Technical Report

Title: Surrogate Trial for MabThera

Date:02/05/2022

Control No: TT237D011

Client : F. Hoffmann-La Roche Ltd

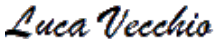
Prepared by:	**Giulia Ferri**
Position:	Process Engineer
Signature:	 Electronically signed by: Giulia Ferri Reason: Author of the GxP document Date: May 2, 2022 17:56 GMT+2
Reviewed by:	**Laura Palmaroli**
Position:	Technology Transfer Project Manager
Signature:	 Electronically signed by: Laura Palmaroli Reason: Reviewer of the GxP document Date: May 2, 2022 18:29 GMT+2
Reviewed by:	**Josefina Di Babbo**
Position:	Process and Cleaning Validation Specialist
Signature:	 Electronically signed by: Josefina Di Babbo Reason: Reviewer of the GxP document Date: May 3, 2022 13:36 GMT+2

Reviewed by:	**Sara Ferretti**
Position:	Industrial Engineering & Client Technical Support

Signature: 

Electronically signed by: Sara Ferretti Reason: Reviewer of the GxP document Date: May 3, 2022 13:49 GMT+2

Reviewed by:	**Luca Vecchio**
Position:	Supervisor, Sterile Department 06

Signature: 

Electronically signed by: Luca Vecchio Reason: Reviewer of the GxP document Date: May 3, 2022 14:49 GMT+2

Reviewed by:	**Fabio Zaffaroni**
Position:	Supervisor, Dispensing Department

Signature 

Electronically signed by: Fabio Zaffaroni Reason: Reviewer of the GxP document Date: May 3, 2022 17:05 GMT+2

Reviewed by:	**Mara Bonanomi**
Position:	Team Leader, An. Support Validation

Signature 

Electronically signed by: Mara Bonanomi Reason: Reviewer of the GxP document Date: May 3, 2022 17:53 GMT+2

Reviewed by:	**Dario La Sala**
Position:	Supervisor, Process Engineering

Signature 

Electronically signed by: Dario La Sala Reason: Reviewer of the GxP document Date: May 3, 2022 18:03 GMT+2

Reviewed by:	**Maria Francesca De Rosa**
Position:	Supervisor, Visual inspection
Signature	 *Electronically signed by: Maria Francesca De Rosa Reason: Reviewer of the GxP document Date: May 4, 2022 15:18 GMT+2*

Approved by:	**Barbara Bassi**
Position:	Sr Manager, Quality System & Compliance
Signature:	 *Electronically signed by: Barbara Bassi Reason: Approver of the GxP document Date: May 5, 2022 09:54 GMT+2*

Client Approval

Approved by:	**Vanessa Cervi**
Position:	Quality Site Manager
Signature:	DocuSigned by: Vanessa Cervi Signer Name: Vanessa Cervi Signing Reason: I approve this document Signing Time: 09-Mai-2022 | 3:23:46 PM CEST 36E395C913944D07A26B0905B96B8A8E

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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 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 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, in Xtrema line 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).

1.1 SCOPE

The scope of this document is to describe the results of the activities executed as per Protocol TT237A011 related to the execution of the two (2) following surrogate trial batches:
- Surrogate Trial A: a surrogate fill of 50 mL Schott vials and Daikyo stoppers and seals that mimics the filling step of MabThera 500mg/ vial presentation manufacturing, along with a preliminary mixing assessment of surrogate solution
- Surrogate Trial B: a surrogate fill of 10 mL Schott vials and Daikyo stoppers and seals that mimics the filling step of MabThera 100mg/vial presentation manufacturing.

The surrogate solution used in both surrogate trials was formulated according to MabThera's buffer composition and preparation provided by the client. Table 1 shows the composition of MabThera formulated bulk and surrogate solution. The surrogate formulation did not foresee any pH adjustment.

