter AQL for Dsigma quality stopper (0,015 % for Major, 0,10% for moderate and 0,25% for minor defect).

MabThera stoppers formal complaint (EN4071222, West QNS 200034794)

On 24th November 2022 Patheon raised a formal complaint to Daikyo (EN4071222, West QNS 200034794) to support the investigation for percentage defects rate found during the visual inspection of MabThera engineering runs on stoppers S10-F210-4 RSV D713 RB2-40 lot number AB001.

Daikyo based the investigation on the vials with particles which were found during manual visual inspection of filled product and subsequently characterized by the external laboratory, in line with Patheon notification.

The details are reported here below:

- product code #363305 : 12 out of 9878 inspected vials
- product code #363306 : 64 out of 1910 inspected vials

Three representative samples from product code 363306 500 mg dosage and three representatives' samples from product code 363305 100mg dosage were sent to an external laboratory to perform a characterization.

Based on Daykio defect detection specification, foreign particles not less than 0.05 mm^2 in size and fibers not less than 0.5mm in length are deemed defects. Patheon analyzed through an external lab the particle's size and communicated them to Daikyo.

Table 58 Defects data shared with Daikyo

PTH code	sample - ID - size
363305	Vial 1 -P1 Cellulose - 700 μm
	Vial 1 -P2 Polyamide/nylon - 500 x 100 μm
	Vial 2 - P1 Polyamide/nylon - 150 x 150 μm
	Vial 2 – P2 Cellulose - >1mm
363306	Vial 3 - P1 Cellulose - >1mm
	Sample 1 – P1 Polyamide and inorganics 300 x 500 μm
	Sample 1 – P2 Cellulose - 200 x 100 μm
	Sample 1 – P3 Silicon rubber - 50 x 100 μm

PTH code	sample - ID - size
	Sample 2 – P1 Silicon rubber - 600 x 100 µm
	Sample 3 – P1 Silicon rubber - 500 x 100 µm

In Daikyo's defect detection specification, foreign particles not less than 0.05 mm² in size and fibers not less than 0.5mm in length are deemed defects. Samples were not sent to the supplier due to fact that the identification test were destructive. Based on PTH outcomes communications and Daikyo's defects size classification, the three particles aforementioned in blue are deemed to be within Daikyo specification.

The supplier investigation is based on Review of manufacturing records and Review of retained samples and the following considerations are based on the documental review.

No anomalies nor deviation for the lot in question were reported by Daikyo. The lot was manufactured in accordance with the established conditions. The 100% manual inspection of molded rubber sheets was performed appropriately. Moreover, the 100% appearance inspection by the vision inspection machine on the stoppers was conducted as per the set procedure after the washing and drying processes. There was no anomaly during the steps described that can explain the generation of defects. Further, no defects were detected during the sampling appearance inspection (500 stoppers) after the vision inspection.

The retained samples (31pcs) for the lot were checked with no defective samples.

The supplier provided with a detailed description of the manufacturing process at their side, confirming the process reported for the previous complaints and the environment where the process is performed in ISO8 class for steps 2 and 3 while points 4 to 10 in ISO7 class.

Supplier (Daikyo) Root cause analysis

Daikyo communicated that the reported particles were attached particles and it is considered that the pre-washing during the washing process is likely to remove foreign particles present on the product surface. To confirm the removal of adherent particles in the pre-washing process, the technical study by the challenge test is conducted, and whether adherent particles (hair, fibers, rubber pieces, and metal fragments) as a defect level can be removed is verified in the pre-washing process. Hence, manufacturing processes where particles could potentially be introduced to products would be during the final finishing processes (cleanroom: ISO class 7) from the vision inspection through packaging after the washing and drying processes.

The production process for each batch takes several weeks to two months to produce one shipping lot due to work-in-progress periods between the processes.

In the process, from step 2 Pre-forming through step 10 Packaging (steps 7 Washing and 10 Packaging as per supplier report are the steps where adherent particles may be generated to rubber stoppers) operators wear appropriate clothing.

For clarification, step 7 of washing for obvious reasons is excluded from the list of processes that may contribute to the generation of the particles.

In detail the operators (from step 2 to 10) wear the cleanroom inner clothes, with a hairnet, a mask, gloves, a cap, an eye shield, the cleanroom garment (overall type), and foot covers, in that order. In addition to going through the air shower, the operators use adhesive rollers and remove foreign particles from the entire surface of their garments before and after entering the cleanroom to ensure that no loose foreign particles are carried into the cleanroom.

A mapping of the reported particle components in the processes has been performed by the supplier regarding the three types of materials (cellulose, silicon and polyamide/nylon) highlighted with the following explanations:

- Light blue clean paper made from cellulose is used as a recording paper in the cleanroom. According to the supplier, the paper minimizes generation of foreign particles and fibers, by using the paper that hardly creates dust (special paper designed to be used in cleanrooms).
- The silicone is sprayed in the RB2 coating process. The review of the manufacturing records found that the operations were performed appropriately, and that the checking and cleaning were performed as per the set procedure.
- Polyamide/nylon is used for hairnets: the operators wear them under the garment and so according to supplier indication they did not expect exposure in the clean room environment

Based on what was reported above polyamide/nylon, cellulose and silicone are used in the process even if mitigation actions from the supplier are explained. So it is not possible to exclude a possible contaminations due to these materials that are intrinsic of Daikyo process.

Daikyo reports that a 100% appearance inspection by the vision inspection machine is performed for final products after the washing and drying. During the inspection, products affected by defects are removed as per supplier defect detection specification (fibers not less than 0.5mm in length and foreign particles not less than 0.05 mm² in size are deemed defects). The size of the seven particles out of the reported ten particles was not less than the specification (refer to Table 58). If such particles were attached to stoppers, the supplier claimed that they could have been detected by the vision inspection machine.

Nonetheless, the vision inspection machine is unable to detect particles that are unrecognized by the camera due to a smaller color difference compared with rubber stoppers,

Taking in considerations the particles found during ENG batches, some foreign particles were similar in color to the stopper or transparent. Therefore Daikyo confirmed that transparent fibers/particles

can be invisible to the qualified visual inspection machine and so be present in the final stoppers bags.

The stopper supplier Daikyo has confirmed that due to limitations of the current automatic visual inspection machine it is not possible to implement improvements for the problems listed above, there is no plan for CAPA in order to overtake the machine limitation in terms of detection of the translucent or transparent particles/fiber

On top of Daikyo investigation, Patheon performed a rinsing study described in the next paragraph highlighting that a high number of particles were present for stopper batches M220112, T217123 and T2210889 after the rinsing of the bags.

Due to that the high number of particles found during the rinse study was found after rinsing the bag itself, was asked to Daikyo to explain how the bags are handled during the process and if the high contamination rate can be explained.

The bag (GLC190 port bag) is manufactured in ISO 7 or higher cleanliness areas with manual operation by operators. Daikyo purchases GLC190 port bag through another supplier and conducts a particulate test on the surface of the inner bag as an incoming inspection. The acceptance standard is 0.8 or less particles of > 50 µm including fibers per 10 cm².

Filling procedure of the bag:

- Operators who fill GLC190 port bags use adhesive rollers to remove foreign substances from their garments before entering the clean room, and then take an air shower before entering the room.

- After vision inspection, stoppers are packaged in a clean PE bag, and the operator introduces the bag into the feeder. When stoppers are loaded into GLC190 port bag, a special loading device is used. The product contact area of the device is wiped with a wiping cloth that has been sprayed with ethanol and treated to prevent fibers from coming out before use. The GLC port bag is preloaded in the loading device, and stoppers are fed into GLC190 port bag via the loading device.

-

Daikyo state that the series of the above operation has been confirmed through a preliminary investigation to ensure that no foreign matter contamination would occur, and procedures have been established based on the results.

It was required to the supplier Daikyo to explain the atypicality of the high contamination rate outcome during the rinse study and to addressed it versus their validated process. Despite the request and the evidence of the rinse study, Daikyo was unable to explain the high contamination found during the rinse study and found no anomalies in the production and storage of the RTP bag.

Supplier (Daikyo) Conclusion

As a result of the supplier investigation, no root cause of the occurrence of the reported particles was identified in the supplier processes based on the documentation review.

Besides that, several points are still unclear and to be addressed by the stopper supplier. Below are listed all the considerations that led to the supplies as the possible root cause:

- the vision inspection machine is unable to detect particles that are unrecognized by the camera due to a smaller color difference compared with rubber stoppers. The stopper supplier Daikyo has confirmed that due to limitations of the current automatic visual inspection machine it is not possible to implement improvements for the problems listed above.
- polyamide and silicone are used in the process so is not possible to exclude a possible contamination due to these materials are intrinsic of Daikyo process.
- the supplier identified the packaging step as possible source of contamination, highlighting the importance for the operators to correctly apply the procedure. The only CAPA proposed by that supplier is applicable to this step. Supplier opened a CAPA on December 14, 2022: Daikyo shared with operators the received complaint for awareness about the possibility that the reported foreign particles could be carried by the operators from the gowning step into the clean room environment. Moreover, the operators were reminded of the importance of following the gowning procedure and using adhesive rollers. Operators were also retrained to follow those procedures without fail for the daily operations and underling to perform room cleaning more carefully.
- No explanation by Daikyo has been provided related to the results (finding of particles/fibers in the bag) of the rinsing study executed at PTH and if these stopper's batches are atypical

Stopper Rinsing study (performed in December 2022 at Patheon on MabThera stoppers)

After the outcome on the engineering productions in order to confirm the root cause, it has been requested to investigate the potential presence of foreign particles in the stopper bags, by performing a rinsing study.

The rinsing study was executed on three lots of the MabThera stoppers (Patheon code 273438) available in Thermo Fisher. Every stopper batch is composed by 36 bags and per each bag there are 4000 stoppers.

The analyzed bags (4000 pieces per bag) were not previously manipulated and are representative of the batches used in productions. The bags were analyzed in the same state in which they arrived from the supplier. Thus, the results obtained refer only to the Daikyo manufacturing process.

Below the detail of the stopper batches used in the study:

- batch M220112

- batch T217123

- batch T2210889

For each batch, three different studies were performed, as agreed with the client:

Study 1: 3 x 11 stoppers were taken from each bag and analyzed as per procedure described below

Study 2: Remaining stoppers into the bag were subjected to the rinsing study, as per procedure described below

Study 3: Once empty, rinsing study was performed on the beta bag as well

All the operations described below were carried out under laminar flow hood.

The glassware used during the analysis was carefully cleaned with Milli-Q Water several times to ensure that the material used was clean and not a source of contamination. Details on the executed study are reported below.

Preparation of Blank sample

Small flasks were used for study 1, whereas 2 L flasks were used for study 2 and 3.

For each of the three study, a separate blank preparation was carried out.

Before starting with the blank preparation, all the glassware was accurately cleaned with milliQ water.

1. All the flasks and backers requested for the rinsing study were carefully cleaned and then each flask was filled with Milli-Q Water.
2. Milli-Q Water contained in the flask was transferred in the backer
3. The backer was shacked to wet all the inner surface
4. The Milli-Q Water was filtered utilizing a filtering system with 0,22 µm filter.

The operations from 1 to 4 were repeated for all the glassware, using the same filter.

The filter was then placed in a specific container closed (Petri plate) and observed at the microscope for ensuring no particle presence.

All the containers utilized for the blank were the same utilized for the stopper test.

Stoppers Analysis

Before proceeding with the opening procedure, the stopper bag was observed externally with a UV lamp for ensuring that no contamination occurred during the test.

The stoppers bag was opened with a clean pair of scissors.

The stoppers were pooled in the same glassware used for the blank.

Study 1

50 mL of Milli-Q Water contained in the flask was poured in the backer in which were present 11 stoppers.

The backer was shacked to wet all the inner surface of the backer and all the stoppers.

The water was filtered utilizing a 0.22 µm filtering system.

The filter was placed in a specific container closed and observed under the microscope, classifying the particles in three categories:

- dimension of particles between 25-50 µm
- dimension of particles between 50-100 µm
- dimension of particles or fiber > 100 µm

It was then calculated the proved clean index (PCI) = $\frac{[(A \times 0,1)+(B \times 0,2)+(C \times 1,0)]}{10}$

The same procedure was performed using a triplicate of set of 11 stoppers.

Study 2

2 L of Milli-Q Water contained in the flask was poured in the backer containing the stoppers.

The backer was shacked to wet all the inner surface of the backer and all the stoppers.

The water was filtered utilizing a 0.22 µm filtering system.

The operations described above were executed for all the remaining stoppers, using the same filter.

The filter was then placed in a specific container closed and observed under the microscope.

Study 3

2 L of Milli-Q Water contained in the flask was poured in the empty stoppers bag

The bag was shacked to wet all the inner surface.

The water was filtered utilizing a 0.22 µm filtering system.

The filter was placed in a specific container closed and observed under the microscope.

Results

In the tables below are summarized the results obtained for each stoppers bag.

Table 59 Batch M220112 - rinsing study results and PCI value

Batch M220112					
	11 stoppers Run_1	11 stoppers Run_2	11 stoppers Run_3	Remaining stoppers	Bag rinse
Particles 25 - 50 um	1	4	1	1	5
Particles 50 - 100 um	0	0	0	0	0
Particles or fibers > 100 um	10	2	6	8	>50

	PCI value	
11 stoppers Run_1	1,01	limit < 3,4
11 stoppers Run_2	0,24	
11 stoppers Run_3	0,61	

Table 60 Batch T217123 - rinsing study results and PCI value

Batch T217123					
	11 stoppers Run_1	11 stoppers Run_2	11 stoppers Run_3	Remaining stoppers	Bag rinse
Particles 25 - 50 um	0	2	3	0	0
Particles 50 - 100 um	0	0	0	0	0
Particles or fibers > 100 um	4	0	10	>50	>50

	PCI value	
11 stoppers Run_1	0,4	limit < 3,4
11 stoppers Run_2	0,02	
11 stoppers Run_3	1,03	

Table 61 Batch T2210889 - rinsing study results and PCI value

Batch T2210889					
	11 stoppers Run_1	11 stoppers Run_2	11 stoppers Run_3	Remaining stoppers	Bag rinse
Particles 25 - 50 um	2	2	2	1	2
Particles 50 - 100 um	0	0	0	0	0
Particles or fibers > 100 um	18	5	6	>50	>50

	PCI value	
11 stoppers Run_1	1,82	limit < 3,4
11 stoppers Run_2	0,52	
11 stoppers Run_3	0,62	

For all the stopper batches investigated within the rinsing study, it can be noticed that there is a higher amount on fibers, in particular with dimension > 100 um, coming from the "beta bag" sample (data from the rinsing of the bag) and "remaining stoppers" sample (data coming from the washing of the 3967 stoppers left in the bag). Fibers with dimension > 100 um are the order of size of most of the particles identified during Engineering runs.

Since all the analyzed batches provided the comparable results, it can be assumed that most of particles remained attached to the bag.

Ten filters coming from the rinsing study, particularly from the blank samples and “remaining stoppers” and “beta bag” samples, were characterized by external laboratory. The characterization confirmed the data collected during the rinsing study, in terms of order of magnitude in the number of particles/fibers founded, and the presence of very few particles in the blank preparation, highlighting no source of contamination, linked due to the execution of the rinsing study itself.

The results of the rinsing study, joint with the information collected about the Supplier processes, highlight the following:

- The bags analyzed were not subjected to any manipulations before the study, and are representative of the stoppers in the status that is delivered by the supplier and used in production. This is supported also by the good outcome of the analysis of the filter from the “blank” samples);
- three (3) bags were used for the rinsing study, from different lots (including also batch M220122 used in both the Engineering batch and Water trial). All bags showed a high amount? of foreign particles found particularly after rinsing of the bag itself.
- the Daikyo investigation report reports that the visual inspection step at has some weaknesses that could potentially explain the high amount of particles detected.

Water trial at maximum batch size (MabThera 100 mg and MabThera 500 mg)

Considering that the engineering trials have been manufactured at half of the minimum batch size foreseen for commercial manufacturing, two water trials at maximum batch size (approximately 70000 pieces for MabThera 100 mg and approximately 14600 pieces for MabThera 500 mg) have been manufactured in December 2022, as part of the investigation.

The activities have been managed according to Technical memo TT237Z051.

All the activities executed for both the two water trials, are identical to the steps performed during the manufacturing of the ENG batches. No variables were therefore added that could impact the results. the results obtained are representative of the standard process.

Below the activities performed:

- cleaning and set up of the Isolator and Xtrema filling line: activities executed as per internal SOP which were the same activities done for the preparation during the engineering trials;
- filling of storage tank RTR344 with water for injection (WFI)
- transfer of the WFI from the storage tank to the filling bag (disposable assemblies equipped with tubing and needles) through Y assembly (equipped with steam thru and Aseptiquik connection) and sterilizing filtration assembly equipped with two sterilizing filters. The assemblies used in the water

trial were the same item of the assemblies used during the engineering trials and also the set up and process steps were the same.

-filling of approximately maximum batch size for MabThera 100 mg foreseen for commercial manufacturing (70312 vials filled MabThera 100 mg)

- cleaning of the Xtrema filling line and Isolator: activities executed as per internal SOP which were the same activities done for the preparation during the engineering trials;

-filling of approximately maximum batch size for MabThera 500 mg foreseen for commercial manufacturing (14628 vials filled MabThera 500 mg)

As per engineering trials, between the two formats, installation of the dedicated formats parts and cleaning and VHP cycle has been executed, as per internal SOP

For the manufacturing of MabThera 100 mg format it has been used the stopper (product code 273438) batch number T217123 and for MabThera 500 mg it has been used the stopper (product code 273438) batch number M220122. The batch M220122 is the same batch used for the manufacturing of both engineering batches.

Finally, the two batches (one batch for MabThera 100 mg and one batch for MabThera 500 mg) have been visually inspected for the following defect "Vial with glass fragment, particles, foreign bodies in the solution", which was the one in scope of the investigation.

Here below are reported the results of the manual visual inspection:

- MabThera 500 mg: 14628 vials have been visually inspected and 6 vials were discarded for presence of particles/fibers. The percentage defect rate is approximately 0,04%
- MabThera 100 mg: 70312 vials have been visually inspected and 3 vials were discarded for presence of particles/fibers. The percentage defect rate is approximately 0,004%

Table 62 summary of engineering batches and water trial

MabThera Batch	Batch size [# vials]	Scrap for particles [%]	Stopper batch number	Number of bags of stopper used (*)
MabThera engineering 50 mL	1910	~ 3%	M220112	~ 1
MabThera engineering 10 mL	9678	~ 0,1 %.	M220112	~ 3
MabThera Water trial 50 mL	14628	~ 0,04%	M220122	~ 4
MabThera water trial 10 mL	70312	~ 0,004%	T217123	~ 18

In conclusion, by combining the table above and the rinsing study outcome it is possible to conclude that the different batch sizes (engineering versus water trials run at maximum batch size) demonstrated a different result in terms of variability, and confirm that the use of several bags can

vary the particles/fibers percentage found, thus indicating a variability of contamination between bags. This phenomena could be linked to particles remaining attached to the bag inner surface (as also indicated from the rinsing study results). Indeed, the detachment of fibers/particles sticked to the beta bag can be likely considered as a variable event.

With the information present to date between the Daikyo's report, the rinse study and water trials carried out on Patheon stoppers, the particle load present in the stopper bag delivered by Daikyo is confirmed as the most likely root cause of the abnormal amount of particles detected during the Engineering run.

- **Product contact materials - Not likely root- cause**

The product contact materials used during the filling step have been evaluated as potential Root Cause (*Stainless steel tank and disposable assemblies*)

The engineering batches foresaw a common compounding and a splitted filling (TT528 for MabThera 500 mg and TT528/1 for MabThera 100 mg) between the two dosages and so the stainless-steel tanks used were the same for each batch.

The materials of the stainless steel tanks and of the disposable assemblies used for the manufacturing of the engineering batches were mapped (see Attachment 2) and the material are: polypropylene, Platinum cured silicone, polysulfone, polycarbonate, PVDF (Polyvinylidene Fluoride), PBT (Polybutylene Terephthalate), PES (Polieteresulfone), polyolefin elastomer, LDPE (low density Polyethylene) and stainless steel.

The assemblies are provided already gamma sterilized by Saint Gobain qualified supplier and they are commonly used also in other manufacturing process of the Xtrema line of Sterile area 6. It was checked if similar reoccurrence was present in other manufacturing process of Sterile area 6 using Saint Gobain assemblies and no reoccurrence was found.

The pipeline is composed by disposable assemblies with sterile to sterile connections and the product is filtered through two sterilizing filters with pore size equal to 0,22 µm.

The only assembly downstream the sterilizing filters is the filling bag (one assembly composed by the surge bag, the tubing and the needles) which is inside a beta bag of Polyethylene (PE). which is packed in a double bag of Polyethylene (PE). The first PE bag is unpacked before entering the filling room (room 843) and the internal PE bag is removed in the filling room (43) according to SOP 000103160 and SOP 000132047. The filling bag is then introduced in the isolator through the RTP port.

The stainless-steel tanks are cleaned and sterilized before the usage, as per SOP-000190518.

All the intrinsic particles/fibers identified (see Characterization of particles sub-paragraph) have a dimension higher than 0,22 µm, so they could not be introduced before the sterilizing filters.

All the material of the tanks and disposable assemblies, except for the silicone, do not correspond with the match of the characterization.

For the silicone, it must be highlighted that all the assemblies upstream the sterilizing filters can be excluded considering the dimension of the particles founded.

The only assembly downstream the sterilizing filters is the filling bag which can be excluded based on the historical data: filling bags supplied by Saint Gobain are already used in the manufacturing of Xtrema filling lines and similar events never happened.

- **Materials – vials- - Not likely root- cause**

The vials involved are:

- vial (50mL): product code 251584 and batch number T218562 for the manufacturing of TT528
- vial (10mL): product code 241582 and batch number T217772 for the manufacturing of TT528/1

The vials are produced by Schott according to Roche dedicated specification and are made of highly resistant borosilicate tubing glass (type I). The AQL check of the involved vials (batch T217772 and product code 241582 for the 10 mL vials, batch T218562 and product code 251584 for the 50 mL vials) performed by Thermo Fisher QC packaging during incoming inspection was conforming:

- vials 10 mL: 0 defective samples founded:
- vials 50 mL: 19 vials founded with scratch- single or multiple non reactive marks found anywhere on the exterior surface of the glass container > 3.0 mm(L) x >0.1 mm (W).

On the Xtrema line, the vials are manually loaded in the vial washer VEGA8 IMA (LFL0013) (details about washing machine are reported in the following paragraph). Vials are conveyed to a rotative carousel where they are washed with WFI and air-blown with compressed air filtered through a 0.22 µm filter. After the washing process, the vials are conveyed into the depyrogenation tunnel 1250FL DH (TST010) (details about depyrogenation tunnel are reported in the following paragraph).

Considering the vials are washed and depyrogenated, a potential root cause for the particles linked to the vials is considered unlikely.

- **Method – not likely root- cause**

Vial washer and depyrogenation tunnel

On the Xtrema line, the vials are manually loaded in the vial washer VEGA8 IMA (LFL0013). Vials are conveyed to a rotative carousel where they are washed with WFI and air-blown with compressed air filtered through a 0.22 µm filter.