Table 1 MabThera formulated bulk and surrogate composition

Excipients/API	Surrogate solution	Mabthera Formulated bulk
Rituximab	NA	10 mg/mL
Sodium citrate dihydrate	7.35 mg/mL	7.35 mg/mL
Sodium chloride	9.0 mg/mL	9.0 mg/mL
PS80	0.7 mg/mL	0.7 mg/mL
Sodium hydroxide or Hydrochloric acid 1N	NA	q.s.ad pH 6.5
WFI	q.s.	q.s.
Density	1.01g/mL	1.01g/mL
Viscosity	0.7 cP at 25ºC 1.2 cP at 5ºC	0.8 cP at 25ºC 1.4 cP at 5ºC

The surrogate solution was used in both surrogate trials of MabThera as it mimics the chemical and physical properties of MabThera's bulk solution, as shown in the table above and as indicated in the memo shared by Roche : "Avastin and Mabthera: Usage of surrogate for mixing trial" dated 10th Dec 2021 (Attachment #9).

In both trials, primary packaging parts were conveyed along the Xtrema filling line, aimed at mimicking the future MabThera drug product fill and stoppering steps for both 100 mg /vial and 500 mg/vial dosage strengths.

Y tubes, sterilizing filtration and filling disposable assemblies foreseen in the MabThera manufacturing process were used to assess their feasibility and handling by personnel.

For each MabThera presentation, 100mg/vial and 500mg/vial, a surrogate trial was performed in order to assess the following characteristics:

- Setup evaluation (common for both surrogate trials):
  - Assess the intended fluid path set-up for formulation assemblies
  - Evaluate the suitability of the disposable assemblies (see Table 3 and 4);
- Process characterization (common for both surrogate trials):
  - tubing fatigue evaluation
  - Evaluate line performance, operational efficiency and then evaluate the filling machine dose accuracy;
  - Evaluate the timings, flows and feasibility of the step from compounding to crimping Machinability of selected primary packaging components in the Sterile 6 Xtrema filling line
  - Machinability of format parts
  - Identify filling parameters to ensure the filling performs as expected. Such parameters will have to be confirmed with product filling trials (engineering run)
  - Evaluate the performance of the crimping machine and the crimping parameters
- Preliminary mixing assessment and homogeneity study (only on Surrogate Trial A)
  - On the Surrogate Trial A (MabThera 500mg/vial presentation) a preliminary mixing assessment of compounding step was evaluated by performing the mixing study proposed by Roche. The scope of this study was to determine the mixing speed and time to be confirmed during the following trial (Engineering Runs and PPQ batches) of MabThera manufacturing. Following mixing study, an homogeneity study was performed to identified minimum mixing speed to be applied for the minimum time to guarantee solution homogeneity. Moreover, these studies are applicable also to Avastin considering that equipment (1100 L tanks) used for buffer preparation and DPS compounding for Avastin and MabThera are identical.

The surrogate trials were not GMP (no microbiological analysis on product and no environmental analysis were executed) and are not intended for human use.

2.0 RESPONSIBILITIES

Process Engineering & Technology Transfer Patheon

- To issue the present Report
- To archive approved Report

Production Patheon

- To review and approve the Report

Quality Assurance (QA) Patheon

- To review and approve the Report

Quality Control (QC) Patheon

- To review and approve the Report

Roche

- To review and approve the Report

3.0 EQUIPMENT AND MATERIALS

In this paragraph, a summary of equipment and materials involved is reported, specifying if equipment and materials pertained to surrogate trial A or to surrogate trial B considering that, as briefly introduced in paragraph 1.1:

- part A): trial involved a surrogate mixing study (two stainless steel tanks), surrogate filtration and filling of the same type of 50 mL vials, stoppers and seals foreseen for commercial manufacturing of MabThera 500mg/vial
- part B): trial involved a preparation of surrogate (one 1100 L tank), surrogate filtration and filling of the same type of 10 mL vials, stoppers and seals foreseen for commercial manufacturing of MabThera 100mg/vial

Major equipment involved in the manufacturing process grouped by process step is reported in Table 2.

Table 2: Equipment used for MabThera part A and part B surrogate trials batch

Process Step	Equipment
Buffer preparation & storage	2x 1100L stainless steel tanks for part A (equipment ID RTR343 and RTR344); 1x 1100 L stainless steel tank for part B (equipment ID RTR343);
	Transpallet for tank movimentation (ID: CAR095)
Sterilizing filtration	Stainless steel trolley for the support of the disposable assembly that incorporates 2 (two) sterilizing filters for redundant 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)

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 3: Primary packaging components[1]

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	Dayko	Dayko	273438
Seals (*)	Seals 20mm RED	Datwayler	Datwayler	273433
Seals (**)	Seals 20 mm GREY	Datwayler	Datwayler	273434

Vials, stoppers, and seals are materials that will be used for both MabThera dosage strengths commercial manufacturing and technology transfer batches. The stopper is 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 are dedicated to 100mg/vial dosage and the 50 mL and grey seals are dedicated to 500mg/vial dosage.

According to procedure Patheon SOP 1602, as the batches were 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.

[1] (*) MabThera 100mg/vial presentation; (**) MabThera 500mg/vial presentation

DISPOSABLE ASSEMBLIES

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

Table 4: Disposable assemblies used for the Surrogate trial part A

Process step	Material description	Patheon code	Supplier code
Transfer of solution between the two tanks during the mixing study	4 (four) Y disposable tubes provided with steam-thru port to be connected to the tank and 2 (two) Aseptiquik ports for the connection to the disposable assembly (*)	273499	SGS03744
	2 (two) Extension tubes for solution transfer between the two tanks	273502	SGS04044
Transfer of solution from SS tank to the filling line and sterilizing filtration	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
	1 (one) disposable extension tube for the connection of the SS tank to the assembly for sterile filtration	273502	SGS04044

(*) For this surrogate trial, steam-thru was replaced by tri-clamp, because it was no possible to receive the assembly with the steam - thru component due to lead time. However, no impact on the trial was present because microbiological tests were not in scope of this trial.

Table 5 Disposable assemblies used for the Surrogate trial part B

Process step	Material description	Patheon code	Supplier code
Transfer of solution from SS tank to sterilizing filtration	1(one) Y disposable tube provided with steam-thru port to be connected to the tank and 2 (two) Aseptiquik ports for the connection to the disposable assembly (*)	273499	SGS03744
	1 (one) disposable extension tube for the connection of the SS tank to the assembly for sterile filtration	273502	SGS04044
	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
Filling	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

(*) For this surrogate trial, steam-thru was replaced by tri-clamp because it was no possible to receive the assembly with the steam - thru component due to lead time. However, no impact on the trial was present because microbiological tests were not in scope of this trial.

All assemblies were provided by the supplier, Saint Gobain, already sterilized by gamma irradiation and ready to use.

As agreed with client, the only assembly which was not gamma irradiated was the filter assemblies (PTH code 273504). Considering that no microbiological analysis was foreseen, no risk was present. 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.

4.0 SURROGATE TRIAL PROCESS DESCRIPTION

The trials included the steps described in detail in the following sections of the document and they were executed on the ST6 area – Xtrema filling line.
Process steps, materials and equipment were dedicated whether the lower or higher dosage was being involved, considering that a preliminary mixing study was executed during higher dosage.
The recipe was set in the filling machine and so dedicated filling parameters were assessed separately taking into consideration their respective target and limits fill weight.

Each step of the whole manufacturing process has been evaluated and the potential Critical Process Parameters (CPP) have been evaluated.

The main objective is to increase the process knowledge, identify the process trend and optimize the control strategy to ensure the stability of potential CPPs over time which will be re-evaluated during Engineering batch and PPQ batches manufacturing.
Moreover, samples were collected during the surrogate trial to get preliminary data on Container Closure Integrity Test (CCIT) applying a validated Blue Dye intrusion method (SIS 1043 and protocol PC0082 Ed.01), performed by Thermo Fisher
Additional samples were inspected through manual visual inspection to identify macro defects and cosmetic defects (Table 36 and Table 37) produced during filling and crimping operations. The manual visual inspection was performed by operators which are qualified to perform a visual inspection on liquid.