The washing machine was qualified as per E878AC01 (IQ) and E878AD01 (OQ) and construction materials are: PEEK, stainless steel and Teflon. As per PQ protocol (E878BE01), the check was performed as per SOP 1395 resulting in the possibility to eliminate fragments with dimension higher than 0,100 mm. Moreover, as additional contaminant also vials treated with sodium chloride were challenged, in order to demonstrate the washing machine capability in terms of dirty removal.

After the washing process, the vials are conveyed into the depyrogenation tunnel 1250FL DH (TST010). After exiting the tunnel, the vials pass through an opening into the isolator and the filling machine. Before the starting of the manufacturing of each batch, the vial washer (LFL0013) and the depyrogenation tunnel (TST010) were prepared as per SOP-000128057. During the manufacturing of the batch, the operators performed periodical check (presence/absence glass) as per SOP-000132006 and no anomalies were observed, as reported in the executed BR.

Briefly, as per SOP-000132006 in the washing machine:

- if the breakage is at the exiting of the machine and the operators see the breakage: the machine is stopped and cleaned, before restarting the production.
- if the breakage is at the exiting of the machine and the operators do not see the breakage (eg: glass found during periodical check): the machine is stopped, the washing machine is empty, cleaned (all vials removed) before restarting the production.

As per SOP-000132006 in the depyrogenation tunnel:

- if the breakage is at the tunnel level and the operators see the breakage: the machine is stopped and the rotary table is empty, before restarting the production.
- if the breakage is at the tunnel level and the operators do not see the breakage (eg: glass found during periodical check): the machine is stopped and the rotary table is empty, before restarting the production. All the vials filled starting from the last conforming check are discharged.

Moreover, it was checked the layout of the machine which is designed in order to space the vials, avoiding the possibility to having friction between the vials. Indeed, the vials are transported by the machine format (infeed and outfeed screws) which is specifically designed to avoid friction.

Considering all the information listed above, a potential root cause for the particles linked to the vial washer (LFL0013) and the Depyrogenation tunnel (TST010) is considered unlikely.

Xtrema sterile 6 filling line, stoppering and gowning

During the set-up activities of the line, executed before the closure of the isolator and starting of VHP cycle, the operators wear Tyvek suit (High density polyethylene), sterile gloves (nitrile), mask (Polyethylene) and glasses (polycarbonate and Thermoplastic rubber frame), as per SOP 000101784.

During the set-up activities, the following materials are used:

- wiped sterile sheets (Patheon code 196512) are used. They are 100% polyester continuous-filament wipe with laser-welded edges to avoid the release of fibers and particles and to ensure the integrity of the cloth during use. They are washed and packaged by the supplier in a controlled contamination environment in a sealed double bag and then are supplied to Patheon already Gamma irradiated;
- stainless steel tweezers

During the manufacturing, the vials after the exiting of the tunnel, are moved automatically by mechanical formats part into the filling station. The filling of MabThera 500 mg and 100 mg is performed through peristaltic pump and so there is not any mechanical part of the filling machine in contact with the product during the filling step.

The stoppers, provided by the supplier to Patheon in a beta bag (GCL 190 25 L Port-bag, made of a film of Tyvek, 4000 pcs/bag) and ready to be sterilized, are sterilized as per SOP-0001100043 (121°C for 40 minutes). Then, they are moved (inside the beta bag) on a stainless-steel trolley and they are inserted in the stopper bowl through the RTP port.

The RTP port avoid any manipulation and activities of envelope cut, as the bag is attacked and opened by the RTP port directly in the isolator, this prevents any generation of fibers by the cut.

The stopper bowl (made of stainless steel) is washed and packed in a Tyvek bag (high-density polyethylene) and then autoclaved. During the set up activities, Tyvek bag is removed, and the bowl is installed inside the isolator as per SOP 000132530.

The machine layout was checked and after the stopper bowl there is a stainless -steel slide which conveys the stoppers from the bowl to the stoppering station. The station layout was checked and it was designed to avoid frictional area

During all the manufacturing of MabThera 500mg and MabThera 100 mg the control of absence of glasses on the line was executed every 30 minutes (+/- 5 min) as per SOP 000128048.

In case of glass breakage and operators do not see the breakage (finding of glass fragments during check) all the vials filled, starting form the last conforming check, are discharged.

The manufacturing documents (executed BR) were checked, and no anomalies were observed.

Moreover during the manufacturing of the batches, the isolator was closed and so there was not any contact between the line and the operators.

Inside the isolator are present the following tools:

- stainless steel scissors, tweezers and beams;
- IPA
- mirror made of glass
- stainless steel clamp

During routine manufacturing they are handled as per SOP 0 00132006.

The stainless steel scissors, tweezers, beams, stainless steel clamp are first enveloped in double cellulose bags and they are sterilized in autoclave. Before entering the isolator (before launching the VHP cycle) the external layer is removed.

Inside the isolator they are so enveloped in a single cellulose bag. When the tool is needed, the cellulose bag is removed below the level of the stopper bowl and the air laminar flow is present which push the air on the bottom of the isolator and moreover near to the hooper non-viable probes are present. The procedure is the same for every batch manufactured on the Xtrema line on which the historical data (details are reported in the dedicated sub-paragraph) confirm no presence of particles in the visually inspected vials.

Moreover, even if around the stopper bowl the tools useful for the set-up activities are present, it has to been highlighted that the operators handle those tools through the dedicated gloves which are present at the two edges of the bowl, avoiding in this way the possibility to pass over the bowl.

The filters of the Depyrogenation tunnel (including the cooling zone) and of the isolator (ISL016) were checked on August 2022 and they were conforming (Protocol E1232AM02 for the tunnel and protocol E1233AM02 for the ISL016 signed on September 2022).

In the washing machine two filters for the water (recirculation water filter and ultrasonic tank recirculation water filter) are present and one filter for the compressed air is present. They are managed as per internal SOP 1251. The filters for the water are replaced every year and the air filter every six months

The details of material composition of the water filters and air filters, with the relative filter's housings, are reported below:

Recirculating water filter, code 196285

- Medium: bonded glass fiber, positive Zeta;
- support/drainage: polyester;

- core/cage: polypropylene;
- o-ring: silicone

and the relative filter housing:

- housing : 316L stainless steel
- o-ring: silicone

Ultrasonic tank recirculation water, code 196575

- Rigimesh filter
- Mesh: stainless steel AISI 304 mesh
- gasket:nitrile

and the relative filter housing:

- housing : 316L stainless steel
- o-ring: silicone

Compressed air filter LFL013, code 195037,

- Membrane: PTFE
- O-rings: silicone

and the relative filter housing:

- housing : 316L stainless steel
- o-rings: silicone

On top of the materials detail reported above, also the status of the filters has been checked.

- Isolator filter test: at the check performed in August 2022, the ISL016 isolator filters were all compliant and none of the filters required preventive replacement.
- Vials washer/tunnel filter test: at the August check, the tunnel and vial washer filters were all compliant and none of the filters needed preventive replacement.

Moreover, the information related to the airflow pattern inside the isolator are reported: isolator is monitored for particles $\geq 0.5 \mu\text{m}/\text{m}^3$, as per Annex 1, with limits at rest and at operational of 3 520. The verification of the particle contamination "non viable" in "at rest condition" were performed on the cold part of the tunnel (TST010-COLD) and was compliant (the reference document is E1232AM02).

During the manufacturing of engineering batches the particle monitoring has been performed as per SOP-000140923 and no events occurred.

Finally, between the filling of MabThera 500 mg and MabThera 100 mg, a VHP cycle was lunched.

Considering all the information listed above, a potential root cause for the particles linked to Xtrema sterile 6 filling line, stoppering and operators is considered unlikely.

- **Machine - Not likely root- cause**

The events involved in this issue are not related to potential problems with the machine used in the production. No anomalies have been recorded.

- **Measurement - Not likely root- cause**

No issues about measurements have been pointed out from the investigation of the suppliers. All the dimensional checks required for incoming stopper batch, have been correctly executed as per ThermoFisher internal SOPs and the dimensional checks resulted all well within the acceptance limits.

As per historical supplier report, the determined AQL levels depend on the size and nature of the fiber and are comply with the specification.

- **Milieu/ Mother nature – Not likely root- cause**

Neither issues related to the environmental conditions (temperature, humidity, pressure and viable/non-viable particle measurements) nor related to the manufacturing environment (Sterile Area 6) have been detected attributable to the defect found. All the cleaning activities for the manufacturing environments and machines at Patheon were properly executed according to the relevant SOP. Besides, no problems unrelated to the Patheon production site that could be linked to the event under investigation, such as faults in the electrical system, have been highlighted.

DEVIATION/ COMPLAINT LOGBOOK

From the point of view of the historicity of finds on the ST6 line concerned, all the 48 batches produced in 2022 were taken into consideration.

The products have two different inspections. For the first product taken into account, the AVI process is applied and there were no out of limits (the limit is 1%) for the camera involved in the inspection of fibers and particles.

The second product, on the other hand, requires SAVI inspection and for all the lots produced, the result is 0 defects for fibers and particles.

Conclusion

Based on the investigation and technical trials performed (brief details listed below as bulleted points), the potential root cause of visible particles detected during the visual inspection is likely due to the trapped particles in the stoppers bags from Daikyo. In light of the results collected, it can be assumed that the use of several bags can vary the percentage of visible particles found in the final product, indicating a potential variability of particle contamination between different bags.

Below is the summary of all assessments performed on the particles/fibers issue :

- the material, design and qualification of Xtrema filling line (vial washer, depyrogenation tunnel, filling and stoppering machine) have been investigated and no source of contamination was identified.
- the gowning procedure, the cleaning, environmental monitoring which are in place for sterile area 6 have been investigated and no source of the contamination was identified.
- an evaluation on the historical data for other products manufactured on the Xtrema filling line in relation to the visible particle contamination revealed no out of conformance trend observed (details are reported in dedicated sub-paragraph);
- the disposable assemblies and stainless-steel tanks were investigated and excluded as a source of contamination for the following reasons:
 - o the tanks and all the assemblies upstream of the sterilizing filters can be excluded as per dimension of particles/fibers (dimension of particles/fibers higher than 0,22um which is the pore size of the sterilizing filters) and usage of sterile to sterile connections;
 - o the assemblies downstream the sterilizing filters (filling bag equipped with tubing and needles) were excluded because same type of assemblies was used for the manufacturing of other product batches without any adverse trend in terms of particles/fibers, as mentioned below)
- a rinsing study on stopper bags was executed, which highlighted that particles could remain most likely attached to the bag inner surface. The bags subjected to the study were not manipulated before, therefore are actually representative of the Daikyo manufacturing process (as also confirmed by the “blank” samples from the study, which resulted in a much lower fiber/particles presence)

Moreover, the rinsing study outcome has been confirmed from the characterization activity carried out at the external laboratory, in which filters from the most contaminated samples were analyzed, confirming the presence of high number of fibers/particles.

- a water trial batch at maximum batch size was produced for the both dosages (for MabThera 500 mg: 14628 visual inspected vials and approximately 0,04% for the defect rate for particles/fibres; for MabThera 100 mg: 70312 visual inspected vials and approximately 0,004% for the defect rate for particles/fibers). The results from the water trial demonstrate (in terms of fibers/particles) that no source are from the filling operations and a likely bag to bag variability of the foreign matter load and distribution;
- Daikyo has been notified for the MabThera engineering trial particles/fibers finding and an official complaint was opened (EN4071222, West QNS 200034794). As outcome from the complaint, the packaging step has been identified as the possible source of contamination (leading to an operators re-training execution) at Daikyo side. In addition, there is a possibility that transparent or similar in color as stopper fibers/particles might not be detected from the

visual inspection machine. Daikyo has confirmed that due to limitations of the current automatic visual inspection machine it is not possible to resolve the above issues immediately.

- A comparison with similar complaint notified to Daikyo from Patheon was assessed for similar event recorded on different filling lines (complaint EN3041221 West QNS 200024332) revealed the re-occurrence of the event and the only improvement suggested was consideration of D-sigma stopper usage.

AQL activities executed on both the Engineering batches have been performed and no defective vials were identified, confirming the good capability of detecting defected units during the manual inspection.

The following mitigation and actions have been identified and will be put in place:

- the stopper batch used in Engineering runs will be put in blocked status before PPQ and not used for any GMP production (neither PPQ production nor following post validation batches);
- for the PPQ batches four different stopper batches will be used (one stopper batch for one PPQs batches, avoiding mixing of stopper batches in one single PPQ batch).
- more information will be collected out of the next production batches in order to monitor particles/fiber contamination trend and take additional actions if needed
- discussion with Daikyo will be continued in order to evaluate further possible improvements

8.15.2.2 AQL on MabThera 100 mg batch

Vials from the inspected batch were randomly sampled as per ISO 2859-1, applying a general inspection level II (plan for normal inspection), as per SOP-000118671, to perform a statistical control (AQL) during the visual inspection activities as per Patheon Monza SOP-000028049 (SOP-1804/F). QC Packaging operators performed the statistical check (AQL)

The sampling plan was defined according to the batch size and the critical defects acceptance = 0 criteria, as per SOP-000028049 (SOP-1804/F) current revision.

The check performed by QC packaging was conforming, finding 0 vials non-conforming.

The defects were classified as explained in table below.

Table 63 Classification of defects

Defect category	Description
m	Minor defects do not impact product performance or compliance; they are often cosmetic in nature, affecting only product appearance or pharmaceutical elegance.

Defect category	Description
M	Major defects carry the risk of a temporary impairment or medically reversible reaction or involve a remote probability of a serious adverse reaction. This classification is also assigned to any defect which causes impairment to the use of the product. These may result in a malfunction that makes the product unusable
C	Critical defects are those that may cause serious adverse reaction or death of the patient if the product is used. This classification includes any nonconformity that compromises the integrity of the container and thereby risks microbiological contamination of the sterile product.

Table below show the AQL that will be applied during the statistical control:

Table 64 AQL Table

	AQL for Critical defects	AQL for Major defects	AQL for minor defects
Acceptance criteria	None Allowed	0.65	2.5
Results	Compliant	Compliant	Compliant

Visible particles test were executed according to USP <790> and Ph.Eur.2.9.20 using an AQL of 0.65. Table 2 A (Single sampling plans for normal inspection) of ISO2859-1 defines the acceptance criteria for each classification of defects (Critical, Major or minor) as per SOP-000028049 (SOP-1804/F). The results were compliant.

8.15.2.3 Sample collection

After visual inspection, the test was conducted in accordance with the sampling plan reported in attachment 1.

During the manual visual inspection, it was executed the sampling as per AQL check (paragraph 8.15.2.3)

No other sampling collection was executed because all the following samples (Container/Appearance, Clarity, Color, pH, Osmolality, Extractable volume (min),Uniformity of dosage unit, Content of protein (by UV), Identity of rituximab (CZE), Purity by SE-HPLC, Purity by IE-HPLC, Visible particles, Subvisible particles, Sterility, Endotoxin) were taken during crimping steps and manual visual inspected as prioritized as compare to the rest of the batch, as per protocol TT237B011

Moreover, during crimping steps were collected and then prioritized in the visual inspection also samples for potency and stability challenge was collected and these vials were analyzed by Roche.

The result for potency were compliant, as per Roche results (attachment #11)

The analytical results from MabThera 100 mg stability studies showed that the results of the side-by-side stressed stability study demonstrate that the MabThera DP (100 mg) manufactured at Patheon Monza changes in a similar way to GNE Rituxan DP Controls with regards to degradation mode, chromatograms, and degradation rates.

Finally, samples exposed to light were collected for MabThera 100 mg. Particularly:

- vials subjected to the second 30 minutes of stoppage during filling of MabThera 100 mg and to light exposure for 30 minutes (attachment #10) in AVI room (room 1116), they finally underwent MVI (under BAO light source) for four times in order to evaluate light impact on product.
- 10 vials collected during filling and later exposed to light for 48 hours in filling room (attachment #11) and then exposed to light (attachment #11) for 30 minutes in AVI room (room 1116), they finally underwent MVI (under BAO light source) for four times in order to evaluate, also in this case, the light impact on product.

The results (RPT-0309669) demonstrated that light exposure from Patheon's routine manufacturing process did not impact MabThera DP quality.

8.16 LINE LOSSES

It was evaluated the lines losses during the manufacturing of these engineering batches.

During the solution preparation room activities, it was lost approximately 1,7 Kg in the bioburden filtration assembly.

Table 65 Line losses during BBR filtration step

Description	weight
BS before BB filter	252,2 Kg
BS after BB filter	249,8 Kg
Sampling	approx. 747,4 g
Losses during BB filtration	approx. 1,7 Kg

During the filling activities, taking into account both formats 50 mL and 10 mL, approximately 4 Kg of BDP was lost.

Considering that it was used a dedicated extension tubing, sterilizing filtration assemblies and filling bag for every format, it can be estimated a line loss of approximately 2 Kg for every format. However, this can be consider a worst case scenario because, in order to split the two formats, it was needed

the closure of the bottom valve after having filled approximately half of the solution in the storage tank: this causes a higher losses in the lines which however are not fully representative of the commercial manufacturing activities considering that for every format a dedicate preparation will be present and no splitting is foreseen.

Table 66 Line losses during filling of MabThera 50 mL and 10 mL

Description	Weight
Storage before 50 mL and 10 mL filling	249,8 Kg
Solution filled during 50 mL (not included vials during needles purging)	123,9 Kg
Solution lost during needles purging 50 mL	approx 2,8 Kg
Solution filled during 10 mL (not included vials during needles purging)	111,8 Kg
Solution lost during needles purging 10 mL	approx 1,4 Kg
Sampling	approx 5,9 Kg
Total losses (sum of filling of 50 mL and 10 mL)	approx 4 Kg

8.17 STORAGE AND SHIPMENT

Upon Visual Inspection completion, the finished product have been currently stored into the Warehouse cold store at 2-8°C. The samples has been shipped under Roche responsibility. After closure of this Engineering Report, any remaining finished products can be discarded.

For the samples shipment set up, the Akylux black boxes coming from production were used. Vials were secured by placing the appropriate alveolar and space filler in case of the 50mL dosage.

9.0 PROCESS HOLDING TIME

9.1 MICROBIOLOGICAL HOLDING TIME

In this paragraph are reported details about the microbiological Holding time (HT) which was supposed to be challenged and the results obtained during these Engineering batches:

- Microbiological grow in buffer tank (Buffer holding time): it was supposed to be challenged approximately 24 hours from the start of WFI addition to the end of buffer addition in compounding tank;
 - It was challenged 33 hours and 45 minutes.
- Microbiological grow in F/T tank (F/T tanks holding time): it was supposed to be challenged approximately 8 hours at room temperature plus approximately 6 days at 2-8°C from end of

cooling phase after thawing to start of BDS pooling in compounding tank; For every F/T tank the total (cumulative) time at room temperature has to be maximum 48 hours. This aspect was not verified directly during this engineering because the same F/T tank will be used for the PPQ. Therefore, cumulative time consumed at room temperature during this engineering run was supposed to be:

$$A + B + C + F + G = \text{approximately 16 hours}$$

where A, B, C, F and G are reported in Table below.

Despite two F/T cycles were executed during this engineering trials, the fulfillment of 16 hours was however achieved:

- total time at room temperature equal to 15 hours and 19 minutes

Table 67 Cumulative holding time at room temperature for the F/T tank during engineering runs

	Start	End
A	End of cooling phase	Entering of the F/T tank in the 2-8°C cell for the HT challenge at 2-8°C, before the BDS pooling
Results first F/T cycle	09/06/2022 11:01	09/06/2022 19:35
Results second F/T cycle	02/07/2022 11:16	02/07/2022 12:30
B	Exit of the F/T tank from the 2-8°C cell (after HT at 2-8°C, before BDS pooling)	Entering of the F/T tank in the 2-8°C cell (after BDS pooling)
Results first F/T cycle	NA (*)	NA (*)
Results second F/T cycle	04/07/2022 11:43	04/07/2022 13:32
C	Exiting of the F/T tank from the 2-8°C cell (HT after the BDS pooling)	Start of re-freezing
Results first F/T cycle	20/06/2022 11:47 (**)	20/06/2022 12:45
Results second F/T cycle	07/07/2022 14:06	07/07/2022 15:01

	Start	End
F (***)	Receiving tank form -25 °C cell before thawing	Start of thawing cycle recipe
Results first F/T cycle	08/06/2022 12:33	08/06/2022 13:22
Results second F/T cycle	01/07/2022 13:12	01/07/2022 13:44
G (***)	End of freezing cycle	Tank received in the elevator
Results first F/T cycle	21/07/2022 13:17	21/06/2022 13:22
Results second F/T cycle	08/07/2022 10:53	08/07/2022 11:16

(*) data not available due to unforeseen re-freezing cycle.

(**) BDS pooling step not performed

(***) this timing was not captured in the protocol TT237B011, however they were tracked during the engineering batch in the dedicated TIR/TOR FORM (FORM-000225917).

After the BDS pooling, additional 3 days at 2-8°C were supposed to be challenged for the F/T tank, before the start of re-freezing.

Therefore, the cumulative holding time at 2-8°C for the F/T tank during engineering run was supposed to be:

$$D + E = \text{approximately 9 days}$$

Where D and E are reported in Table below.

In the table below, it is reported the data considering the two F/T cycle executed.

Table 68 Cumulative holding time at 2-8°C for the F/T tank during engineering runs

	Start	End
D	Entering of the F/T tank in the 2-8°C cell for the HT challenge at 2-8°C, before the BDS pooling	Exit of the F/T tank from the 2-8°C cell (after HT at 2-8°C, before BDS pooling)
Results first F/T cycle	09/06/2022 19:35	NA (**)

	Start	End
Results second F/T cycle	02/07/2022 12:30	04/07/2022 11:43
E	Entering of the F/T tank in the 2-8°C cell (after BDS pooling)	Exiting of the F/T tank from the 2-8°C cell (HT after the BDS pooling)
Results first F/T cycle	NA (**)	20/06/2022 11:47
Results second F/T cycle	04/07/2022 13:32	07/07/2022 14:06

(**) BDS pooling step not performed

The cumulative timing at 2-8°C spent during this engineering run (D+E), which should have been approximately 9 days, it was 15 days. This was due the unplanned second F/T cycle which was executed during the engineering runs. At the donor site the cumulative timing validated at 2-8°C is 60 days. If the 60 days are divided by the three possible F/T cycles , the result is 20 days which is higher than the maximum time (15 days) spent at Thermo Fisher.

- Microbiological growth in compounding tank (compounding holding time): < 24 hours at room temperature from start of BDS pooling to end of bioburden reduction filtration.
 - It was challenged 19 hours and 59 minutes.
- Microbiological growth in storage tank (Storage holding time): approximately 24 hours at room temperature from the beginning of bioburden filtration. At the end time at room temperature, other approximately 24 hours at 2-8°C was supposed to be challenged (start is attachment of 1100L tank to glycol for cooling down). Finally other additional time at 2-23°C was evaluated (this additional time starts from start the filling of first vials to the end of sterilizing filtration for the filling of the last vial and it will be experimental evaluated with the results of the engineering batches). The end of this holding time is the end of sterilizing filtration which can be assumed equal to end of filling. As per filter validation, the maximum contact time for sterilizing filter is 72 hours.