4.1 PROCESS OVERVIEW

The main process steps to be applied for the MabThera surrogate trails were dedicated to **Part A** (preliminary mixing study and filling of 500 mg/vial presentation) and **Part B** (filling of 100 mg/vial presentation).
The main process steps for **Part A** were:

- *Buffer stock solutions prepared in 10L glass bottles.*
- *Preparation of buffer solution (1100L) in SS buffer tank by pooling the quantities of excipients needed*
- *Mixing study using two (2) SS tanks and according to mixing study proposal (as reported in protocol TT237A011, Surrogate trial for MabThera)*
- *Transfer 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 (performed through bubble point test and according to filters' supplier certificate)*
- *Sterilizing filtration of the buffer solution through 2 (two) 0.22µm sterilizing filters by nitrogen pressure (pressure monitoring in place).*

- *Vials filling driven by peristaltic pumps*
- *Vials stoppering and crimping*
- *Post-use integrity test of both the sterilizing filters (performed through bubble point test and according to filters' supplier certificate)*

The main process steps for **Part B** were:

- *Buffer stock solutions prepared in 10L glass bottles.*
- *Preparation of buffer solution (1100L) in SS buffer tank by pooling the quantities of excipients needed*
- *Mixing of the buffer solution with mixing parameters determined in the Surrogate Trial part A*
- *Transfer of the SS buffer 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 (performed through bubble point test and according to filters' supplier certificate)*
- *Sterilizing filtration of buffer solution through 2 (two) 0.22µm sterilizing filters by nitrogen pressure (pressure monitoring in place).*
- *Vials filling driven by peristaltic pumps*
- *Vials stoppering and crimping*
- *Post-use integrity test of both the sterilizing filters (performed through bubble point test and according to filters' supplier certificate)*

The present surrogate trials exercise simulated the manufacturing operations (with the exception of thawing/freezing, BDS pooling , bioburden filtration step and 100% visual inspection) from the stainless-steel storage tank to the crimping step.
A more detailed description of the main steps simulated during the present surrogate run exercise will be covered in the next paragraphs.

4.2 SOLUTION PREPARATION OPERATIONS

Objectives:

- Mixing study to preliminarily evaluate minimum and maximum mixing speed and homogeneity study to identify minimum mixing speed to be applied for the minimum time for guarantee the homogeneity of the maximum batch size for the bulk solution (executed only for Surrogate Trial Part A)
- Simulate the set-up operations
- Evaluate the suitability of the disposable assemblies in terms of dimensions and connections
- On the field training of manufacturing personnel

4.2.1 Part A

The assemblies supplied in the solution preparation room for the installation were:

- N°4 (four) assemblies "Y" (273635) that allow the connection of the SS tanks (pooling and storage tanks) through the extension tube.

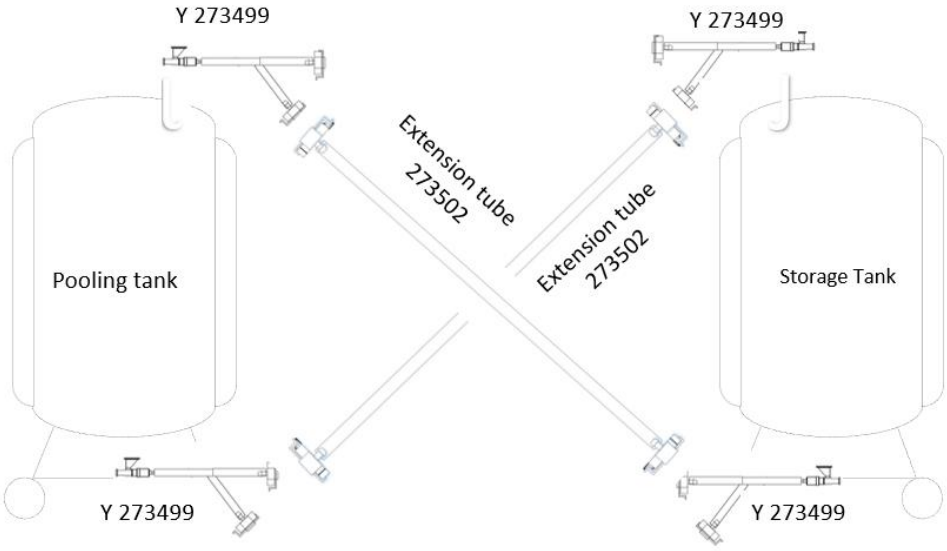
Considering that for this trial, a steam-thru connection was not available and was replaced by a tri-clamp by Stain Gobain, the Y assemblies were installed after CIP/SIP of tanks and then connected to the disposable assemblies. For the engineering and following trials, the Y assembly will have the steam-thru and it will be installed before SIP of tanks and then connected to the disposable assemblies.

- N°2 (two) “extension tubes” assemblies (273502) to connect to the Y assemblies of the two tanks, allowing the solution transfer between SS tanks during mixing study and solution transfer into the filling line

Moreover, stainless steel J-tubes were installed inside the two tanks before performing the CIP/SIP operations. They are used to limit the possible foam formation during the solution transfer into the tanks and during excipients transfer inside the tank.

The setup in solution preparation room is shown in the Figure 1 below.

Figure 1: Assemblies setup in solution preparation room (Surrogate Trial part A)



All the activities to be performed in the solution preparation room (room 823 of Sterile Area 6) were carried out in Grade C area at room temperature (19-23°C).

4.2.1.1 Surrogate preparation

The surrogate solution to be used in both MabThera's surrogate trials had the same formulation and preparation of the MabThera's buffer solution, except the surrogate preparation did not foresee any pH adjustment. Indeed, given the described objectives of the trials, pH adjustment was not seen as a value added step to be performed. MabThera's buffer solution was chosen as the surrogate solution as it mimics the physical and chemical properties of Mabthera final bulk solution (Table 1).

Moreover, MabThera buffer solution has a very similar formulation characteristic, in terms of density and viscosity, to Avastin. Therefore, Mabthera buffer was chosen as proper to perform the mixing characterization which will applied also to Avastin (TT237A021). More details are reported in the memo "Avastin and Mabthera: Usage of surrogate for mixing trial" dated 10th December 2021 provided by Roche.

Moreover, the equipment (1100 L tanks) used for buffer preparation and DPS compounding for Avastin and MabThera are identical thus making the mixing study applicable to both products.

In dispensing, in grade D area under laminar airflow, according to specific Patheon SOP 0010, the following excipients were weighted and partitioned:

- Sodium chloride (weighted salt): target ± 5% = 9,000 Kg ± 0,045 Kg
- Sodium Citrate Dihydrate (weighted salt): target ± 5% = 7,400 Kg ± 0,037 Kg
- PS80: target ± 5% = 0,703 Kg ± 0,004 Kg

Then, the dispensing, according to Patheon SOP 0009, provided the excipients to the sterile area 6 department (room 823) where the surrogate solution was prepared.

Polysorbate 80 stock solution

Polysorbate 80 was prepared using a 10 L glass bottle, according to the recipe reported in Table 6. The dispensing added 703 g in the glass bottle that was then delivered to sterile area 6 where WFI (7497,5 g) was added to obtain the final concentration. The PS80 solution was mixed using a magnetic stirring bar, applying a stirring speed not causing splashes or foam of the fluid, until completely dissolved (Table 7). Visual checks confirmed the homogeneity of PS80 solution.

Table 6 Recipe for PS80 stock solution – to be prepared in 10L glass bottle

Component	Quantity (grams)
PS80	703 g ± 0.5 %
WFI to be added to get final weight	q.s
Total PS80 stock solution formulation	8200 g ± 1%

The main parameters recorded during the preparation of the PS80 stock solution are listed in the table below.

Table 7 PS-80 Solution preparation

Parameter	Results	pCPP (Y/N)
Start of PS 80 solution preparation (date dd.mm.yyyy, time hh:mm)	09/02/2022 08:44	No
PS 80 solution start of mixing (time hh:mm) and initial mixing speed	09/02/22 09:55 300 rpm	No
PS 80 solution mixing speed modification and start of mixing at the new mixing speed	NA (*)	No
PS 80 solution end of mixing (time hh:mm)	09/02/22 10:45	No
End of PS 80 solution preparation (date dd.mm.yyyy, time hh:mm)	09/02/22 10:45	No
Mixing time	1h and 59 minutes	No

(*) for this trial, it was evaluated to not adjust the initial time and to perform the trial with the initial speed.