It was challenged:

- 24 hours and 7 minutes at room temperature
- 24 hours at 2-8°C
- 2 hours and 24 minutes during the filling of MabThera 50 mL
- 3 hours and 24 minutes during the filling of MabThera 10 mL

- The filling holding time is 48 hours per media fill. During engineering, this time was respected for both formats filling.

9.2 PHYSICAL-CHEMICAL HOLDING TIME

The maximum cold chain interruption at Roche side for the manufacturing of MabThera from the starting of BDS pooling to the end of visual inspection activities is 147 hours. The time at 8-25 °C for the thawed BDS before BDS addition is 48 hours. The 147 hours are referring to manufacturing of specific batch, whereas the 48 hours is cumulative time for the F/T tank (maximum three F/T cycles are admissible). Same approach was applied also in Patheon, as agreed with the client.

During engineering trials, the fulfillment of the requirement of the 48 hours was achieved, as reported in the tables below. Moreover, the cumulative TOR consumed during these engineering trials was also less than 16 hours which can be considered the maximum value for single F/T cycle.

Table 69 Cumulative TOR consumed during engineering runs for the F/T tank

	Time
Cumulative TOR consumed during first F/T cycle	10 hours and 26 minutes
Cumulative TOR consumed during second F/T cycle	4 hours and 53 minutes
Total TOR consumed during engineering batches	15 hours and 19 minutes

For the TOR starting from the pooling of BDS until returning at 2-8°C cell after visual inspection, the values obtained (Table 56) are due to the holding time challenge in the sterile area and due to the visual inspection.

During the night shift, manual visual inspection is not performed. During these engineering runs, it was ongoing the equilibration study for each format and so the pallet remained at room temperature also during night. Moreover, for the 100 mg format, it was needed more than one day to conclude the visual inspection and in order to optimize the activities and considering that enough TOR was

left, the pallet was not put back at 2-8°C also the other nights. Starting from PPQs batches, every pallet will be put at 2-8°C cell during night in order to save TOR.

However, during engineering trials, the fulfillment of the requirement of the 147 hours was achieved for both batches, as reported in the tables below.

Table 70 TOR consumed during manufacturing of MabThera 50 mL (starting form BDS addition)

		MabThera 50 mL	
		TOR consumed in Sterile 6	Total TOR consumed in sterile 6 + VI
Batch	TOR consumed in VI		
Batch	53 hours and 53 minutes	35 hours and 44 minutes	89 hours and 37 minutes
Sample	53 hours and 36 minutes	28 hours and 41 minutes	84 hours and 17 minutes

Table 71 TOR consumed for manufacturing of MabThera 10 mL (starting form BDS addition)

		MabThera 10 mL	
		TOR consumed in Sterile 6	Total TOR consumed in sterile 6 + VI
Batch	TOR consumed in VI		
Batch	55 hours and 27 minutes	77 hours	131 hours and 27 minutes
Sample	55 hours and 27 minutes	12 hours and 35 minutes	68 hours and 2 minutes
Sample S_L_100	55 hours and 27 minutes	23 hours and 55 minutes	79 hours and 22 minutes
Samples LIGHT_48_100	96 hours and 50 minutes	12 hours and 15 minutes	109 hours and 5 minutes

The physiochemical cumulative holding time for one F/T tank is 60 days at 2-8°C from the start of first thawing (start is the first thawing of the tank and the end is the transfer of the BDS to the compounding tank of the last manufacturing).

During these engineering trials it was cumulative consumed approximately 15 days (details are reported in the table below) which is less than the 60 days available at Roche side.

Table 72 TIR consumed on the F/T tank considering the two F/T cycles

Time

Cumulative TiR consumed during first F/T cycle	256 hours and 12 minutes (10 days, 16 hours and 12 minutes)
Cumulative TiR consumed during second F/T cycle	119 hours and 47 minutes (4 days, 23 hours and 47 minutes)
Total TiR consumed during engineering batches	375 hours and 59 minutes (15 days, 15 hours and 59 minutes)

11.0 LIST OF ACRONYMS

Table 73 List of acronyms

Reference Acronyms	Meaning Reference #
HT	Holding time
HMI	Human machine interface
MVI	Manual visual inspection
IPC	In process control
MBR	Master Batch Record
pCPP	Potential critical process parameter
VPHP	Vapor phase Hydrogen Peroxide
OEL	Occupational Exposure Limit
BDS	Bulk drug substance
BS	Bathc size
BB	Bioburden
BDP	Bulk drug product
DP	Drug product
TOR	Time out of refrigerator

10.0 CONCLUSION

The MabThera engineering runs (one common compounding, filling of MabThera 500 mg, filling of MabThera 100 mg) can be considered successfully completed. Indeed, a good manufacturing

process performance was confirmed as well as smooth materials processability along the Xtrema filling line. Moreover, the disposable assemblies suitability and fluid path set up were confirmed appropriate.

Thawing/Freezing of the BDS

The thawing and freezing process was successfully completed twice. All the potential critical process parameter were fulfilled, and the samples taken met the acceptance criteria confirming the good applicability of the freezing and thawing steps.

To emptying the recirculation tube, it is suggested to use a dedicated tool (similar to the one already used at the donor site) to squeeze manually the tube. Moreover, if possible (when thawing recipe is finished), before the above listed manual step, it is suggested to perform a pre-step with the peristaltic pump, inverting the rotational speed.

Buffer preparation

The buffer preparation was successfully completed. The mixing range (100-200) rpm identified during the surrogate trial (TT237D011) was successfully applied also during the engineering batches. During engineering runs, the timing between the addition of one excipient and the following one was based on conformity of the visual check dissolution and the timing was recorded.

From a practical point of view, it is recommended to mix:

- at least 10 minutes after the addition of sodium chloride;
- at least 20 minutes after the addition of sodium citrate dihydrate.
- at least 20 minutes after the rinsing of the PS80
- at least 2 hours from the first WFI addition

For every stock solution, the mixing ranges were identified. For the engineering runs, the HCl was added in small aliquots for a total of 1500mL.

Considering that:

- during engineering runs its was added 1500 mL of HCl, reaching 6,7 pH
- data from the donor site foreseen the addition of approximately 1700 mL;

it is recommended to perform the pH adjustment starting with a first aliquot of 800 mL and using a reducing amount for the following ones (100 mL), to reach approximately the target value of pH (6,5).

All analytical results confirmed the good process established for the buffer preparation.

BDS pooling

In the BDS pooling steps, the set up of the operations, the assembly connection and the usage of the peristaltic pump were tested, confirming good results. The range of the peristaltic pumps during pooling was identified between 20 and 50 rpm. All analytical results confirmed the good process

established for the pooling step. It is recommended to pooling the BDS at 50 rpm considering that no impact on the BDS is foresaw and moreover at 50 rpm the pooling step will be faster and so potentially less TOR will be consumed for the F/T.

Buffer dilution and compounding operation

The calculation for the compounding activities was successfully performed by the operators. The results of the protein content analysis confirmed the good applicability of the steps.

Mixing stress study

After the pooling of the BDS and buffer dilution in the compounding tank a mixing stress study was executed. During the surrogate trial (TT237D011) the mixing range identified was 150 rpm-250 rpm and the minimum mixing time identified was 15 minutes.

To perform a stress study, the BDS was mixed for a maximum of 45 minutes at the maximum velocity identified during surrogate trial (250 rpm). Samples for purity were taken at every time point (every 15 minutes). For every time points the results were comparable, showing that the BDP can be mixed at 250 rpm until 45 minutes without any impact on the quality of the product. Moreover, results of the visible and subvisible particles were comparable between time zero (before mixing) and after mixing, confirm the applicability of the process parameters identified.

Bulk homogeneity

After the mixing and during the transfer from the compounding tank to the storage tank, it was taken samples representative of the bottom, middle and top of the tank to confirm the bulk homogeneity. The results confirmed that after the mixing steps the bulk was successfully homogeneous.

Bioburden reduction filtration and storage

The bioburden reduction filtration from the compounding to the storage tank was successfully performed and the post use test of the bioburden filter was conforming and the operative activities of the test were performed smoothly. It is recommended to perform the bioburden reduction filtration at 0,6 abr.

Sterilizing filtration and filter flush study

The sterilizing filtration assembly confirmed good results in terms of the integrity of both sterilizing filters (both pre-use and post-use) in both trials.

In both trials, all the utilities filters (post use tests) were conforming. No recurrences of the non-conformances happened during the surrogate trial (Surrogate trial report TT237D011) were present, confirming the effectiveness of the corrective actions identified during surrogate trials and implemented during the engineering trials.

The filter flush study of sterilizing filters identified that at least 1000 mL of solution need to be flush, however to have the protein content at the target of the acceptance criteria is recommended to flush 2000 mL.

Filling/stoppering

Filling accuracy was assessed overtime during the execution of both formats (MabThera 500mg and MabThera 100 mg) and good capability of the process was observed ($P_{pk}/Cpk \geq 1,33$).

MabThera 500 mg

For the engineering of MabThera 500 mg, several machines eject due to filling bags emptiness were present in the last part of the trial. The emptiness of the filling bags were caused by too low pressure in the storage tank during the last part of the trial, not compatible with the high amount of solution exiting from the filling bags during the filling.

During the first part of the trial (pressure > 0,50 bar) no similar occurrence were present and the filling process did not show adverse trend in the machine rejects. Moreover, no foam was present in the filling bags and the samples taken were conforming.

All the samples taken from filling homogeneity were conforming confirming the homogeneity of the solution among all the filling process.

It is recommended to set the target of the pressure in the storage tank at 0,6 bar (same pressure applied also during the bioburden reduction filtration step).

It is also recommended to have the working range of the filling bag at 50%-80% (values applied during this engineering trial).

Morevoer, an optimization during the filling was performed on the height of the needle to minimize foam creation.

Finally, it is recommended to have in the recipe at least 20 purging cycle with a purging volume per needle equal to 26 mL

No adverse trend in the stoppering machine rejects.

MabThera 100 mg

All the samples taken from filling homogeneity were conforming confirming the homogeneity of the solution among all the filling process.

During the filling of MabThera 100mg the pressure in the storage tank was set at 0,3 bar, but considering that for MabThera 500 mg it is recommended to set the target of the the pressure in the storage tank at 0,6 bar (same pressure applied also during the bioburden reduction filtration step), it is recommended to set the same value (target =0,6 bar) also for MabThera 100 mg to simplify the

practical operations. In particular, this value has to be set on the HMI by the operators before the starting of the filling.

Moreover, an optimization during the filling was performed on the height of the needle to minimize foam creation.

Finally, it is recommended to have in the recipe at least 14 purging cycle with a purging volume per needle equal to 12 mL

No adverse trend in the stoppering machine rejects.

Crimping

Crimping performance was evaluated through a CCI test and visual inspection. CCIT (vacuum decay method) executed on samples collected during both formats resulted in conforming (no sign of leakage). Visual inspection did not identify an adverse trend for defect related to crimping process. Thus, also the target values for the height of the crimping and pressure of the crimping are applicable. The maximum and minimum values for the height of the crimping and for the pressure of the crimping were confirmed being applicable during the surrogate trials run at maximum batch size (TT237D011). It is recommended to perform the crimping steps at the target value (100 KPa and 68 mm for MabThera 500mg and 90KPa and 46,5 mm for MabThera 100mg).

Equilibration study

It is recommended to estimate an equilibration time of approximately 15 hours for MabThera 500 mg and 14 hours for MabThera 100 mg.

Moreover, no bubbles were present on the vials during the checks performed during the equilibration activities confirm the good applicability also of the previous one manufacturing steps.

Visual inspection

For the visual inspection step of MabThera 500 mg and MabThera 100 mg engineering batches, out of all defects listed in the defect list, the following points were deeply investigated (based on the defective units obtained) :

- Cracked vials (body, bottoms): this defect was identified only for MabThera 500 mg prioritized samples inspected. This kind of defect could be linked to some friction between adjacent vials, in extracting some vials manually from the rest of the box. However, it must be noted that this type of activity was needed only for sampling activities which will be not performed during routine batches.
- Surface scratch on vial body (length > 1 cm, width > 2 mm): this type of defect (rounded scratches) was linked to a guide in stainless-steel present in the vials washing machine during engineering runs. Before the manufacturing of MabThera PPQs batches, the guide has been replaced in PPEK

materials (this implementation has been already put in place for other products manufactured on Xtrema filling line with good results)

- Vial with glass fragment, particles, and foreign bodies in the solution: the percentage defect rate found was 0,1 % for MabThera 100 mg and approximately 3% for MabThera 500 mg. The potential root cause of this defect was likely identified (as discuss in detail in paragraph 8.15.2.1) in particles trapped inside the stoppers bag coming from the manufacturing process steps at Daikyo supplier. The investigation lead also to the conclusion that the use of several bags can influence the percentage found in the final product, indicating a potential variability of contamination between bags. It has to be highlighted that AQL activities executed on both the Engineering batches have been performed and no defective vials were identified, confirming the good capability of detecting defected units during the manual inspection operations, decreasing a qualitative impact on the PPQ exercise. Finally, more information will be collected with the production of next batches in order to monitor particles/fiber trend and the discussion with Daikyo will be continued in order to evaluate further possible improvements.

VPHP study

The results of the study (Roche report RPT-0309731) demonstrated that residual VPHP from the decontamination process is sufficiently removed by the routine process at Thermo Fisher and no further controls are needed to mitigate drug product exposure to hydrogen peroxide. Furthermore, because MabThera 500 mg (50 mL in 50 cc vial) utilizes the same filling process with a larger fill volume and larger pump tubing, the results generated in the 100 mg (10 mL in 10 cc vial) configuration can be considered worst-case compared to the 500 mg configuration.

Leachable study and elemental impurity analysis study

The results (Roche report RPT-0304717) demonstrated that the levels of organic leachables and elemental impurities of the tested process equipment and components are below levels of toxicological significance.

Therefore, the leachables profile of the tested process equipment and components was considered acceptable from a toxicological perspective.

PS80 concentration and content of protein

The results (RPT-0308922) on the first filled vials samples (both for PS80 and protein concentrations) taken during MabThera 100 mg were comparable with the Mabthera bulk for fill control. This demonstrated that filter flush (~2L) and filling needle purge (24 stroke volumes ~1.92L) used during the engineering run is sufficient and does not impact PS80 and protein content concentrations after sterile filtration and DP filling.

Because MabThera 500mg filling process uses the same type and number of filters, and a

larger fill volume and larger diameter pump tubing, all results generated in the 100mg configuration can be considered worst-case compared to the 500mg DP process.

Light study

The results (RPT-0309669) demonstrated that light exposure from Thermo Fisher's routine manufacturing process did not impact Mabthera DP quality.

Stability

The analytical results from MabThera 500 mg and MabThera 100 mg stability studies showed that the results of the side-by-side stressed stability study demonstrate that the MabThera DP (500 mg & 100mg) manufactured at Patheon Monza changes in a similar way to GNE Rituxan DP Controls with regards to degradation mode, chromatograms, and degradation rates.

Potential critical process parameter summary

The process parameters and the potential critical process parameters identified in the surrogate trial (Protocol TT237A011 and Report TT237D011) and engineering protocol (TT237B011) are summarized in the table below.

Table 74 potential critical process parameters

Process step	Parameter	Results	Requirements	pCPP (Y/N)
Thawing	Drug substance weight in Freeze/Thaw tanks	1° cycle: Weight ≥51,5 Kg 2° cycle: Weight ≥51,5 Kg	307,2 Kg≥ Weight ≥ 51,5 Kg	Y
	Number of freeze/thaw cycle	2 cycle	N° cycles≤3	Y
	Heat transfer fluid temperature set point	1° cycle Conforming 2° cycle: Conforming	Target = 23°C Temperatur e≤26°C	Y
		1° cycle Conforming	Target = 21 rpm [19-23 rpm]	Y

Process step	Parameter	Results	Requirements	pCPP (Y/N)
	Recirculation Mixing peristaltic pump rpm	2° cycle: Conforming		
	Recirculation mixing duration	1° cycle Conforming	Time ≥ 10 hours	Y
		2° cycle: Conforming		
	Thaw operation duration	1° cycle Conforming	Time ≥ 17 hours	Y
		2° cycle: Conforming		
Freezing	Drug substance weight in Freeze/Thaw tanks	1° cycle: Weight ≥ 51,5 Kg	Weight ≥ 51,5 Kg	Y
		2° cycle: Weight ≥ 51,5 Kg		
	Number of freeze/thaw cycle	3 cycle	N° cycles ≤ 3	Y
	Heat transfer fluid temperature set point	1° cycle Conforming	Target: -50°C Temperature ≤ 26°C	Y
		2° cycle: Conforming		
	Freeze operation duration	1° cycle Conforming	Target = at least 19 hours	Y
		2° cycle: Conforming		
HCl solution preparation	Mixing time	15 minutes	Time ≥ 15 minutes	N
	Mixing speed	150 rpm 250 rpm	Target = 200 rpm [150 – 250] rpm	N
NaOH solution preparation	Mixing time	14 minutes	Time ≥ 15 minutes (*)	N

Process step	Parameter	Results	Requirements	pCPP (Y/N)
	Mixing speed	220 rpm 330 rpm	Target = 280 rpm [220-330] rpm	N
PS80 solution preparation	Mixing time	1 hour and 58 minutes	Time ≥ 2 hours	N
	Mixing speed	350 rpm 450 rpm	Target = 400 rpm [350-450] rpm	N
Buffer preparation	Mixing rate (RPM)	100 rpm [during surrogate TT237D011] 100-200 rpm	100 rpm (100-200 rpm)	N (**)
	Mixing time	8 hours and 52 minutes [during surrogate TT237D011] 1 hours and 53 minutes]	Time ≥ 2 hours	N (**)
	Quantity of sodium chloride to be compounded	8845,1 g	8840,0 g (8751,6-8928,4) g	N
	Quantity of sodium citrate dihydrate to be compounded	7224,9 g	7220,0 g (7147,8-7292,2) g	N
	Quantity of PS80 solution to be compounded	7996,3 g	8000,0 g (7920,0 -8080,0) g	N
	Quantity of HCl to be compounded (for pH adjustment)	1500 mL	qs (2)	N
	Final weight of the tank	991,2 Kg	991,0 Kg [981,2-1000,8]Kg	Y

Process step	Parameter	Results	Requirements	pCPP (Y/N)
Compounding	Mixing rate (RPM) after having reach the final weight of the tank	200 rpm [during surrogate TT237D011 Identified 150 rpm and 250 rpm]	200 rpm [150-250]rpm	Y
	Mixing time after having reach the final weight of the tank	15 minutes	Time ≥ 15 minutes	Y
	Mixing temperature	20°C	20°C [15-25]°C	Y
	Initial weight of compounding tank (after BDS pooling)	46,4 Kg	$Q^1 \pm 0,4$ Kg	Y
	Peristaltic pump setting during BDS pooling	20 rpm 50 rpm	Target = 40 rpm [20-50] rpm	N
	Pressure in buffer tank for buffer transfer in compounding tank	0,5 bar	Target = 0,5 bar [0,5 – 1] bar	N
Blending	Mixing rate (RPM)	250 rpm [during surrogate TT237D011 mixed at 150 rpm]	(150-250)rpm	Y
	Final weight of compounding tank	253,0Kg	$A^3 \pm 0,4$ Kg	Y
	Mixing temperature	18°C	Target at 20°C [15-25] °C	Y

Process step	Parameter	Results	Requirements	pCPP (Y/N)
	Mixing time	45 minutes. Time points at= 15,30 and 45 minutes [same time points checked during surrogate TT237D011]	[15-45] minutes.	Y
Bioburden reduction filtration	Filtration pressure	P = 0,6 bar	P=0,6 Barg Range [0,5-1barg]	Y
	Volume to surface area ratio (batch size / filter area)	342,19 Kg/m ²	Record (***)	Y
	Filter integrity test post use	3500 mbar	Conform if pressure ≥ 3450 mbar	Y
Sterilizing filtration	Filter flush with product at the beginning of the sterilizing filtration	2000 mL	2000 mL	Y
	Filtration ΔP on filters	ΔP ≤ 1 barg	ΔP ≤ 1 barg	Y
	Volume to surface area ratio (*)	342,19 Kg/m ²	≤345,9 Kg/m ² for the minimum BS	Y
	Pressure in the storage tank during filling	0,43 ≤ P ≤ 0,63 bar for MabThera 500 mg 0,2 ≤ P ≤ 0,4 bar for MabThera 100 mg	0,6 bar ± 0,1 bar	N

Process step	Parameter	Results	Requirements	pCPP (Y/N)
	Filtration time	2h and 36 minutes for MabThera 500mg	≤ 48 hours (as per media fill)	Y
		3hours and 54 minutes for MabThera 100 mg		
	Temperature during filtration	Temperature between range [2-25°C] for MabThera 500 mg	Temperature between range [2-25°C]	Y
		Temperature between range [2-25°C] for MabThera 100 mg		
	Pre-Use Sterilizing Filter I Integrity test	3800 mabr for MabThera 500mg	Conform if pressure ≥ 3450 mbar	Y
		3850 mabr for MabThera 500mg		
	Pre-Use Sterilizing Filter II Integrity test	3900 mabr for MabThera 500mg	Conform if pressure ≥ 3450 mbar	Y
		4000 mabr for MabThera 500mg		
	Post-use Sterilizing Filters I Integrity test	3450 mabr for MabThera 500mg	Conform if pressure ≥ 3450 mbar	Y
		3550 mabr for MabThera 500mg		

Process step	Parameter	Results	Requirements	pCPP (Y/N)
	Post-use Sterilizing Filters	3700 mbar for MabThera 500 mg	Conform if pressure \geq 3450 mbar	Y
	II Integrity test	3550 mbar for MabThera 100mg		
Filling	Filling velocity MabThera 500 mg	100 vials/min. [80-100]vials/min	[90 vials/min – 100 vials/min] The minimum filling speed changed from 80 vials/min (requirement as per media fill) to 90 vials/min as per water trial optimization	Y
	Fill weight MabThera 500 mg	Target=51620 mg Action limit [52620- 50900]mg Alert limit [51880- 51360]mg	Target=51620 mg Action limit [52620- 50900]mg Alert limit [51880- 51360]mg	Y
	Filling bag working range MabThera 100 mg	50% (min) - 80%(max) Target =5Kg	50% (min) - 80%(max) Target =5Kg	N
	Filling bag working range MabThera 500 mg	50% (min) - 80%(max) Target =5Kg	50% (min) - 80%(max) Target =5Kg	
	Filling velocity MabThera 100 mg	180 vials/min 80 vials/Min as per media fill requirement	[80 vials/min – 180 vials/min]	Y

Process step	Parameter	Results	Requirements	pCPP (Y/N)
	Fill weight MabThera 100 mg	Target=10630 mg Action limit [11030-10400]mg Alert limit [10740-10520]mg	Target=10630 mg Action limit [11030-10400]mg Alert limit [10740-10520]mg	Y
Crimping	Crimping Pressure MabThera 500mg	Engineering at 100 KPa [During surrogate TT237D011 teste 90 KPa and 120 KPa]	100 KPa [90-120]KPa	Y
	Crimping Pressure MabThera 100mg	Engineering at 90 KPa [During surrogate TT237D011 teste 80 KPa and 100 KPa]	Target = 90 KPa [80-100]KPa	Y
	Crimping Height MabThera 500 mg	Engineering at 68mm [During surrogate TT237D011 teste 67,5 mm and 68,5 mm]	68 mm [67,5-68,5]mm	Y
	Crimping Height MabThera 100 mg	Engineering at 46,5 mm [During surrogate TT237D011 teste 46 mm and 47 mm]	Target = 46.5 mm [46-47]mm	Y

1. The amount of BDS is calculated based on the batch size and protein content of the DS reported in the CoA. Calculations are reported in the MBR

2. quantity of HCl to be added is quantum satis (q.s) to reach approximately the pH target 6,5 (range between 6,2 and 6,8).

3. The final amount of the compounding tank is calculated based on the batch size, the actual amount of BDS pooled. Calculations are reported in the MBR.

(*) During the engineering run the end of mixing was defined based on the conforming visual check. Considering that the mixing took 14 minutes, after the engineering batch it is recommended to mix at least for 15 minutes (it was increase of 1 minute with respect to the one obtained during engineering to simplify the manufacturing instruction).

(**) the mixing velocity and mixing time for the buffer preparation before the final WFI addition was considered as pCPP in the protocol (TT237B011) but after the execution of the trial they were downgraded to PP considering that no impact on the buffer preparation is expected, which instead could be expected in the mixing speed and time (after the last WFI addition) which are still considered as pCPP.

(***) It depends on the maximum batch size which will be confirmed during engineering runs

Microbiological holding time summary

In the table below is reported the summary of the microbiological holding time challenged in the sterile area 6.

Table 75 Microbiological holding times

Holding time	Start	End	Time
Buffer tank	Start of WFI addition	End of buffer addition in compounding tank	33 hours and 45 minutes
Compounding tank	BDS pooling	End of bioburden reduction filtration	19 hours and 59 minutes
Storage tank	Beginning of bioburden reduction filtration	End of sterilizing filtration ≡ end of filling	24 hours and 7 minutes at room temperature
			24 hours at 2-8°C
			2 hours and 24 minutes (**)
			3 hours and 24 minutes (**)
Filling holding time	Start of filling	End of filling	48 hours (*)

(*) timing validated per media fill. During these engineering runs, it was checked that this timing was respected for both formats.

(**) these timings are a first experimental evaluation and they have to be considered related to a filling of half of the minimum batch size which is not foreseen during commercial manufacturing.

In the table below, it is reported the summary of the microbiological holding time challenged on the F/T tank. Even if the F/T tank was freeze and thaw twice, the timing challenged can be considered as a cumulative of a one cycle. For every freeze/thaw cycle, the timing at room temperature and at 2-8°C were tracked in the BR and using the FORM-000225917 (one form is compiled for every cycle). Starting from the PPQs, the F/ along with the FORM-000225917, another dedicated form (FORM-000314056) will be also present: in this form it will be reported the specific TIR/TOR consumed for the F/T tank for the manufacturing of one batch (data reported in the FORM-000225917) summarized the TIR/TOR consumed in every cycle to track the timing for the F/T tank across multiple usage.

Table 76 TIR/TOR for the F/T tank

Holding time	Time for one F/T cycle
Room temperature	15 hours and 19 minutes
2-8°C	15 days

Finally, a complete characterization of the holding times will be performed with the PPQ batches where holding time will be challenged again with minimum and maximum batch size.

12.0 RELATED DOCUMENTS

Table 77 List of related documents

Document	Reference #	Responsible	Approval
Change Control for introduction of MabThera	191938	Laura Palmaroli	TF/Roche
Technology Transfer Plan of Avastin and MabThera	TTP23701	Laura Palmaroli	TF/Roche
Surrogate trial protocol for MabThera	TT237A011	Giulia Ferri	TF/Roche
Surrogate trial report for MabThera	TT237D011	Giulia Ferri	TF/Roche
Summary of Light mapping data for Xtrema line Sterile Area 6	TT237Z011	Giulia Ferri	TF
MabThera engineering protocol	TT237B011	Giulia Ferri	TF/Roche

Document	Reference #	Responsible	Approval
Addendum I to MabThera engineering protocol	Addendum I to TT237B011	Giulia Ferri	TF/Roche
Light Assessment for MabThera 500 mg and 100mg	TT237F011	Giulia Ferri	TF/Roche
Sterilization equivalency evaluation between stoppers 273716, 273437, 273438, 273929 and 273711	O927BI01	Angelica Torchia	TF

13.0 DOCUMENT INFORMATION

Table 78 Revision History

Version	Reason for change	Author	Date
01	Issue for approval	Giulia Ferri	27/03/2023
02	Issue the final report including the results of stability analysis executed at Roche	Giulia Ferri	10/08/2023

ATTACHMENT 1: ANALYTICAL RESULTS

Table 79 end of thawing – Purity samples

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
PUR-SE-FT	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Purity by SE-HPLC	Monomer ≥ 97.5 area%	98.1%	RC0615 Ed 01
PUR-IE-FT	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Purity by IE_HPLC	Fc Peak 25.0 – 31.0 area% Fab Peak 60 – 65 area%	Fc Peak = 28,1% Fab Peak = 63%	RC0615 Ed 01

Table 80 end of thawing - chemical samples

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
ID_CZE-FT		ID-CZE	positive identity (corresponds)	Corresponds	RC0615 Ed 01
COC-FT	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T <u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo	Color/opalescence	Description clear to opalescent Ph. Eur. Opalescent Value max. Ref. III Clarity/ Opalescence in NTU nc ≤ 18.0 NTU *NC = non certificated (not on CoA)	< Ref. IV < Y7	RC0615 Ed 01
CONTENT OF PROTEIN - FT		Content of protein (by UV)	50-60 mg/mL	57 mg/mL	RC0615 Ed 01
OSMO-FT		Osmolality	324 – 396 mOsmol/kg	369 mOsmol/kg	RC0615 Ed 01
PH-FT		pH	6.2 – 6.8	6.5	RC0615 Ed 01

Table 81 end of thawing - Roche samples

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria		Results	Reference
POT_FT	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Potency by bioassay	0.8-1.3E5 U/ml	NA (**)		NA (**)
LEAC_CTRL_FT	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Leachable	Report results (refer to Roche study report)	NA (*)		NA (*)
LEAC_CTRL_FT_BIS	<u>When:</u> At the end of thawing (second cycle) <u>Quando:</u> alla fine dello scongelo (secondo ciclo) <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Leachable	Report results (refer to Roche study report)	See report RPT-0304717		RPT-0304717

(*) Due to additional F/T cycle, considering that after the first thaw there was a re-freeze without the manufacturing of the DP on the line, it was agreed to re-taken (after the second thaw) the control for the leachable study (LEACH_CTRL_FT_BIS) and for this reason the sample taken after the first thaw (LEAC_CTRL_FT) was not needed to be analyzed anymore.

(**) The sample was not tested since the donor site does not perform testing on the DS but only on the DP, and the sampling at beginning, middle and end of the DP crimping process are considered the ones representative for the purpose of this study

Table 82 end of thawing – endotoxin and bioburden – T0 for holding time study in F/T tank

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_FT_T0	<p><u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo</p> <p><u>Where:</u> From the sampling assembly connected to the F/T tank</p> <p><u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T</p>	Endotoxin	≤ 55,0 EU/ mL	<0,4 EU/ml	EXE000266393
HT_BB_FT_T0	<p><u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo</p> <p><u>Where:</u> From the sampling assembly connected to the F/T tank</p> <p><u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T</p>	Bioburden	≤10 CFU / 10 mL	0CFU/10ml	EXE000266393
HT_ENDO_FT_T0_BIS	<p><u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo</p> <p><u>Where:</u> From the sampling assembly connected to the F/T tank</p> <p><u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T</p>	Endotoxin	≤ 55,0 EU/ mL	2,4 EU/ml	EXE000266393
HT_BB_FT_T0_BIS	<p><u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo</p> <p><u>Where:</u> From the sampling assembly connected to the F/T tank</p> <p><u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T</p>	Bioburden	≤10 CFU / 10 mL	0CFU/10ml	EXE000266393

Table 83 Holding time study in F/T tank at room temperature – endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_FT_T1 T1 = circa dopo 8 h da quanto F/T tank è stato staccato dalla stazione di scongelo/ approx 8 hours after F/T tank is detached from the thawing station	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Endotoxin	For information only	<0,3 EU/ml	EXE000266393
HT_BB_FT_T1 T1 = circa dopo 8 h da quanto F/T tank è stato staccato dalla stazione di scongelo/ approx 8 hours after F/T tank is detached from the thawing station	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Bioburden	For information only	0CFU/10ml	EXE000266393

Table 84 Holding time study in F/T tank at 2-8°C before BDS pooling– endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_FT_T2 <i>T2 =circa 6 giorni dopo T1/ approx. 6 days after T1</i>	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Endotoxin	For information only	NA	NA
 <i>T2 =circa 6 giorni dopo T1/ approx. 6 days after T1</i>	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Bioburden	For information only	NA	NA

Samples not taken due to unforeseen freezing without having performed the activities in solution preparation room

Table 85 Holding time study in F/T tank at 2-8°C after BDS pooling (*) – endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_FT_T3 T3 = circa 3 giorni dopo il pooling della BDS/ approx 3 days after BDS pooling (*)	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Endotoxin	For information only	2,3 EU/ml	EXE000266393
HT_BB_FT_T3 T3 = circa 3 giorni dopo il pooling della BDS/ approx 3 days after BDS pooling (*)	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Bioburden	For information only	0 CFU/10mL	EXE000266393

(*) Samples were not taken after the BDS pooling due to unforeseen freezing without having performed the activities in solution preparation room. They were taken before the first refreezing on 20/06/2022

Table 86 Holding time study in F/T tank at 2-8°C after BDS pooling (*) – endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_FT_T3_BIS T3 = circa 3 giorni dopo il pooling della BDS/ approx 3 days after BDS pooling	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Endotoxin	For information only	2,8 EU/ml	EXE000266393
HT_BB_FT_T3_BIS T3 = circa 3 giorni dopo il pooling della BDS/ approx 3 days after BDS pooling	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelo <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Bioburden	For information only	0 CFU/10mL	EXE000266393

(*) samples taken after the holding time challenge at 2-8°C after the BDS pooling and before the second refreezing (on 07/07/2022)

Table 87 Holding time study in F/T tank at 2-8°C before BDS pooling (*)– endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_FT_T4 T4=dopo facoltativo stoccaggio a 2-8°C prima del pooling della BDS / after facultative storage at 2-8°C before BDS pooling	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelato <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Endotoxin	For information only	3,2 EU/ml	EXE000266393
HT_BB_FT_T4 T4 =dopo facoltativo stoccaggio a 2-8°C prima del pooling della BDS / after facultative storage at 2-8°C before BDS pooling	<u>When:</u> At the end of thawing <u>Quando:</u> alla fine dello scongelato <u>Where:</u> From the sampling assembly connected to the F/T tank <u>Dove:</u> Dall'assembly di campionamento connesso al serbatoio F/T	Bioburden	For information only	0 CFU/10mL...	EXE000266393

(*) samples taken on 04/07/2022 in solution preparation room before the BDS pooling

Table 88 Holding time study in F/T tank at room temperature after BDS pooling (*)– endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_FT_T5 T5=dopo il pooling della BDS ()/ after BDS pooling (approx 5 h after exiting from 2-8°C cell)	When: At the end of thawing Quando: alla fine dello scongelo Where: From the sampling assembly connected to the F/T tank Dove: Dall'assembly di campionamento connesso al serbatoio F/T	Endotoxin	For information only	6,6 EU/ml	EXE000266393
HT_BB_FT_T5 T5=dopo il pooling della BDS (after BDS pooling (approx 5 h after exiting from 2-8°C cell)	When: At the end of thawing Quando: alla fine dello scongelo Where: From the sampling assembly connected to the F/T tank Dove: Dall'assembly di campionamento connesso al serbatoio F/T	Bioburden	For information only	0 CFU/10mL	EXE000266393

(*) samples taken after the BDS pooling in sterile area on 04/07/2022

Table 89 confirmation of pH adjustment – IPC binding

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
pH-IPC	<p><u>When:</u> At the end of pH adjustment, to confirm the pH of buffer</p> <p>Quando: alla fine dell'aggiustaggio del pH del buffer, per confermare il pH del nuffer</p> <p><u>Where:</u> From RTR342 tank</p> <p>Dove: Serbatoio RTR342</p>	pH	6.2-6.8	6,7	N4739 P090

Table 90 end of buffer preparation - chemical samples

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
pH-BU	<u>When:</u> At the end of buffer preparation <u>Quando:</u> alla fine della preparazione buffer <u>Where:</u> From Novaseptum bag at bottom of RTR342 tank <u>Dove:</u> Sacca Novaseptum sul fondo del serbatoio RTR342	pH	6.2-6.8	6.7	RC0615 Ed.01
OSMO-BU	<u>When:</u> At the end of buffer preparation <u>Quando:</u> alla fine della preparazione buffer <u>Where:</u> From Novaseptum bag at bottom of RTR342 tank <u>Dove:</u> Sacca Novaseptum sul fondo del serbatoio RTR342	Osmolality	324 – 396 mOsmol/kg	357 mOsm/kg	RC0615 Ed.01

Table 91 end of buffer preparation - Roche samples

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Refernce
PS80	<p><u>When:</u> At the end of buffer preparation <u>Quando:</u> alla fine della preparazione buffer</p> <p><u>Where:</u> From pipette from top of RTR342 tank <u>Dove:</u> Pipetta dalla sommità del serbatoio RTR342</p>	Polysorbate 80	For information only	Refer to Roche report RPT-0308922	RPT-0308922

Table 92 end of buffer preparation -endotoxin and bioburden – T0 for holding time study in buffer tank

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_BU_T0	<p><u>When:</u> At the end of buffer preparation <u>Quando:</u> alla fine della preparazione buffer</p> <p><u>Where:</u> From Novaseptum syringe at middle of RTR342 tank</p> <p><u>Dove:</u> Siringa Novaseptum a metà del serbatoio RTR342 tank</p>	Endotoxin	≤0,25 EU/mL	<0,10 EU/ml	EXE000266393
HT_BB_BU_T0	<p><u>When:</u> At the end of buffer preparation <u>Quando:</u> alla fine della preparazione buffer</p> <p><u>Where:</u> From Novaseptum bag at bottom of RTR342 tank</p> <p><u>Dove:</u> Sacca Novaseptum sul fondo del serbatoio RTR342</p>	Bioburden	For information only	0 CFU/10ml	EXE000266393

Table 93 Holding time study in buffer tank at room temperature – endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_BU_T1 T1 = circa 24 da T0/ after approx. 24 h from T0	<u>When:</u> At the end of buffer preparation, <u>Quando:</u> alla fine della preparazione buffer <u>Where:</u> From Novaseptum syringe at middle of RTR342 tank <u>Dove:</u> Siringa Novaseptum a metà del serbatoio RTR342 tank	Endotoxin	For information only	<0,10 EU/ml	EXE000266393
HT_BB_BU_T1 T1 = circa 24 da T0/ after approx. 24 h from T0	<u>When:</u> At the end of buffer preparation <u>Quando:</u> alla fine della preparazione buffer <u>Where:</u> From Novaseptum bag at bottom of RTR342 tank <u>Dove:</u> Saccia Novaseptum sul fondo del serbatoio RTR342	Bioburden	For information only	0CFU/100ml	EXE000266393

Table 94 End of compounding – before mixing – visible and subvisible particles

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
VP_BU	When: At the end of compounding, before mixing <u>Quando:</u> alla fine del compounding, prima di agitare	Visible particles Ph. Eur. 2.9.20, JP <6.06>	For information only	Complies	RC0615 Ed.01
SVP_BU	Where: From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank	Subvisible particles (SAM- 0102796 Version 2.0, USP <788>; Ph.Eur.2.9.19, JP<6.07>)	For information only	$\geq 2 \mu\text{m}$: 364 particles/mL $\geq 5 \mu\text{m}$: 55 particles/mL $\geq 10 \mu\text{m}$: 6 particles/mL $\geq 25 \mu\text{m}$: 0 particles/mL	RC0615 Ed.01

Table 95 End of compounding – during mixing – stress/degradation study

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
SS_T1_IE	<p><u>When:</u> after 15 minutes of mixing <u>Quando:</u> dopo 15 minuti di agitazione <u>Where:</u> From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank</p>	Purity by IE-HPLC	Fc Peak 25.0 – 31.0 area% Fab Peak 60 – 65 area%	Fc Peak = 28,1% Fab Peak = 63%	RC0615 Ed.01
SS_T1_SE	<p><u>When:</u> after 15 minutes of mixing <u>Quando:</u> dopo 15 minuti di agitazione <u>Where:</u> From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank</p>	Purity by SE-HPLC	Monomer ≥ 97.5 area%	99.1%...	RC0615 Ed.01
SS_T2_IE	<p><u>When:</u> after 15 minutes of mixing, after T1 sampling <u>Quando:</u> dopo 15 minuti di agitazione, dopo i campionamenti T1 <u>Where:</u> From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank</p>	Purity by IE-HPLC	Fc Peak 25.0 – 31.0 area% Fab Peak 60 – 65 area%	Fc Peak = 28,0% Fab Peak = 63%	RC0615 Ed.01
SS_T2_SE	<p><u>When:</u> after 15 minutes of mixing, after T1 sampling <u>Quando:</u> dopo 15 minuti di agitazione, dopo i campionamenti T1 <u>Where:</u> From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank</p>	Purity by SE-HPLC	Monomer ≥ 97.5 area%	99.1%	RC0615 Ed.01
SS_T3_IE	<p><u>When:</u> after 15 minutes of mixing, after T2 sampling <u>Quando:</u> dopo 15 minuti di agitazione, dopo i campionamenti T2 <u>Where:</u> From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank</p>	Purity by IE-HPLC	Fc Peak 25.0 – 31.0 area% Fab Peak 60 – 65 area%	Fc Peak = 28,1% Fab Peak = 63%	RC0615 Ed.01
SS_T3_SE	<p><u>When:</u> after 15 minutes of mixing, after T2 sampling <u>Quando:</u> dopo 15 minuti di agitazione, dopo i campionamenti T2 <u>Where:</u> From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank</p>	Purity by SE-HPLC	Monomer ≥ 97.5 area%	99.1%	RC0615 Ed.01

Table 96 End of compounding – at the end of mixing – Roche samples

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
LEACH_CU	<p><u>When:</u> At the end of mixing in RTR343 <u>Quando:</u> alla fine dell'agitazione nel serbatoio RTR343</p> <p><u>Where:</u> From Novaseptum bag at bottom of RTR343 tank</p> <p><u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank</p>	Leachable	Report results (refer to Roche study report)	NA (*)	NA (*)

(*) This sample were not analyzed because it was an extra control sample as per Roche document VAL-0212060 and the sample taken at the end of the F/T was representative as control sample),

Table 97 End of compounding – at the end of mixing – chemical samples

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
pH_CU		pH	6.2-6.8	6.7	RC0615 Ed.01
APP_CU		Appearance	liquid	Liquid	RC0615 Ed.01
COLOR_CU	<p><u>When:</u> At the end of mixing in RTR343 <u>Quando:</u> alla fine dell'agitazione nel serbatoio RTR343</p> <p><u>Where:</u> From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank</p>	Color/opalescence	Description colorless to pale yellow Ph. Eur. Color Scale not more colored than Y6	Colorless < Y7	RC0615 Ed.01
PROTEIN CONTENT_CU		Protein content	9.2 – 10.8 mg/mL	10.1 mg/mL	RC0615 Ed.01
OSMO_CU		Osmolality	324 – 396 mOsmol/kg	357 mOsmol/kg	RC0615 Ed.01

Table 98 End of compounding – at the end of mixing – visible and subvisible particles

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
VP_CU	<u>When:</u> At the end of mixing in RTR343 <u>Quando:</u> alla fine dell'agitazione nel serbatoio RTR343	Visible particles Ph. Eur. 2.9.20, JP <6.06>	For information only	Complies	RC0615 Ed.01
SVP_CU	<u>Where:</u> From Novaseptum bag at bottom of RTR343 tank <u>Dove:</u> Sacca novaseptum sul fondo del serbatoio RTR343 tank	Subvisible particles (SAM- 0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>)	For information only	$\geq 2 \mu\text{m}$: 630 particles/mL $\geq 5 \mu\text{m}$: 58 particles/mL $\geq 10 \mu\text{m}$: 1 particles/mL $\geq 25 \mu\text{m}$: 0 particles/mL	RC0615 Ed.01

Table 99 End of compounding and end of mixing – bulk homogeneity

Sample	Sample point & timing/ Punto di campionamento & quando	Parameter	Acceptance Criteria / Target	Results	Reference
BUH_BOTTOM	At the beginning of the BB reduction filtration from the bottom of the compounding tank	Protein content Target [9.2 – 10.8 mg/mL]	PVT stage 1 : individual values 98.0-102.0% PVT stage 2: - individual values 95.0-105.0% - each location mean (n=3) 98.0-102.0% - overall SD ≤ 3.0%	101.8%	RC0615 Ed.01
BUH_MIDDLE	At the middle of the BB reduction filtration from the bottom of the compounding tank			101.9%	
BUH_TOP	At the end of the BB reduction filtration from the bottom of the compounding tank			102.0%	

Table 100 Holding time study in compounding tank at room temperature –bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_BB_CU	<p>When: At the end of bioburden reduction filtration ($T \sim 22$ h) Quando: alla fine della filtrazione bioburden ($T \sim 22$ h)</p> <p>Where: From Novaseptum bag at bottom of RTR343 tank</p> <p>Dove: Sacca Novaseptum sul fondo del serbatoio RTR343</p>	Bioburden	For information only	< 1CFU/100ml	EXE000266393

Table 101 Holding time study in storage tank at room temperature – endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_ST_T0	<p><u>When:</u> After 24 hours from the beginning of bioburden reduction filtration</p> <p>Quando: Dopo 24 ore dall'inizio della filtrazione bioburden</p> <p><u>Where:</u> From Novaseptum syringe at middle of RTR344 tank</p> <p>Dove: Siringa Novaseptum a metà del serbatoio RTR344 tank</p>	Endotoxin	Action if >1.0 EU/mL (*)	<0,3 EU/ml	EXE000266393
HT_BB_ST_T0	<p><u>When:</u> After 24 hours from the beginning of bioburden reduction filtration</p> <p>Quando: Dopo 24 ore dall'inizio della filtrazione bioburden</p> <p><u>Where:</u> From Novaseptum bag at bottom of RTR344 tank</p> <p>Dove: Sacca Novaseptum sul fondo del serbatoio RTR344</p>	Bioburden	≤ 10 CFU/100 mL	0CFU/100 mL	EXE000266393

(*) Acceptance criteria updated based on Memo “In process Controls (IPC) and Shelf Life Specification Information for MabThera 100 mg/10ml & 500mg/50ml” attached

Table 102 Holding time study in storage tank at 2-8°C – endotoxin and bioburden

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
HT_ENDO_ST_T1	<p><u>When:</u> After 24 hours after T0 <u>Quando:</u> Dopo 24 ore dopo T0</p> <p><u>Where:</u> From Novaseptum syringe at middle of RTR342 tank</p> <p><u>Dove:</u> Siringa Novaseptum a metà del serbatoio RTR342 tank</p>	Endotoxin	For information only	<0,3 EU/ml	EXE000266393
HT_BB_ST_T1	<p><u>When:</u> After 24 hours after T0 <u>Quando:</u> Dopo 24 ore dopo T0</p> <p><u>Where:</u> From Novaseptum bag at bottom of RTR344 tank</p> <p><u>Dove:</u> Sacca Novaseptum sul fondo del serbatoio RTR344</p>	Bioburden	For information only	0CFU/100ml	EXE000266393

Table 103 Control for VPHP samples -Roche samples

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
CTRL_VPHP	<p><u>When:</u> Before start of sterilizing filtration Quando: Prima dell'inizio della filtrazione sterilizzante</p> <p><u>Where:</u> From Novaseptum bag at bottom of RTR344 tank</p> <p><u>Dove:</u> Sacca Novaseptum dal fondo del serbatoio RTR344 tank</p>	VPHP	NA	Roche Report RPT-0309731	RPT-0309731

Table 104 Sterilizing filters flush study – Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Acceptance criteria	Results	Reference
FF_1_500	<p><u>When:</u> At the beginning of sterilizing filtration <u>Quando:</u> All'inizio della filtrazione sterilizzante</p> <p><u>Where:</u> From sampling assembly connected downstream the second sterilizing filter <u>Dove:</u> Dall'assembly di campionamento collegato valle del secondo filtro sterilizzante</p>	For information only	4.5 mg/mL	RC0615 Ed.01
FF_2_500	<p><u>When:</u> At the beginning of sterilizing filtration, after FF_1_500 <u>Quando:</u> All'inizio della filtrazione sterilizzante, dopo FF_1_500</p> <p><u>Where:</u> From sampling assembly connected downstream the second sterilizing filter <u>Dove:</u> Dall'assembly di campionamento collegato valle del secondo filtro sterilizzante</p>	For information only	8.4 mg/mL	RC0615 Ed.01
FF_3_500	<p><u>When:</u> At the beginning of sterilizing filtration, after FF_2_500 <u>Quando:</u> All'inizio della filtrazione sterilizzante, dopo FF_2_500</p> <p><u>Where:</u> From sampling assembly connected downstream the second sterilizing filter <u>Dove:</u> Dall'assembly di campionamento collegato valle del secondo filtro sterilizzante</p>	For information only	9.4 mg/mL	RC0615 Ed.01
FF_4_500	<p><u>When:</u> At the beginning of sterilizing filtration, after FF_3_500 <u>Quando:</u> All'inizio della filtrazione sterilizzante, dopo FF_3_500</p> <p><u>Where:</u> From sampling assembly connected downstream the second sterilizing filter <u>Dove:</u> Dall'assembly di campionamento collegato valle del secondo filtro sterilizzante</p>	For information only	9.8 mg/mL	RC0615 Ed.01
FF_5_500	<p><u>When:</u> At the beginning of sterilizing filtration, after FF_3_500 <u>Quando:</u> All'inizio della filtrazione sterilizzante, dopo FF_3_500</p> <p><u>Where:</u> From sampling assembly connected downstream the second sterilizing filter <u>Dove:</u> Dall'assembly di campionamento collegato valle del secondo filtro sterilizzante</p>	9.2 – 10.8 mg/mL	10.0 mg/mL	RC0615 Ed.01

Table 105 Start of filling Mab 500 – leachable, visible particles and filling homogeneity

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
BL_500	<p><u>When:</u> First 2 vials after calibration <u>Quando:</u> Prime 2 vials dopo calibrazione</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Leachable	Report results (refer to Roche study report)	See Roche report RPT-0304717	RPT-0304717
VP_B_500	<p><u>When:</u> after vials of sampling BL_500 <u>Quando:</u> dopo il campionamento BL_500</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Visible particles Ph. Eur. 2.9.20, JP <6.06>	Practically free from particles	Complies	RC0615 Ed.01

Table 106 Leachable - First tubing hold up volume sample – Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
ML_500	<p><u>When:</u> middle of filling, approx. 19 and 20. <u>Quando:</u> a metà del riempimento, approx vials 19 e 20.</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Leachable	Report results (refer to Roche study report)	See Roche report RPT-0304717	RPT-0304717

Table 107 Leachable – approximately after 2L of solution – approx. vials 41 and 42 - Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
2LL_500	<p><u>When:</u> after approximately 2L of solution (approx. vials 41-42)</p> <p><u>Quando:</u> dopo circa 2 L di soluzione (approx vials 41-42)</p> <p><u>Where:</u> from Xtrema unloading</p> <p>Dove: Dallo scarico buoni linea Xtrema</p>	Leachable	Report results (refer to Roche study report)	See Roche report RPT-0304717	RPT-0304717

Table 108 Table 87 Filling homogeneity –samples to be taken approximately every 60 vials filled

ID N	Parameter	Acceptance Criteria / Target	Results	Reference
FH_500_1	Protein content (to evaluate filling homogeneity)	PVT Stage 1: Individual values 75.0 – 125.0% Meets 90% confidence/95% coverage for n=602 PVT Stage 2: All Individual values 75.0 –125.0% Meets 90% confidence/95% coverage for n=120	101.0%	RC0615 Ed.01
FH_500_3			101.5%	
FH_500_5			101.5%	
FH_500_7			100.8%	
FH_500_8			101.8%	
FH_500_11			101.5%	
FH_500_13			100.8%	
			101.3%	
			101.4%	
			100.2%	
			101.6%	
			101.3%	
			100.6%	
			101.8%	
			101.6%	
			101.6%	
			101.4%	
			101.2%	
			101.4%	
			101.4%	
			101.7%	

ID N	Parameter	Acceptance Criteria / Target	Results	Reference
FH_500_15			101.2%	
FH_500_17			101.7%	
FH_500_19			101.9%	
FH_500_21			101.3%	
FH_500_23			101.5%	
FH_500_25			101.8%	
FH_500_27			101.4%	
FH_500_29			101.4%	
			101.4%	
			101.9%	
			101.3%	
			101.7%	
			101.4%	
			101.3%	
			101.2%	
			101.1%	
			101.2%	
			101.1%	
			101.6%	
			101.2%	
			101.3%	
			101.4%	
			101.3%	
			101.5%	
			101.4%	

ID N	Parameter	Acceptance Criteria / Target	Results	Reference
FH_500_31			101.2%	
FH_500_33			101.0%	
FH_500_35			101.5%	
FH_500_37			101.3%	
FH_500_39			101.4%	
			100.3%	
			101.5%	
			101.3%	
			100.9%	
			101.7%	
			101.5%	
			103.2%	
			101.9%	
			101.2%	
			101.3%	

Table 109 End of first filling – endotoxin and bioburden -Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
ENDO_EFF_500	<u>When:</u> at end of filling format 500 <u>Quando:</u> Alla fine del riempimento formato 550 <u>Where:</u> From samopling assembly before first sterilizing filter <u>Dove:</u> Dall'assembly di campionamento prima del primo filtro sterilizzante	Endotoxin	Action if > 1.0 EU/mL (*)	<0,3 EU/ml	EXE000266393
BB_EFF_500	<u>When:</u> at end of filling format 500 <u>Quando:</u> Alla fine del riempimento formato 550 <u>Where:</u> From samopling assembly before first sterilizing filter <u>Dove:</u> Dall'assembly di campionamento prima del primo filtro sterilizzante	Bioburden	≤ 10 CFU/100 mL	0CFU/100ml	EXE000266393

(*) Limit in accordance with memo "memo In process Controls (IPC) and Shelf Life Specification Information for MabThera 100 mg/10ml & 500mg/50 " attached

Table 110 Start of filling Mab 100 – leachable, polysorbate concentration, VPHP testing, content of protein, visible particles and filling homogeneity

ID. N.	Sample point & timing/ Punto di campionamento & quando	Volume/ Quanto	Parameter/ Parametro	Acceptance criteria	Results	Reference
BV_100_L	<u>When:</u> First 40 vials after calibration <u>Quando:</u> Prime 40 vials dopo calibrazione	Vials #1, 2, 3, 20, 21, 22, 25, 26, 31, 32	Leachable	Report results (refer to Roche study report)	See Roche report RPT-0304717	RPT-0304717
BV_100_PS			Polysorbate concentration	For information only	Roche report RPT-0308922	RPT-0308922
BV_100_VP	<u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	30 out of first 40 excluded the BV_100_L	VPHP testing	For information only	See Roche report RPT-0309731	RPT-0309731
BV_100_UV			Content of protein (by UV)	For information only	Roche report RPT-0308922	RPT-0308922
VP_B_100	<u>When:</u> after vials of sampling ML_100 <u>Quando:</u> dopo il campionamento ML_100 <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	10	Visible particles Ph. Eur. 2.9.20, JP <6.06>	Practically free from particles	Practically free from particles	RC0615 Ed.01

Table 111 Leachable - After First tubing hold up volume sample – Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
ML_100	<p><u>When:</u> vials 41- 50 approximalty <u>Quando:</u> vials da 41 a 50 aprrox.</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Leachcables	Report results (refer to Roche study report)	See Roche report RPT-0304717	RPT-0304717

Table 112 Leachable – approximately after 2L of solution – approx. vials 201-210 Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
2LL_100	<p><u>When:</u> after approximately 2L of solution (approx. vials 201 – 210)</p> <p>Quando: dopo circa 2 L di soluzione (vials 201-210 approx)</p> <p><u>Where:</u> from Xtrema unloading</p> <p>Dove: Dallo scarico buoni linea Xtrema</p>	Leachables	Report results (refer to Roche study report)	See Roche report RPT-0304717	RPT-0304717

Table 113 After 30 min stop – VPHP – Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
VPHP_S_100	<u>When:</u> After the 30 min planned stop <u>Quando:</u> Dopo il fermo pianificato di 30 minuti <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	VPHP testing	For information only	See Roche report RPT-0304717	RPT-0304717

Table 114 Filling homogeneity –samples to be taken approximately every 280 vials filled

Sample	Parameter	Acceptance Criteria / Target	Results	Reference
FH_100_1	Protein content (to evaluate filling homogeneity)	PVT Stage 1: Individual values 75.0 – 125.0% PVT Stage 2: All Individual values 75.0 –125.0% Meets 90% confidence/95% coverage for n=602 All Individual values 75.0 –125.0% Meets 90% confidence/95% coverage for n=120	100.8%	RC0615 Ed.01
FH_100_3			100.8%	
FH_100_5			101.0%	
FH_100_7			101.0%	
FH_100_9			101.4%	
FH_100_11			101.7%	
FH_100_13			100.9%	
FH_100_15			101.5%	
			101.5%	
			101.2%	
			101.0%	
			101.3%	
			101.2%	
			101.4%	
			101.1%	
			101.6%	
			100.9%	
			101.2%	
			100.8%	
			101.3%	
			101.5%	
			100.7%	
			101.5%	
			101.4%	

Sample	Parameter	Acceptance Criteria / Target	Results	Reference
			101.8%	
FH_100_17			101.6%	
FH_100_19			103.5%	
FH_100_21			100.6%	
FH_100_23			101.1%	
FH_100_25			101.5%	
FH_100_28			101.6%	
FH_100_31			102.1%	
FH_100_35			101.5%	
			106.1%	
			101.2%	
			100.7%	
			101.4%	

Sample	Parameter	Acceptance Criteria / Target	Results	Reference
	FH_100_37		101.2%	
FH_100_37			101.7%	
			101.4%	
			101.6%	
			101.6%	
			101.8%	
FH_100_40			101.7%	

Table 115 Vials for light exposure - after 30 minutes stoppage

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
S_L_100	<u>When:</u> After the 30 min planned stop Quando: Dopo il fermo pianificato di 30 minuti <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	light exposure (**)	For information only	Roche report RPT-0309669	RPT-0309669

(**) vials were exposed approximately 30 minutes in AVI visual inspection room (room 1116). And finally they underwent 4 times in MVI

Table 116 Vials for light exposure - 48 hours in filling room

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
LIGHT_48_100	<u>When:</u> All'inizio del riempimento Quando: At the beginning of the filling <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	light exposure (**)	For information only	Roche report RPT-0309669	RPT-0309669

(**) vials were exposed approximately 48 hours in Xtrema filling room (room 843), then 30 minutes in AVI visual inspection room (room 1116). And finally they underwent 4 times in MVI

Table 117 End of second filling – endotoxin and bioburden -Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
ENDO_EFF_100	<p>When: at end of filling format 100 Quando: Alla fine del riempimento formato 100</p> <p>Where: From samopling assembly before first sterilizing filter Dove: Dall'assembly di campionamento prima del primo filtro sterilizzante</p>	Endotoxin	For information only	<0,3 EU/ml	EXE000270724
BB_EFF_100	<p>When: at end of filling format 100 Quando: Alla fine del riempimento formato 100</p> <p>Where: From samopling assembly before first sterilizing filter Dove: Dall'assembly di campionamento prima del primo filtro sterilizzante</p>	Bioburden	For information only	0CFU/100ml	EXE000270724

Table 118 Crimping Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
CCIT_500	<p><u>When:</u> random during crimping Quando: random durante la ghiatura</p> <p><u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema</p>	Vacuum decay	No leakage is detected (all vials compliant)	125/125 complies	RC0615 Ed.01

Table 119 Crimping Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
CONT_APP_500	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Container/Appearance	Container: Flip-Off Cap red Seal silver Appearance: liquid	Complies	RC0615 Ed.01
CLARITY_500	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Clarity	Description: clear to opalescent Ph. Eur. Opalescent Value: max. Ref. III	Slightly opalescent Opalescent Value: < ref. II	RC0615 Ed.01
COLOR_500	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Color	Description: colorless to pale yellow Ph. Eur. Color Scale: not more colored than Y6	Color Scale < Y7	RC0615 Ed.01
PH_500	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	pH	6.2 – 6.8	6.7	RC0615 Ed.01
OSMO_500	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Osmolality	324 – 396 mOsmol/kg	358 mOsmol/kg	RC0615 Ed.01

Table 120 Crimping II Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
EXT_500	<u>When:</u> random during crimping Quando: random durante la ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	Extractable volume (ml)	Ph. Eur./USP/JP: corresponds	51 mL	RC0615 Ed.01
UNI_500	<u>When:</u> random during crimping Quando: random durante la ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	Uniformity of dosage unit	Compliant according to USP <905> and JP <6.02	Compliant	RC0615 Ed.01
COP_500	<u>When:</u> random during crimping Quando: random durante la ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	Content of protein (by UV)	9.2 – 10.8 mg/mL	10.1 mg/mL	RC0615 Ed.01
iD_500	<u>When:</u> random during crimping Quando: random durante la ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	Identity of rituximab (CZE)	positive identity (corresponds)	Corresponds	RC0615 Ed.01
PU_SE_500	<u>When:</u> random during crimping Quando: random durante la ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	Purity by SE- HPLC	Monomer ≥ 97.5 area%	99.1%	RC0615 Ed.01
PU_IE_500	<u>When:</u> random during crimping Quando: random durante la ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	Purity by IE- HPLC	Fc Peak 25.0 – 31.0 area% Fab Peak 60 – 65 area%	Fc Peak = 28.0% Fab Peak = 63%	RC0615 Ed.01

Table 121 Crimping III Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Acceptance criteria	Results	Reference
VP_500	<u>When:</u> random during crimping Quando: random durante la ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	Practically free from particles	Complies	RC0615 Ed.01
SVP_500	<u>When:</u> random during crimping Quando: random durante la ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	Particles $\geq 2\mu\text{m}$ per Container : report Particles $\geq 5 \mu\text{m}$ per Container : report Particles $\geq 10 \mu\text{m}$ per Container : ≤ 3000 Particles $\geq 25 \mu\text{m}$ per Container : ≤ 300	$\geq 2 \mu\text{m}$: 17350 particles per container $\geq 5 \mu\text{m}$: 3600 particles per container $\geq 10 \mu\text{m}$: 250 particles per container $\geq 25 \mu\text{m}$: 0 particles per container	RC0615 Ed.01
STER_B_500 STER_M_500 STER_E_500	<u>When:</u> at the beginning, middle and end of crimping Quando all'inizio, metà e fine della ghieratura <u>Where:</u> from Xtrema unloading Dove: Dallo scarico buoni linea Xtrema	no growth (corresponds)	conforming	EXE000266393

Table 122 Crimping IV Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptanc e criteria	Results	Reference
ENDO_B_500 ENDO_M_500 ENDO_E_500	<p><u>When:</u> at the beginning, middle and end of crimping <u>Quando</u> all'inizio, metà e fine della ghieratura</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Endotoxin	≤1.0 EU/ml (*)	<0,3 EU/ml	EXE000266392

(*) Limit in accordance with client specification

Table 123 Crimping Roche sample Mab 500

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
POT_B_500	<u>When:</u> at the beginning of crimping <u>Quando</u> all'inizio della ghiatura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Potency by Bioassay	0.8 – 1.3 E5 U/mL	See attachment #10	See attachment #10
POT_M_500	<u>When:</u> at the middle of crimping <u>Quando</u> a metà della ghiatura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Potency by Bioassay	0.8 – 1.3 E5 U/mL	See attachment #10	See attachment #10
POT_E_500	<u>When:</u> at the end of crimping <u>Quando</u> a fine della ghiatura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Potency by Bioassay	0.8 – 1.3 E5 U/mL	See attachment #10	See attachment #10
STAB_500	<u>When:</u> random during crimping <u>Quando</u> random durante ghiatura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Stability	NA	See Roche report RPT-0313475	See Roche report RPT-0313475

Table 124 Crimping Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
CCIT_100	<p><u>When:</u> random during crimping <u>Quando:</u> random durante la ghierratura</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Vacuum decay	No leakage is detected (all vials compliant)	315/315 complies	RC0615 Ed.01

Table 125 Crimping Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
CONT_APP_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghiratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Container/Appearance	Container: Flip-Off Cap red Seal silver Appearance: liquid	Complies	RC0615 Ed.01
CLARITY_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghiratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Clarity	Description: clear to opalescent Ph. Eur. Opalescent Value: max. Ref. II	Slightly opalescent Opalescent Value: < ref. II	RC0615 Ed.01
COLOR_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghiratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Color	Description: colorless to pale yellow Ph. Eur. Color Scale: not more colored than Y6	Color Scale < Y7	RC0615 Ed.01
PH_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghiratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	pH	6.2 – 6.8	6.7	RC0615 Ed.01
OSMO_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghiratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Osmolality	324 – 396 mOsmol/kg	360 mOsmol/kg	RC0615 Ed.01

Table 126 Crimping II Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
EXT_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xrema unloading <u>Dove:</u> Dallo scarico buoni linea Xrema	Extractable volume	Ph. Eur./USP/JP: corresponds Min : 10 or 50 mL tbd	10 mL	RC0615 Ed.01
UNI_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xrema unloading <u>Dove:</u> Dallo scarico buoni linea Xrema	Uniformity of dosage unit	Compliant according to USP <905> and JP <6.02>	Compliant	RC0615 Ed.01
COP_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xrema unloading <u>Dove:</u> Dallo scarico buoni linea Xrema	Content of protein	9.2 – 10.8 mg/mL	10.1 mg/mL	RC0615 Ed.01
ID_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xrema unloading <u>Dove:</u> Dallo scarico buoni linea Xrema	Identity of rituximab (CZE)	positive identity (corresponds)	Corresponds	RC0615 Ed.01
PU_SE_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xrema unloading <u>Dove:</u> Dallo scarico buoni linea Xrema	Purity by SE-HPLC	Monomer ≥ 97.5 area%	99.1%	RC0615 Ed.01
PU_IE_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la ghieratura <u>Where:</u> from Xrema unloading <u>Dove:</u> Dallo scarico buoni linea Xrema	Purity by IE-HPLC	Fc Peak 25.0 – 31.0 area% Fab Peak 60 – 65 area%	Fc Peak = 28,1% Fab Peak = 63%	RC0615 Ed.01

Table 127 Table 106 Crimping III Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
VP_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la gheratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Visible particles	Practically free from particles	Complies	RC0615 Ed.01
SVP_100	<u>When:</u> random during crimping <u>Quando:</u> random durante la gheratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Subvisible particles	Particles $\geq 2\mu\text{m}$ per Container : report Particles $\geq 5\mu\text{m}$ per Container : report Particles $\geq 10\mu\text{m}$ per Container : ≤ 3000 Particles $\geq 25\mu\text{m}$ per Container : ≤ 300	$\geq 2\mu\text{m}$: 6650 particles per container $\geq 5\mu\text{m}$: 1940 particles per container $\geq 10\mu\text{m}$: 280 particles per container $\geq 25\mu\text{m}$: 0 particles per container	RC0615 Ed.01
STER_B_100 STER_M_100 STER_E_100	<u>When:</u> at the beginning,middle and end of crimping <u>Quando:</u> all'inizio, metà e fine della gheratura <u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema	Sterile	no growth (corresponds)	conforming	EXE000314269

Table 128 Crimping IV Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
ENDO_B_100 ENDO_M_100 ENDO_E_100	<p><u>When:</u> at the beginning, middle and end of crimping <u>Quando</u> all'inizio, metà e fine della ghieratura</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Endotoxin	≤1.0EU/ml (*)	<0,3 EU/ml	EXE000314269

(*) Limit in accordance with client specification

Table 129 Crimping Roche sample Mab 100

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
POT_B_100	<p><u>When:</u> at the beginning of crimping <u>Quando</u> all'inizio della gheratura</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Potency by Bioassay	0.8 – 1.3 E5 U/mL	See Attachemnt 11	See Attachemnt 11
POT_M_100	<p><u>When:</u> at the middle of crimping <u>Quando</u> a metà della gheratura</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Potency by Bioassay	0.8 – 1.3 E5 U/mL	See Attachemnt 11	See Attachemnt 11
POT_E_100	<p><u>When:</u> at the end of crimping <u>Quando</u> a fine della gheratura</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Potency by Bioassay	0.8 – 1.3 E5 U/mL	See Attachemnt 11	See Attachemnt 11
STAB_100	<p><u>When:</u> random during crimping <u>Quando</u> random durante gheratura</p> <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	Stability	NA	See Roche report RPT-0313475	See Roche report RPT-0313475

Table 130 VPHP study – additional samples collected during water trail as per Technical memo TT237Z051

ID. N.	Sample point & timing/ Punto di campionamento & quando	Parameter/ Parametro	Acceptance criteria	Results	Reference
NA	<p><u>When:</u> at the beginning of filling (including calibration phase of filling pumps)</p> <p>First 250 vials from good:</p> <ul style="list-style-type: none"> vials from 1 to 242 (good from calibration phase). Vials labeled sequentially; 8 vials from the first filled in production (from 243 to 250). Vials labelled sequentially. <p>- Discarded units from calibration step (73 units)</p> <p><u>Quando</u> all'inizio del riempimento (inclusa la fase di calibrazione delle pompe di riempimento)</p> <p>Primi 250 flaconi dai buoni:</p> <ul style="list-style-type: none"> Flaconi dal 1 al 242 (buoni dalla fase di calibrazione). Flaconi etichettati sequenzialmente; 8 flaconi dai primi di modalità produzione (flaconi da 243 a 250). Flaconi etichettati sequenzialmente <p><u>Where:</u> from Xtrema unloading <u>Dove:</u> Dallo scarico buoni linea Xtrema</p>	VPHP testing	For information only	See Roche report RPT-0309731	RPT-0309731

ATTACHMENT 2 – MAPPING OF MATERIAL FOR PRIMARY PACKING, DISPOSABLE ASSEMBLIES AND STAINLESS-STEEL TANKS

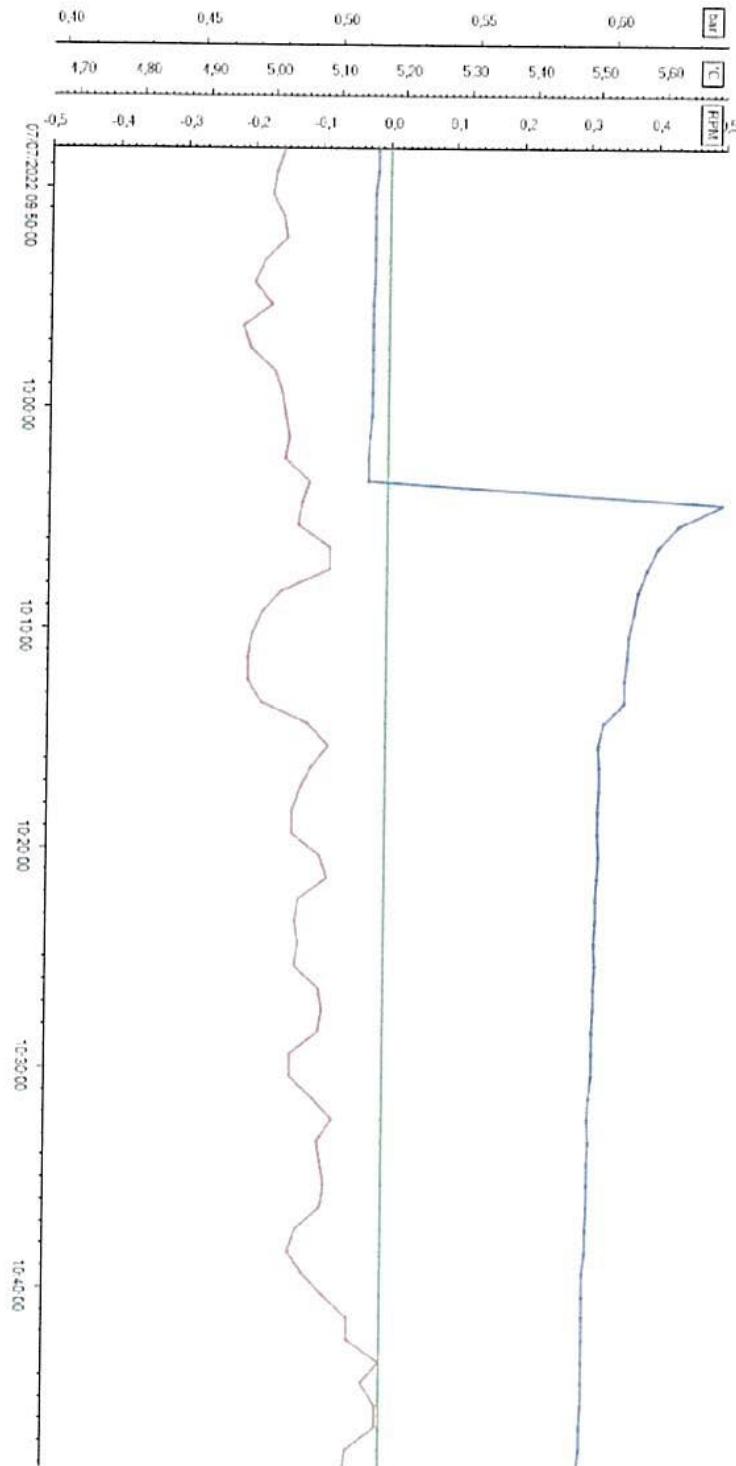
Table 131 Mapping of material

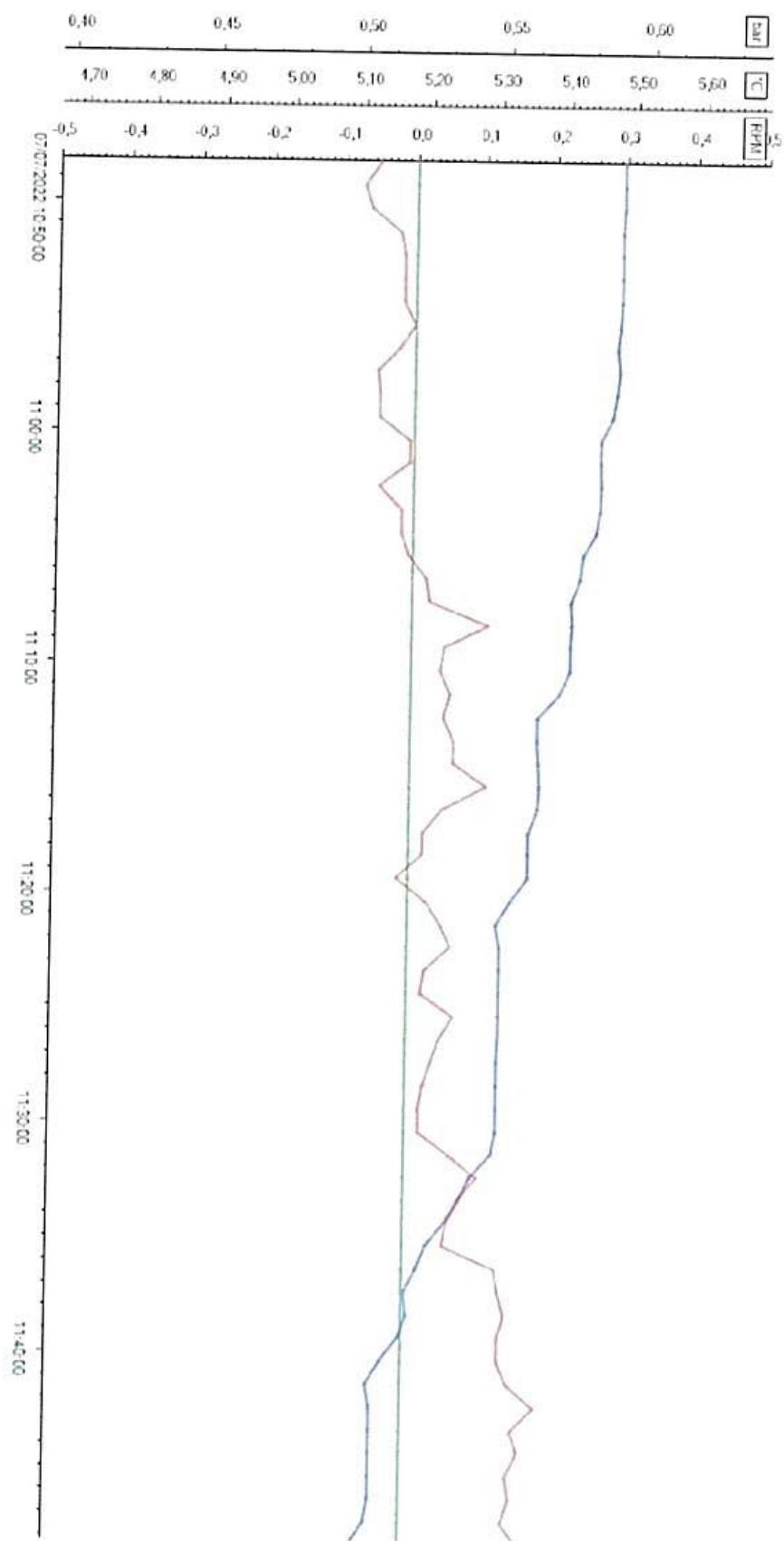
Process Phase	Patheon reference	Disposable assembly description	Materials			Product in contact		Type of use	Process Conditions		Temperature (°C)	
			Material	Description use of the material	Manufacturer Name	Material of construction	Type of product in contact		Cleaning / rinsing	Sterilization		
BULK PREPARATION / STORAGE	RTR342	F/T tank recirculation tubing and BDS pooling	Buffer tank	Product dedicated tank in which the buffer solution is prepared and stored	Novinox	Stainless steel AISI 316 L	Buffer	Liquid	RU	Yes	SIP	RT
	RTR343		Compounding tank	Product dedicated tank in which the bulk solution is prepared	Novinox	Stainless steel AISI 316 L	Final bulk	Liquid	RU	Yes	SIP	2-25°C
	RTR344		Storage tank	Product dedicated tank in which the bulk solution is stored	Novinox	Stainless steel AISI 316 L	Final bulk	Liquid	RU	Yes	SIP	2-25°C
TRANSFER AND FILLING PART	273493	Y assembly	Tri-clamp	Connector	Saint Gobain	PP and Pt cured silicone	BDS	Liquid	SU	No	Irradiated	2-25°C
			Pt cured silicone tubing	Tube	Saint Gobain	Silicone	BDS	Liquid	SU	No	Irradiated	2-25°C
			Connector	Connector	Saint Gobain	PPAF	BDS	Liquid	SU	No	Irradiated	2-25°C
	273499	Y assembly	Steam-thru connection	Connector	Saint Gobain	Polysulfone	Buffer/Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Pt cured silicone tubing	Tube	Saint Gobain	Silicone	Buffer/Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Connector	Connector	Saint Gobain	PPAF	Buffer/Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Aseptiquik connector	Tube	Saint Gobain	Polycarbonate	Buffer/Final bulk	Liquid	SU	No	Irradiated	2-25°C

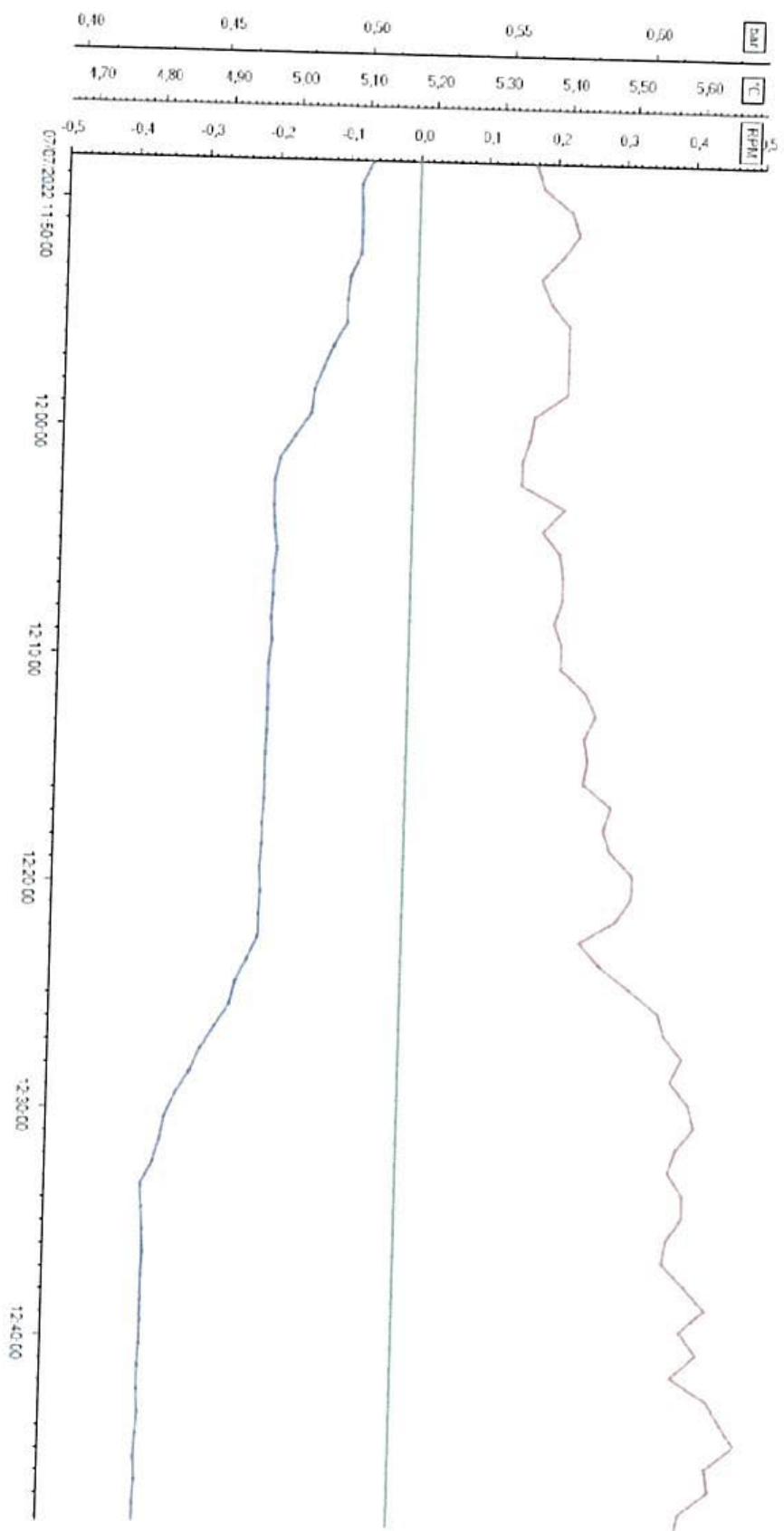
Process Phase	Patheon reference	Disposable assembly description	Materials				Product in contact		Type of use	Process Conditions		
			Material	Description use of the material	Manufacturer Name	Material of construction	Type of product in contact	State of the product		Cleaning / rinsing	Sterilization	Temperature (°C)
	273502	Extension tubing	Aseptiquik connector	Connector	Saint Gobain	Polycarbonate	Buffer/Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Pt cured silicone tubing	Tube	Saint Gobain	Silicone	Buffer/Final bulk	Liquid	SU	No	Irradiated	2-25°C
	273497	Bioburden reduction filtration assembly	Aseptiquik connector	Connector	Saint Gobain	Polycarbonate	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Pt cured silicone tubing	Tube	Saint Gobain	Silicone	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Connector	Connector	Saint Gobain	PPAF	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Filter	Filter	Saint Gobain	PP/PVDF	Final bulk	Liquid	SU	No	Irradiated	2-25°C
	273504	Sterilizing filtration assembly	Aseptiquik connector	Connector	Saint Gobain	Polysulfone	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Connector	Connector	Saint Gobain	Polysulfone	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Pt cured silicone tubing	Tube	Saint Gobain	PPAF	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Filter	Filter	Saint Gobain	PP/PVDF	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Pendotech	pressure sensor	Saint Gobain	Polysulfone	Final bulk	Liquid	SU	No	Irradiated	2-25°C

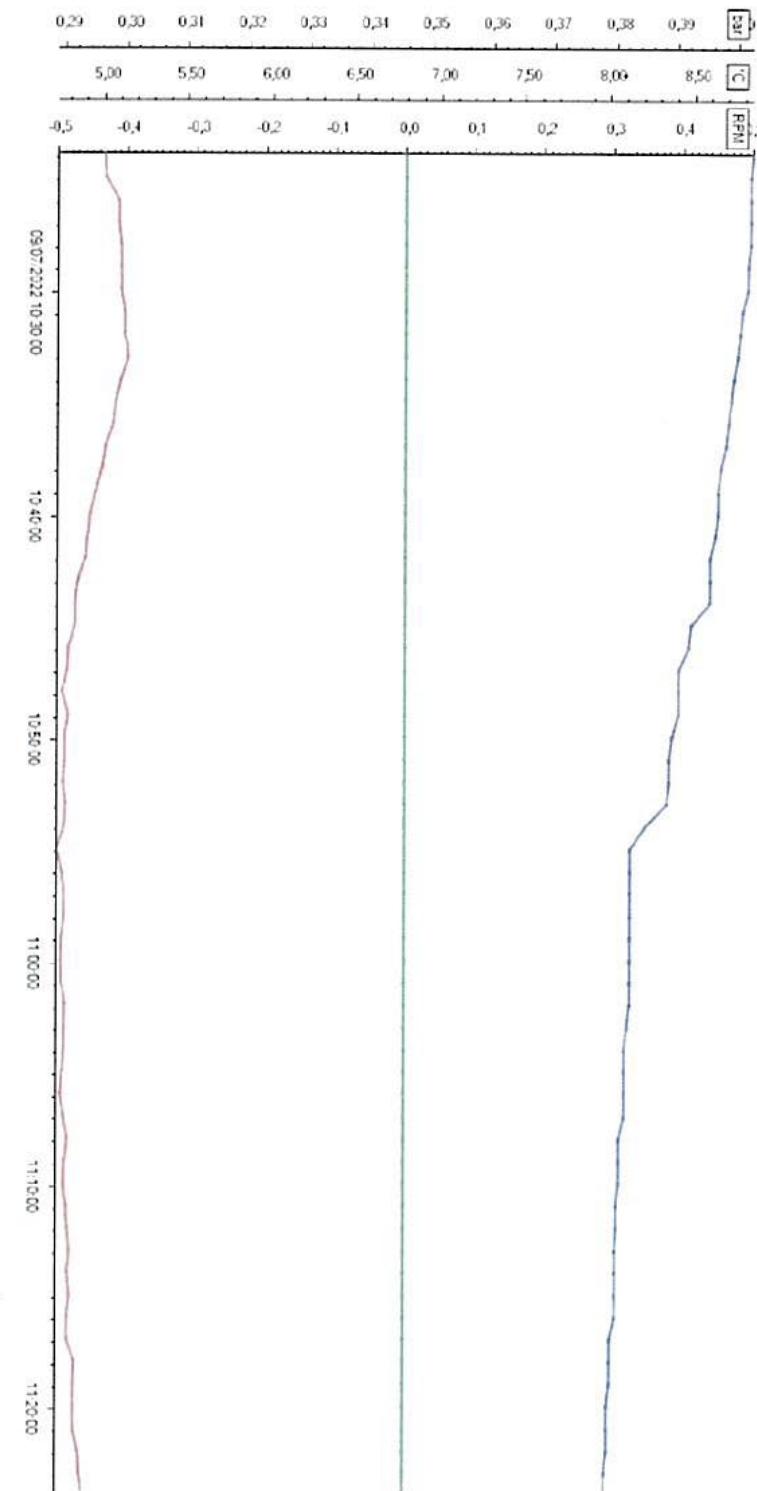
Process Phase	Patheon reference	Disposable assembly description	Materials				Product in contact		Type of use	Process Conditions		Temperature (°C)
			Material	Description use of the material	Manufacturer Name	Material of construction	Type of product in contact	State of the product		Pre-treatment	Cleaning / rinsing	
		Filling bag MabThera 100 mg	Vent filter	Filter	Saint Gobain	PBT/PC/PES	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			WFI filter	Filter	Saint Gobain	PP/PES	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Tri clamp connection	Connector	Saint Gobain	PP and Pt cured silicone	Final bulk	Liquid	SU	No	Irradiated	2-25°C
	273508	Filling bag MabThera 100 mg	Pt cured silicone tubing	Tube	Saint Gobain	Silicone	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Filling bag	surge bag	Saint Gobain	LDPE	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Connector	Connector	Saint Gobain	Polyolefin elastomer	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Needle	needle	Saint Gobain	stainless-steel	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Aseptiquik connector	connector	Saint Gobain	Polycarbonate	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Needle bag	bag	Saint Gobain	PP	Final bulk	Liquid	SU	No	Irradiated	2-25°C
	273510	Filling bag MabThera 500mg	Pt cured silicone tubing	Tube	Saint Gobain	Silicone	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Filling bag	surge bag	Saint Gobain	LDPE	Final bulk	Liquid	SU	No	Irradiated	2-25°C

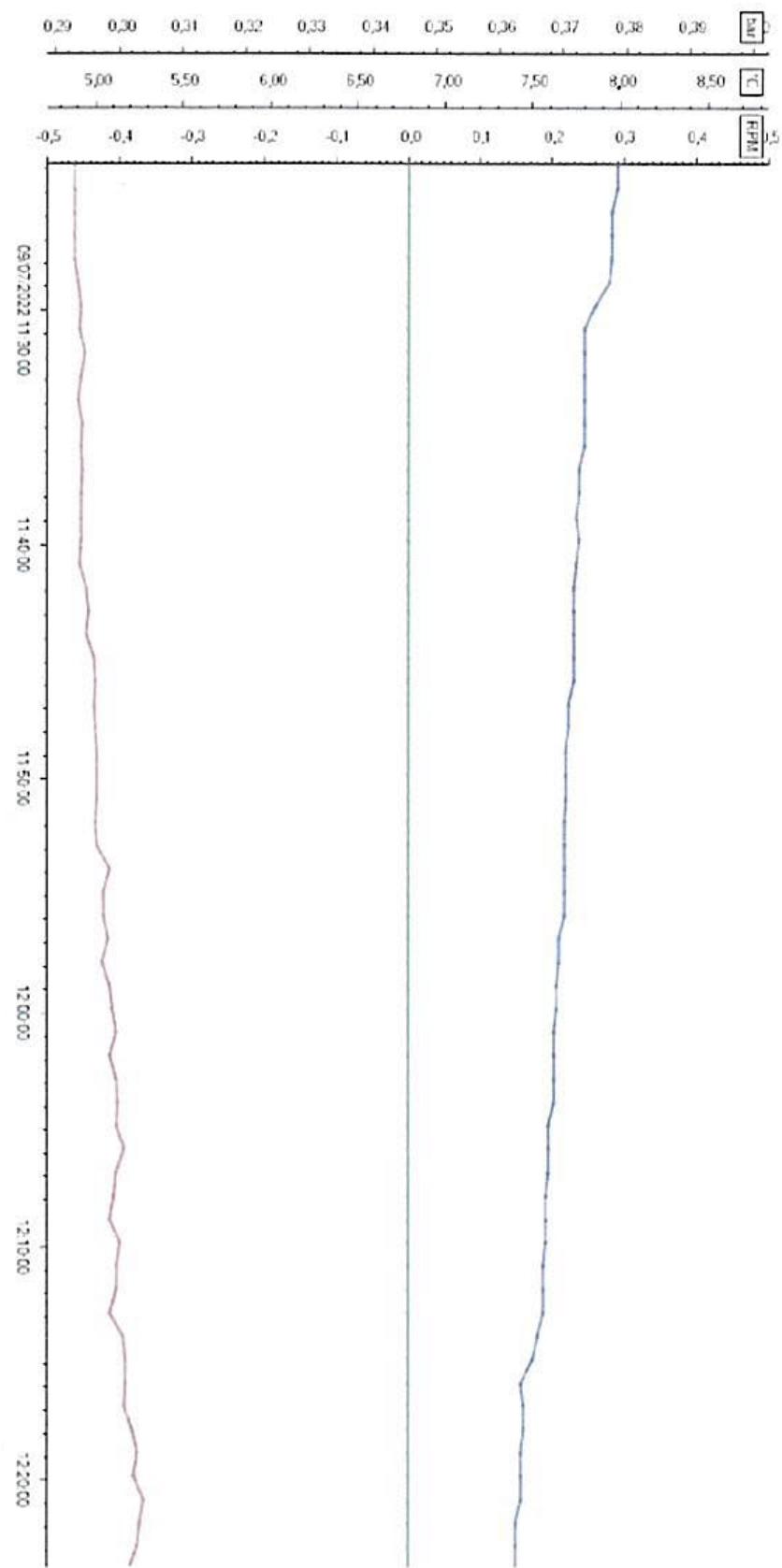
Process Phase	Patheon reference	Disposable assembly description	Materials				Product in contact		Type of use	Process Conditions		Temperature (°C)
			Material	Description use of the material	Manufacturer Name	Material of construction	Type of product in contact	State of the product		Cleaning / rinsing	Sterilization	
273314	Tee assembly	Connector	Connector	Connector	Saint Gobain	Polyolefin elastomer	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Needle	needle	Saint Gobain	stainless-steel	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Aseptiquik connector	connector	Saint Gobain	Polycarbonate	Final bulk	Liquid	SU	No	Irradiated	2-25°C
			Needle bag	bag	Saint Gobain	PP	Final bulk	Liquid	SU	No	Irradiated	2-25°C
	Tee assembly	Pt cured silicone tubing	Tube	Saint Gobain	Silicone	Final bulk	Liquid	SU	No	Irradiated	2-25°C	
		Connector	Connector	Saint Gobain	PVDF	Final bulk	Liquid	SU	No	Irradiated	2-25°C	
		Aseptiquik connector	Connector	Saint Gobain	Polycarbonate	Final bulk	Liquid	SU	No	Irradiated	2-25°C	
Final container			Rubber Stopper	Stopper	West Daykio	S10-F210-4 ETFE (ethylene tetra fluoro ethylene) D713 (formulation) RB2-40 (coating)	Final bulk	Liquid	SU	No	Autoclave	2-25°C
			Schott (borosilicate tubing glass)	Vials	Schott	FIOLAX Glass Type I	Final bulk	Liquid	SU	No	Depyrogenation tunnel	2-25°C

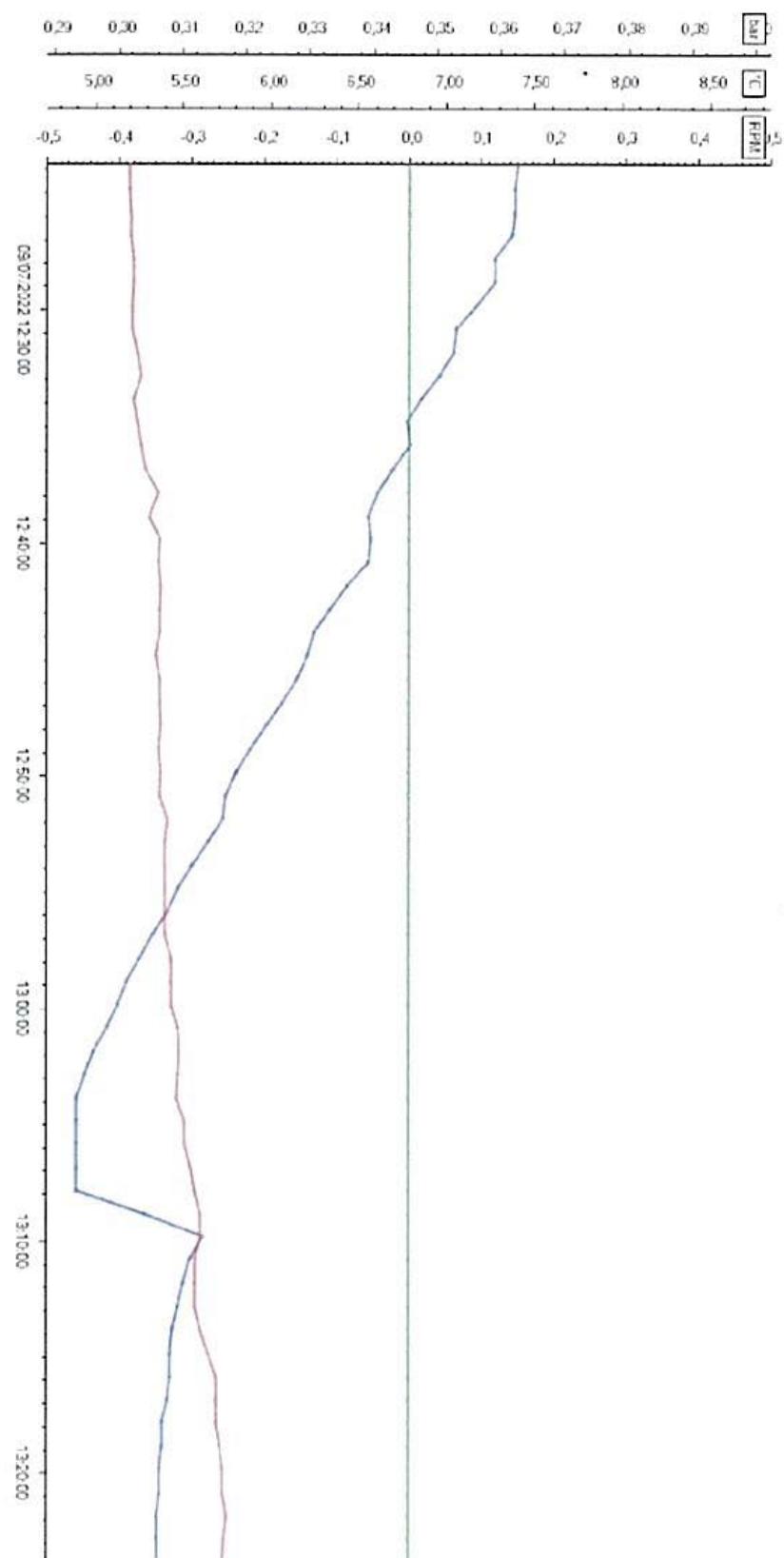
**ATTACHMENT 3 – PRESSURE IN THE STORAGE TANK DURING FILLING –
MABTHERA 500MG**

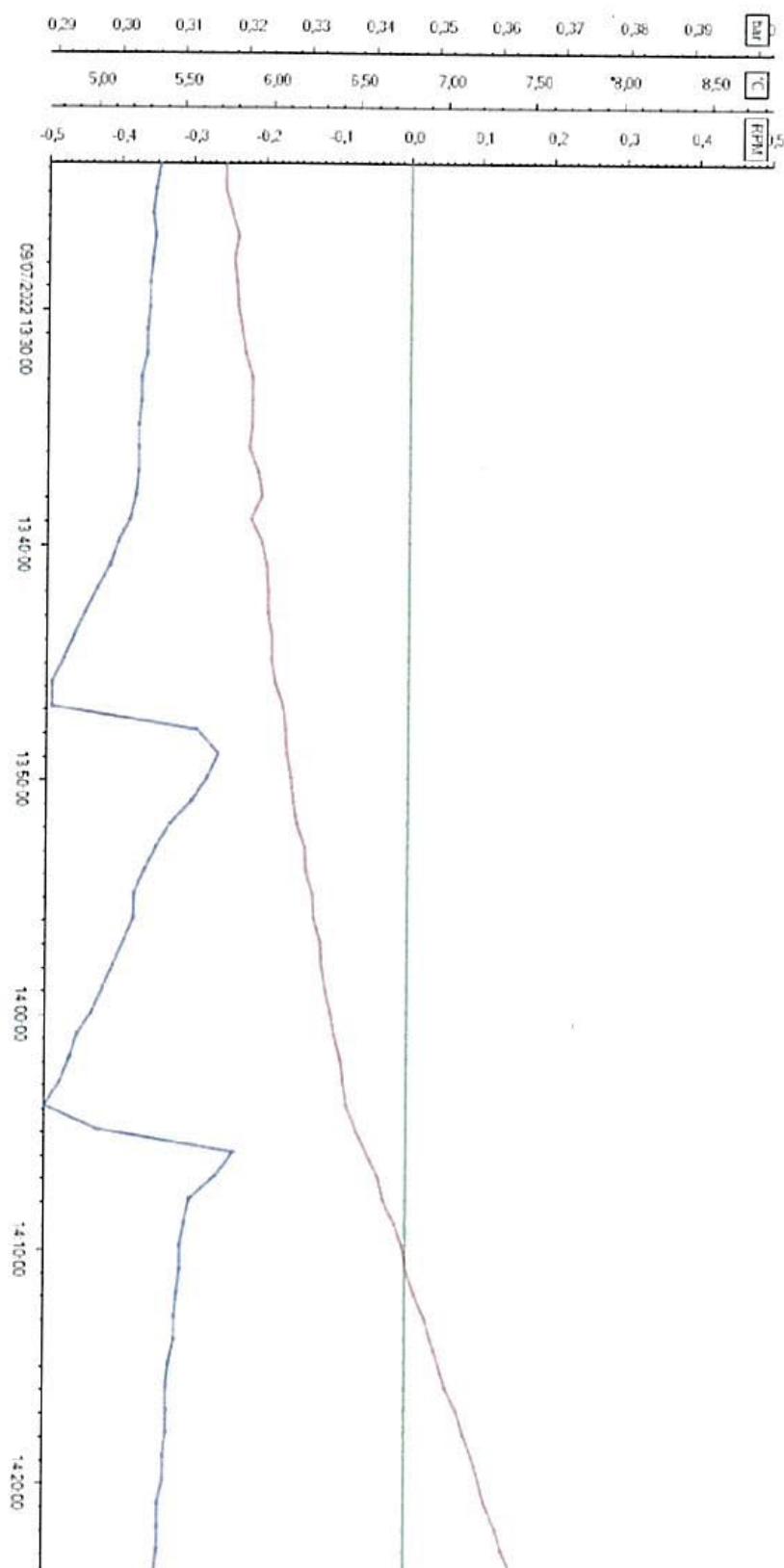


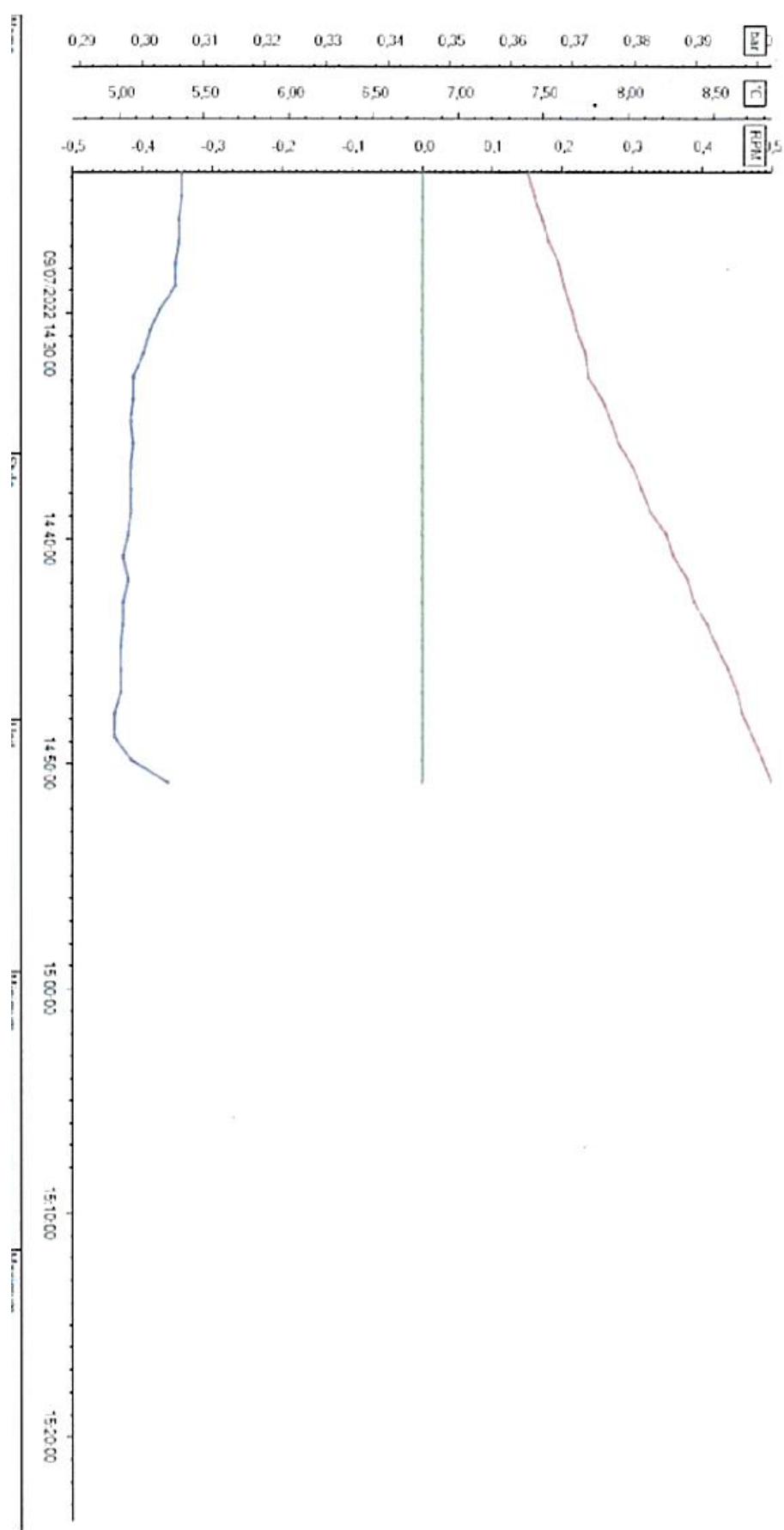


**ATTACHMENT 4 PRESSURE IN THE STORAGE TANK DURING FILLING –
MABTHERA 100MG**









ATTACHMENT 5 – INVESTIGATION FORM – INCORRECT WAREHOUSE LOADING AND BOXES LABELING_TT237B011

ATTACHMENT "MABTHERA 100MG/10 ML ENG DA SPERL VIALS: INCORRECT WAREHOUSE LOADING AND BOXES LABELING_TT237B011" - INVESTIGATION FORM - TT237

ATTACHMENT "MABTHERA 100MG/10 ML ENG DA SPERL VIALS: INCORRECT WAREHOUSE LOADING AND BOXES LABELING_TT237B011" - INVESTIGATION FORM

The present document can be filled-in in the paper version or in the electronic version.

The approved version will be attached to the final report.

Product: 352520 MabThera 100mg/10 mL ENG da sperl Lot: TT528/1

DESCRIPTION OF THE EVENT

According to Engineering protocol TT237B011 and addendum and relative MBR, the production of MabThera engineering batches had to be intended as one common compounding step, splitted in two filling and crimping steps, one for each of the following format:

- MabThera 500 mg/50 mL ENG da sperl, associated to product code 352521
- MabThera 100 mg/10 mL ENG da sperl, associated to product code 352520

In order to manage the split in production, one single MBR/order was launched as code 352521 whereas the 100 mg/10 mL format (product code 352520) had to be considered as a secondary product, following the indication reported in the MBR.

In terms of boxes labeling, the MBR provided instructions for labeling the two crimped formats with the right associated product code.

Moreover, the process foresaw the prioritization in terms of manual visual inspection for the samples, managed by TT237B011 add. 01.

At the end of the production of the Engineering batches, MabThera 500 mg/50 mL formats crimped vials (both vials to be visual inspected and samples already inspected by VI department) were correctly uploaded in the warehouse system associated to the product code 352521 and the batch number TT528

In terms of labeling, boxes associated to this format were correctly labelled reporting the following information: product code 352521 and batch TT528.

MabThera 100 mg/10 mL format crimped vials were supposed to be loaded in the warehouse system with the product code 352520 (following the secondary product indication in the MBR). Nevertheless, during the warehouse system uploading after the crimping step, vials coming from this format were erroneously associated to the product code 352521. In order to identify the 100 mg/10 mL vials, a different batch number (TT528/1) was assigned.

In terms of labeling, final product boxes to be visual inspected were correctly labelled as product code 352520 and batch TT528/1 (following the instruction in the MBR), whereas samples already subjected to manual visual inspection (both for internal chemical and microbiological analyses and for client's analyses) have been erroneously labeled by the visual inspection department with the product code 352521 but keeping the correct batch number TT528/1.

Therefore, two events are managed by the present investigation form:

- Event 1: Incorrect loading in the warehouse system of the MabThera 100 mg/10 mL crimped vials to be visually inspected, associated to the code 352521 rather than 352520;
- Event 2: Incorrect loading and box labeling of the MabThera 100 mg/10 mL samples, already visually inspected, associated to the code 352521 rather than 352520

INVESTIGATION AND EVALUATION

For the manufacturing of the MabThera 500 mg/50 mL and MabThera 100 mg/10 mL, one MBR had to be issued considering that one common compounding step was foreseen.

The MBR was issued with the code of the 500mg/50 mL format (product code 352521), whereas the format 100 mg/10 mL (product code 352520) had to be considered as a secondary product.

Prior to starting batches visual inspection activities, it has been noted that all the crimped vials were uploaded in the system associated to the product code 352521, despite the different formats.

ATTACHMENT "MABTHERA 100MG/10 ML ENG DA SPERL VIALS: INCORRECT WAREHOUSE LOADING AND BOXES LABELING_TT237B011" - INVESTIGATION FORM – TT237

Based on the occurred events, it has been assessed no impact for the following reasons:

Event 1 - Incorrect loading in the warehouse system of the MabThera 100 mg/10 mL crimped vials:

- the treatability of the product is guaranteed by the batch number which is different between the two formats (TT528 and TT528/1);
- physical labels applied on final product boxes to be visual inspected have the correct data in terms of batch number and product code;
- engineering batches are not GMP

Event 2: Incorrect loading and box labeling of the MabThera 100 mg/10 mL samples, already inspected by visual department

- Physical labels applied on samples boxes after VI activity report ID as per sampling plan and batch number TT528/1 in order to ensure the right samples identification;
- the treatability of the product is guaranteed by the batch code which is different between the two formats (TT528 and TT528/1);
- engineering batches are not GMP

ROOT-CAUSE

Both the events described above can be considered due to an error during the 100 mg/10 mL format warehouse system uploading after the crimping step. It has to be highlighted that the split production is not common practice and it is applicable only for not GMP batches.

CONCLUSION

In order to manage the discrepancy in terms of product code associated to the crimped MabThera 100mg/10 mL to be visual inspected by VI department, the right information prior to start the visual inspection of the crimped units has to be provided and reported below.

Particularly, the following units will be inspected by visual inspection department:

- 352521 MabThera 500 mg/50 mL ENG da spel TT528, 1482 units, associated to VT74247001001001
- 352521 MabThera 500 mg/50 mL ENG da spel TT528/1, 9248 units, associated to VT74534001001001

After the visual inspection activities, the units will be uploaded in the warehouse system as follows:

- 352521 MabThera 500 mg/50 mL ENG da spel TT528 units, to be associated to the code 363308 MABTHERA 500MG/50ML ENG
- 352521 MabThera 500 mg/50 mL ENG da spel TT528/1 units, to be associated to the code 363305 MABTHERA 100MG/10ML ENG

For what regards the event 2, the present investigation form will be referenced in QC laboratory dedicated documentation in order to keep track of the wrong product code reported on the vials boxes/internal documentation.

ATTACHMENT "MASTHERA 100MG/10 ML ENG DA SPERI VIALS: INCORRECT WAREHOUSE LOADING AND BOXES LABELING_TT237B011" - INVESTIGATION FORM – TT237

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ATTACHMENT 6 - INVESTIGATION FORM – LEAKAGE DURING THAWING PRODUCT RECIRCULATION

ATTACHEMNT "LEAKAGE DURING PRODUCT RECIRCULATION PHASE- FIRST THAWING TT237B011"-

INVESTIGATION FORM

The present document can be filled-in in the paper version or in the electronic version.

The approved version will be attached to the final report.

Product : 352521

Lot.: TT528

DESCRIPTION OF THE EVENT

During the thawing cycle of MabThera engineering batch (product code 352521, batch TT528), at the beginning of the recirculation phase (at 20:53 on 08/06/2022) a leakage between the two-recirculation tubes (SGS04049) assembled in series (through tri-clamp connection) was detected by dispensing operators. As immediate action, the operators clamped with pinch clamps the upstream and downstream section of the connection. Then, the operators opened the tri clamp and detected the gasket which was misplaced. The gasket was adjusted (without touching it, moving the extremity of the two tubing), the tri-clamp reclosed, and the pinch clamps removed.

All these operations were performed under LAF, wearing sterile gloves.

After this correction, no leakage was observed.

INVESTIGATION AND EVALUATION

The BDS is supplied to Thermo Fisher by Roche in frozen F/T tanks which have to be thawed for the manufacturing of MabThera drug product. The thawing cycle is composed by two phases:

- a first phase in which the product is thawed inside the F/T tank;
- a second phase in which the product is thawed inside the F/T tank and meantime the product recirculates from the bottom to the top of the F/T tank. The recirculation is performed through two disposable tubes (connected in series through tri-clamp connections) and peristaltic pump. This step is performed to gain product homogeneity in the F/T tank.

In the thawing step at Thermo Fisher, there are three tri-clamp connections which are:

- one between the first disposable tubing and the top valve of the F/T tank;
- one between the second disposable tubing and the bottom valve of the F/T tank;
- one which connects the two disposable tubing in series

During the first thawing performed during engineering run, all the connections were performed at the beginning of the thawing cycle on 08/06/22 by the same operator. Out of the three connections, only the connection between the two disposables showed a leakage, the other two tri-clamp connections did not show any leakage.

Before the engineering run, Thermo Fisher qualified the two thawing stations (TW0001 and TW0002) for the thawing of the F/T tank for manufacturing of MabThera and Avastin.

To qualify the thawing station, multiple thawing cycles were performed, and it was never experienced a leakage from the tri-clamps connections.

Moreover, during the engineering run, another cycle of thawing was performed (cycle started on 01/07/2022). During this run no leakage was observed.

Finally, based on the information listed above and moreover considering that:

- after thawing a total of three sterilizing grade filtering steps are present;
- all bioburden analysis performed on the samples taken after every time points identified for the microbiological evaluation were conforming (0CFU/10 mL),

- Endotoxin results were conforming
- it has been assessed no impact on the quality of the product.

ROOT-CAUSE

The event described above can be considered due to an error during the assembling of the two disposable tubing through tri-clamp connections.

Moreover considering that this event did not occur in the previous one cycles of qualification and in the following thawing cycle of the engineering run, it can be considered a narrowed event.

CONCLUSION

Based on the information considered above, no further actions in the short term can be considered needed.

Indeed, in the long term, the suggested corrective action is the increasing of the tubing length of the recirculation tubing in order to reduce the tri clamps connections needed for the thawing cycle. Indeed, the current configuration foreseen the presence of two recirculation tubing connected by a tri-clamp connection which will be removed in the future configuration as it will foresee the presence of a single recirculation tubing whose length is equal to the sum of the lengths of two current recirculation tubing.

This action is suggested to be implemented before the manufacturing of commercial lots and it will be captured in the post qualification change.

Written by: Giulia Ferri	Signature/ Date:  Electronically signed by: Giulia Ferri Reason: Author of the GxP document Date: Dec 14, 2022 08:59 GMT+1
Reviewed by: Laura Palmaroli	Signature/Date:  Electronically signed by: Laura Palmaroli Reason: Reviewer of the GxP document Date: Dec 14, 2022 08:59 GMT+1
Reviewed by: Fabio Zaffaroni	Signature/Date:  Electronically signed by: Fabio Zaffaroni Reason: Reviewer of the GxP document Date: Dec 14, 2022 08:40 GMT+1
Approved by: Emanuele Fois	Signature/Date:  Electronically signed by: Emanuele Fois Reason: Approver of the GxP document Date: Dec 14, 2022 08:59 GMT+1

ATTACHMENT 7 - INVESTIGATION FORM – BLACK OUT EVENTS DURING FREEZING CYCLE

ATTACHEMNT "BLACK OUT DURING FIRST FREEZING CYCLE TT237B011"- INVESTIGATION FORM

The present document can be filled-in in the paper version or in the electronic version.

The approved version will be attached to the final report.

Product: 352521

Lot: TT528

DESCRIPTION OF THE EVENT

During the first freezing cycle of MabThera engineering run (product code 352521 and batch TT528), launched on 20/06/22 at 12:45 am (Batch report 352521 MABTHERA TT528 1 FREEZE), 7 black-out events occurred (see table below).

One black out happened during the purging phase, the other 6 during the first freezing step (of these, 4 events occurred one straight after the other and can be considered as a single event).

Black out Time occurrence	Resume cycle Time occurrence	On HOLD duration	Step of the recipe black out happened	Rec per step duration	Each step Start/End time	Silicon oil temperature setpoint	Silicon oil temperature probe (inlet)	Silicon oil temperature probe (outlet)	Product Temperature probe
13:00	13:23	00:23	Purging step	At least 120 min	Start: 20.06.2022 12:55:58 End: 20.06.2022 13:39:39	NA	NA	NA	NA
13:41	15:45	02:04	Primary Freeze	At least 11h 30 min	Start: 20.06.2022 13:39:49 End: 21.06.2022 04:28:55	-50°C	Alarm High: 26°C Warning High: 30°C Warning Low: -60°C	NA	Warning Low: -55°C
18:49	18:51	00:01							
18:51	18:55	00:03							
18:55	18:55	00:00							
18:56	18:58	00:01							
19:53	21:03	01:10							

After the black out, the power supply was restored immediately, and the recipe was automatically put on hold by the machine. The restore from an holding phase is foreseen to need an input from the operators on the HMI. Holding time is not the time while the power supply was missed, because it was resume immediately (few seconds), but it was the time needed by the operators to resume the recipe.

For every black out events report in table above, the start time is the starting of hold step and the end time is the restoring of the recipe executed by the operators.

During the purging phase the circuit is purging in order to get ready to perform the freezing steps of the recipe.

The first freezing is supposed to last at least 11 hours and 30 minutes and the total duration for the involved cycle was 14 hours and 47 minutes, so the time duration (at least 11 hours and 30 minutes) (pCPP listed in the protocol TT237B011) was respected.

The secondary freezing is supposed to last at least 7 hours and 30 minutes and it lasted 7 hours and 30 minutes (no black out events during secondary freezing step).

INVESTIGATION AND EVALUATION

During engineering run, to freeze the BDS, the 300 L F/T tank which contains the BDS was connected (20/06/2022) to one of the two Zeta thawing stations (Thawing station 2 TWU002 SN S453427) in room 2204.

The TWU002 freezing process consist in a heat transfer fluid (Silicon oil) which is tempered in the station and goes to the jacketed of the F/T tank and back to the station to freeze the BDS contained in the tank. The freeze of the 300 L US F/T tank used in the engineering run was performed according to the qualified recipe (FREEZE_300L).

The pCPP applicable (as per engineering protocol TT237B011) for the involved process step are reported in the table below.

Parameter	Requirements	Results	pCPP (Y/N)
Drug substance weight in Freeze/Thaw tanks	Weight \approx 51,5 Kg	228,5 Kg	Y
Number of freeze/thaw cycle	N* cycles \geq 3/Record	2 (*)	Y
Heat transfer fluid temperature set point	Setpoint = -50°C Temperature \geq 26°C	Conform (see graph)	Y
Freeze operation duration (first freezing + secondary freezing)	Time \approx 19 hours	22 hours and 17 minutes	Y

(*) first freezing cycle is the one executed at D8 manufacturing site

In the graph below it is reported the trend of the temperature of the silicon oil during the freezing cycle. In particular the red line is related to the temperature of the silicon oil when entering the F/T tank and the blue line is related to the temperature of the silicon oil when exiting the F/T tank. The black line is instead the temperature of the product measured by the thermocouple inserted in the dedicated holding of the F/T tank. Nevertheless, the temperature of the product is not listed as potential pCPP, it is however displayed and described as outcome of the process.

The target of the temperature of the silicon oil during the freezing process was set at -50°C as per validated recipe "FREEZE_300L".

From the trend, it is possible to see that some peak in the temperature of the silicon oil is present both at inlet and outlet temperature graph of the F/T tank during:

- Purging step
- First freezing step

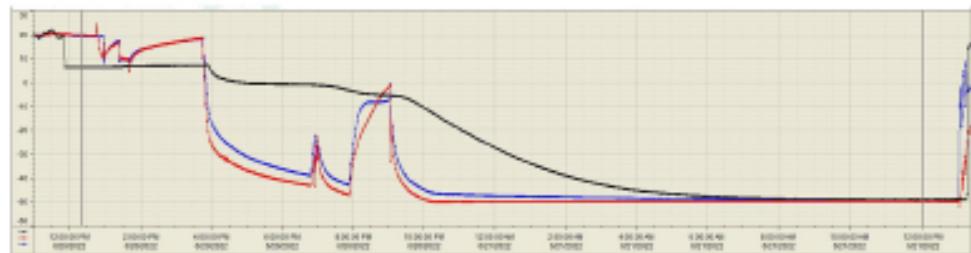
The peaks were caused by the blackout events in the facility during the cycle running. After the blackout events, the power supply was guaranteed immediately but the machine put on hold the cycle and needed an input from the operators to resume the cycle. For every blackout events, the operator restored the cycle.

However, there was no alarm regarding the silicon oil product temperature during all cycle.

Moreover, the product temperature (black line) never exceeded 8°C during the holding phase.

Finally, considering that the recipe is time based and if an holding happens during the cycle, the time of the holding is summed up to the overall time of the phase. It was also respected the pCPP of the duration of the cycle.

The cycle was conforming and the BDS was correctly frozen



In May 2022, a dedicated intervention for increasing the power supply was carried out. The blackouts event happened straight after this activity. It has been performed a check of the power cabinet and a malfunctioning was identified.

Moreover, during the engineering run, it was performed in total:

- 2 thawing cycles
- 2 freezing cycles

and during the other two thawing cycles and the other freezing cycle, no similar event occurred.

Finally, based on the information listed above and moreover considering that:

- all the pCPP reported in the protocol TT237B011 was respected;
- the product temperature never exceed 8°C

It has been assessed no impact on the quality of the product.

ROOT-CAUSE

The event described above can be considered due to a supply failure (malfunctioning of the power cabinet) during the involved freezing step.

Moreover considering that:

- these un-expected events did not occur in the other two thawing cycles and other freezing cycle executed during the engineering runs;
- no similar reoccurrences in the Monza site before the activities executed in May and after the fixing intervention on the power cabinet

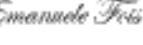
they can be considered a narrowed events.

CONCLUSION

Based on the information listed in the previous one section, and in particular considering that:

- these un-expected occurrences can be considered narrowed;
- no impact on the product quality of the BDS contained in the F/T was assessed;
- all the pCPP listed in the protocol TT237B011 (table 10) were respected
- analytical results on the BDS met the acceptance criteria (RC0615 Ed.01);

no further actions (other than the fixing activities executed on the power cabinet) are considered needed.

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Reviewed by: Laura Palmaroli	Signature/ Date:  Electronically signed by: Laura Palmaroli Reason: Reviewer of the GxP document Date: Dec 14, 2022 09:21 GMT+1
Reviewed by: Fabio Zaffaroni	Signature/ Date:  Electronically signed by: Fabio Zaffaroni Reason: Reviewer of the GxP document Date: Dec 14, 2022 09:40 GMT+1
Approved by: Emanuele Fols	Signature/ Date:  Electronically signed by: Emanuele Fols Reason: Approver of the GxP document Date: Dec 14, 2022 09:59 GMT+1

ATTACHEMNT 8 - MEMO IN PROCESS CONTROLS (IPC) AND SHELF LIFE SPECIFICATION INFORMATION FOR MABTHERA 100 MG/10ML & 500MG/50

Memo



To:	Enza Schlosaro, (Patheon Italia S.p.A part of Thermo Fisher Scientific)	Copies:	Vanessa Cervi (Roche), Operations Manager Jürgen Lang (Roche) GxP Quality Supply Manager
From:	Shrinivas Tata (Roche)	Roche GxP Quality Supply Manager	
Date:	16 November 2022		

In process Controls (IPC) and Shelf Life Specification Information for MabThera 100 mg/10ml & 500mg/50ml

This memo serves as to provide information on IPC controls and Shelf Life currently registered for the US, Japan & EU market for MabThera 100 and 500mg Drug Product. Refer to Page 2 & 3 for details.

Additional specification or some changes in specification may be adopted for MabThera 100 and 500mg Drug Product manufactured at Patheon Italia which will be independently registered. All specifications will be reviewed and approved by Roche.

Sincerely
SHRINIVAS TATA

DocuSigned by:
Shrinivas Tata
A small blue shield-shaped icon with a white 'D' inside, representing DocuSign.
Signer Name: Shrinivas Tata
Signing Reason: I approve this document
Signing Time: 18-Nov-2022 | 5:08:04 AM PST
BA4B6485229D4B89A4AB5FAD68D525C5

In process Controls (IPC) and Shelf Life Specification Information for
MabThera 100 & 500mg



**1) FOR US/Japan (IPC Specifications MabThera 100 & 500mg)
IPCs of Rituximab Drug Product Manufacturing**

Process Step	In Process Control-	Type of Limit	Limit
Thawing of Drug Substance	Bioburden	Action limit	>10 CFU/10 mL
Preparation of Dilution Buffer	Osmolality	Action limit	<324 or >396 mOsmol/kg
	pH	Action limit	<6.2 or >6.8
	Bioburden	Action limit	>10 CFU/10 mL
	Endotoxin	Action limit	>0.25 EU/mL
Formulated Bulk	Osmolality	Action limit	<324 or >396 mOsmol/kg
	pH	Action limit	<6.2 or >6.8
Bioburden Reduction Filtration	Bioburden (before filtration)	Action limit	>10 CFU/10 mL
	Filter integrity testing (before and after filtration)	Action limit	<3450 mbar (bubble point with water) <2790 mbar (bubble point with product)
Inline Sterile Filtration-	Bioburden(before filtration)	Acceptance criterion	≤10 CFU/100 mL
	Endotoxins (before filtration)	Action limit	>1.0 EU/mL
	Filter integrity testing (before and after filtration)	Action limit	<2790 mbar (bubble point with product) <2790 mbar (bubble point with product)
Aseptic Vial Filling and Stoppering	Fill volume (by weight control)	Action limit	10 mL: <10.40 or >11.03 g/vial 50 mL: <50.90 or >52.62 g/vial
Finished Product after Secondary Packaging and Labeling	Identity of rituximab by CZE	Acceptance criterion	Positive identity

Abbreviations: AQL = acceptance quality limit; CFU = colonyforming unit; CZE = capillaryzone electrophoresis; EU = endotoxin unit.

In process Controls (IPC) and Shelf Life Specification Information for
MabThera 100 & 500mg



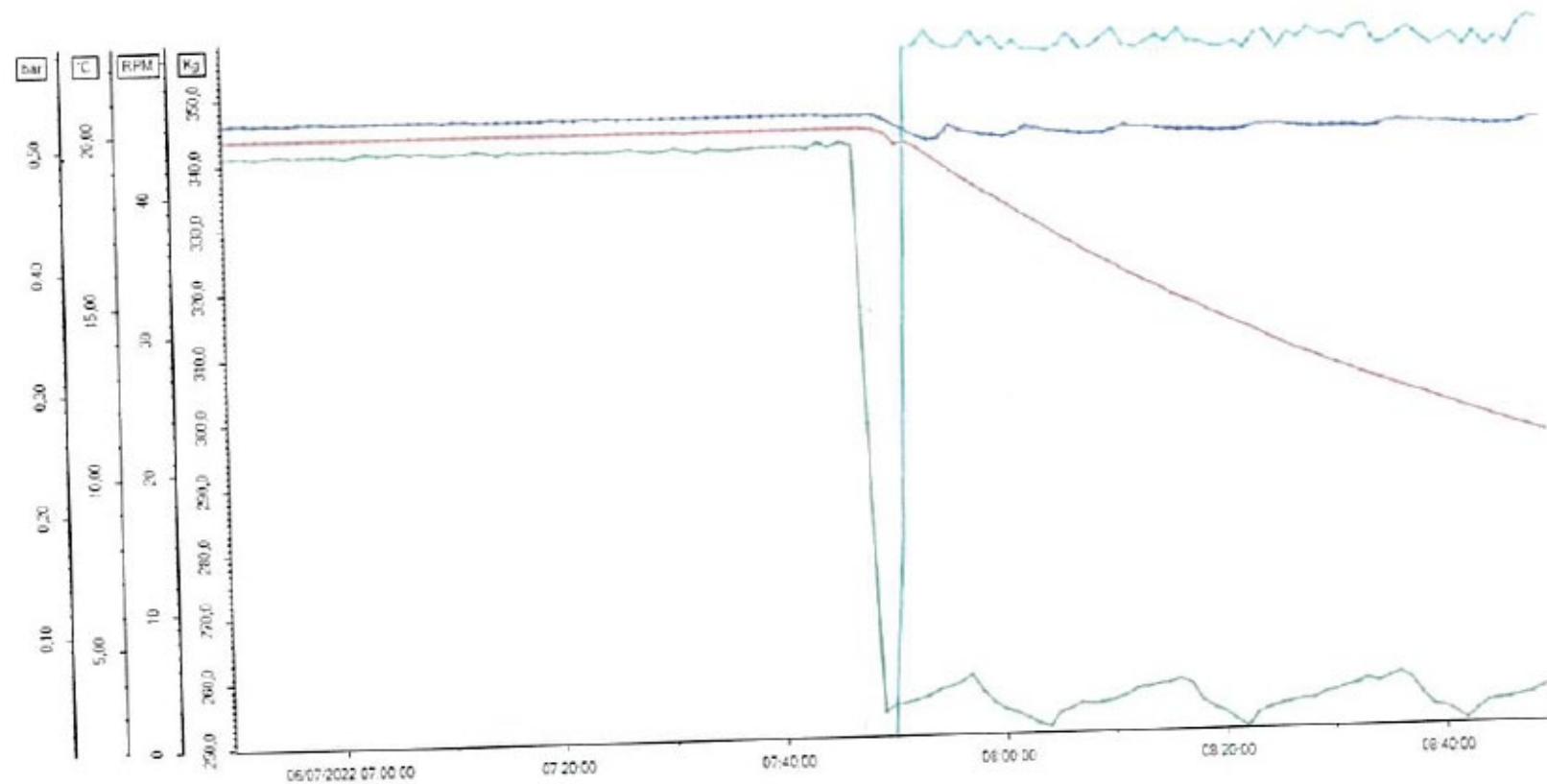
2) FOR EU (IPC Specifications MabThera 100 & 500mg)

Process step	In-process control parameter	Acceptance criteria
Compounding of MabThera' bulk drug product solution	pH	6.5 ± 0.3
1= 0.22 µm filtration	Filter integrity before and after filtration Bioburden	according to filter specifications Max. 10 CFU/ 100 ml (after filtration)
2= 0.22 µm filtration	Filter integrity before and after filtration	According to filter specifications
Aseptic filling	Fill weight	* 100 mg / 10ml vials: limits: 10.1 – 11.5 g/vial (target: 10.6 g/vial) * 500 mg / 50ml vials: limits: 50.6 – 53.0 g/vial (target: 51.6 g/vial)
Completed bulk vials	100 % final inspection for non-compliant vials	Complies

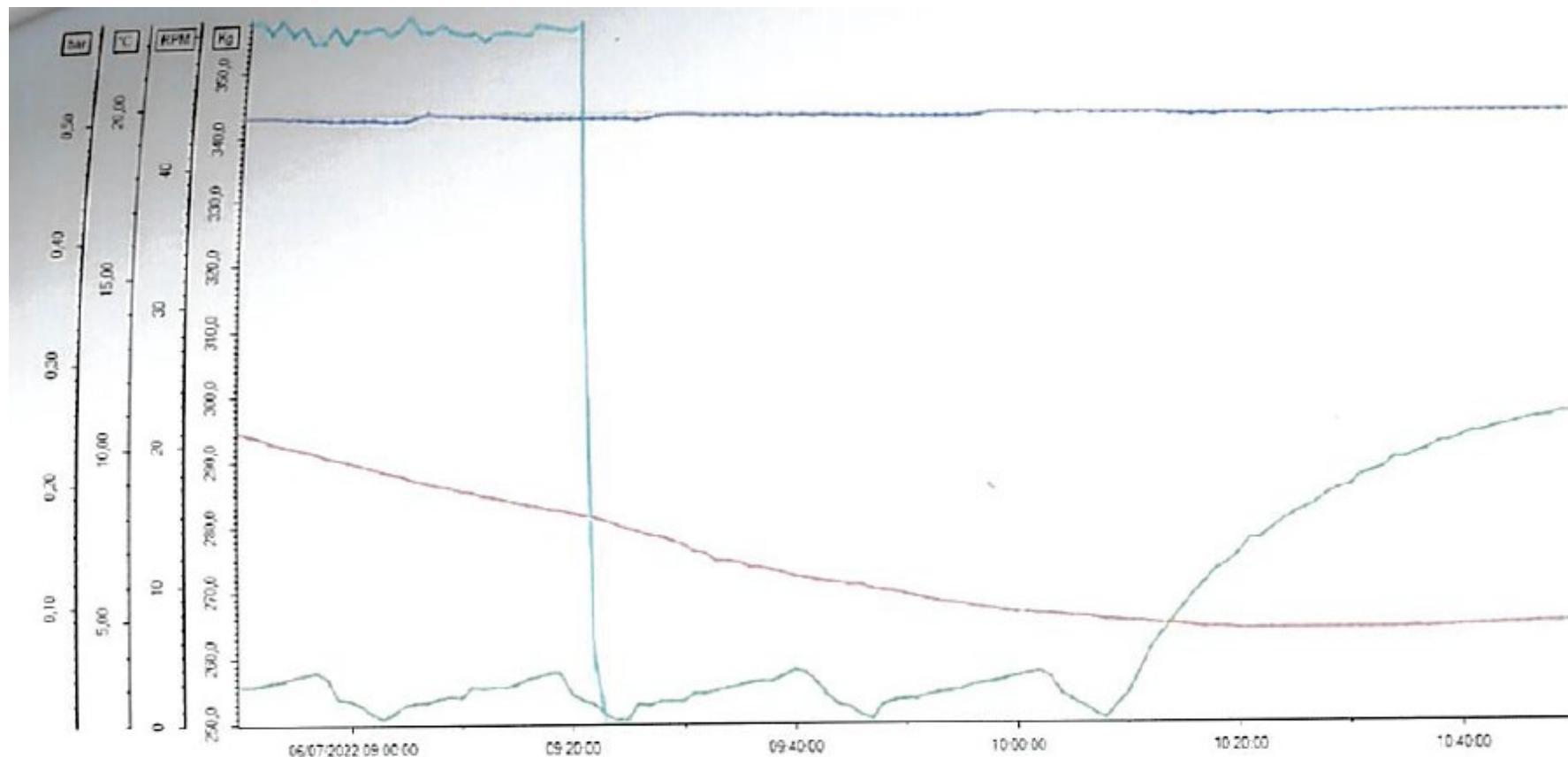
3) STORAGE CONDITIONS AND SHELF LIFE

Drug product recommended storage conditions: 2°C–8°C, protected from light

Shelf life at recommended storage conditions: 36 months

ATTACHMENT 9: TEMPERATURE PROFILE DURING COOLING DOWN AT 2-8°C FOR THE STORAGE TANK

Red: Temperature trend; Blu: Pressure trend; Grey: mixer speed trend



Red: Temperature trend; Blu: Pressure trend; Grey: mixer speed trend

ATTACHMENT 10: POTENCY RESULTS FOR MABTHERA 500 MG

PT Bioteesting
Bioassay

Ergebnisbewertung Bioassay
MW /Aktivität [%] / CV% / Satralizumab

Druckdatum: 09.11.2022 12:25

1. Eingabe der Proben

Analysennummer	27268317
Projekt	Mabthera
Zusatzinfo	PDS-500mg
Standard Aktivität (%)	n.a.
Akzeptanzkriterium CV%	15
Testanweisung	SAM-0104062 Version 5.0
Einheiten (z.B. 10 ⁴ U/ml)	x10 ⁵ U/ml

2. Eingabe der Daten

Versuchsnummer	Aktivität	Aktivität %
	x10 ⁵ U/ml	
MH01_Mab22032_p1	1.0637	
MH01_Mab22033_p1	1.1022	
MH01_Mab22034_p1	1.0535	

3. Ergebnisse

Mittelwert ungerundet:	1.073133333
Ganzzahl:	—

4. Berechnung Satralizumab ✓ N/A

Konz. Std.	
Aktivität	
U/ml	
U/mg	

5. Bewertung

Seabw: 0,0257
CV%: 2,393357019
CV% gerundet: 2

Prüfung CV% entspricht ✓

Akzeptanzkriterium CV%
s 15

Bemerkungen: ✓ N/A

09.Nov.2022 Up
erstellt: Datum, Unterschrift

09.NOV. 2022 VK
geprüft: Datum, Unterschrift

ATTACHMENT 11: POTENCY RESULTS FOR MABTHERA 100 MG

Anlagen 09 Nov. 2022 U3
 PT Biotesting
 Bioassay

Ergebnisbewertung Bioassay
 MW /Aktivität [%] / CV% / Satralizumab

Druckdatum: 09.11.2022 12:28

1. Eingabe der Proben

Analysennummer	27266317
Projekt	Mabthera
Zusatzinfo	PDS-100mg
Standard Aktivität (%)	n.a.
Akzeptanzkriterium CV%	15
Testanweisung	SAM-0104862 Version 5.0
Einheiten (z.B. 10^4 U/mg)	$\times 10^4$ U/ml

2. Eingabe der Daten

Versuchsnummer	Aktivität	Aktivität %
	$\times 10^4$ U/ml	
MH01_Mab22032_p1	1,0705	
MH01_Mab22033_p1	1,1555	
MH01_Mab22034_p1	1,1058	

3. Ergebnisse

Mittelwert ungerundet:	1,1106
Grenzwert:	...

4. Berechnung Satralizumab *X N/A*

Konz. Std.	
Aktivität	
U/ml	
U/mg	

5. Bewertung

Stabw: 0,0427
 CV%: 3,845021629
 CV% gerundet: 4

Akzeptanzkriterium CV%
 Prüfung CV% entspricht ✓ s 15

Bemerkungen *X N/A*

09 Nov 2022 U3
 erstellt: Datum, Unterschrift

09 NOV. 2022 U3
 geprüft: Datum, Unterschrift