All the excipients were pooled in the stainless-steel tank for the compounding of the surrogate solution (Figure 2).

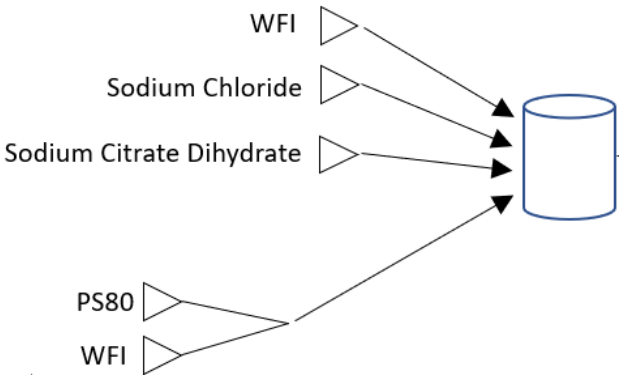


Figure 2 Surrogate preparation in stainless-steel pooling tank

Water for injection (WFI) and Nitrogen (N_2) were supplied by Patheon to the Sterile area 6 department through dedicated utilities.

After having filled the stainless-steel pooling tank with MabThera surrogate solution (1010,4 Kg), according to Table 8, the solution was mixed for excipients dissolution and homogeneity.

The main parameters recorded, during the preparation, are listed in Table 9. After the addition of the excipients, the mixing velocity was increased from the initial one identified with the water (100 rpm) until 200 rpm in order to ensure the right visual check to confirm the dissolution of excipients.

Table 8 Final surrogate formulation in the SS tank (1010 kg)

Components addition	Quantity (grams)
WFI	891800 g ± 1 %
Sodium Chloride (weighted salt)	9000 g ± 1 %
Sodium Citrate Dihydrate (weighted salt)	7400 g ± 1 %
Polysorbate 80 stock solution	8200 g ± 1 %
WFI to rinse PS80 stock solution bottle	2100 g ± 1 %
WFI to be added to get the final weight	q.s.
Total surrogate formulation	1010000 g ± 1%

Table 9 Surrogate solution preparation

Parameter	Results	pCPP (Y/N)
Initial weight of pooling tank	0 (Tare)	No
Start of Surrogate solution preparation (date dd.mm.yyyy, time hh:mm)	09/02/22 08:51	No
Start of mixing in pooling tank (date dd.mm.yyyy, time hh:mm)	09/02/22 09:15	No
Mixing rate (RPM)	100 rpm	Yes
Quantity of sodium chloride to be compounded	8991,4 g [8910,00-9090,00]g	No
Quantity of sodium citrate dihydrate to be compounded	7399,5 g [7326,00-7474,00]g	No
Quantity of PS80 solution to be compounded	8200,5 g [8118,00-8282,00]g	No
Mixing rate (RPM) after excipients addition	100 – 200 rpm	Yes
End of Surrogate solution preparation (date dd.mm.yyyy, time hh:mm)	09/02/22 11:08	No
Final weight of pooling tank	1010,4 Kg [999,9 - 1020,1]Kg	Yes
Mixing temperature	20 °C (19-23)°C	Yes
End of mixing in pooling tank (date dd.mm.yyyy, time hh:mm)	09/02/22 11:08	No
Mixing time (from WFI addition)	1 h and 53 min	Yes

Once, the surrogate solution was confirmed, by visual check, appropriate and all excipients appeared dissolved, sample to test the osmolality was taken from the RTR343 tank.

Results of osmolarityis 355 mOsm/Kg which is inside the acceptance range (324-396 mOsm/kg), confirming the good preparation of the buffer.

Then, a mixing study was performed in order to preliminary characterize mixing time and speed of bulk solution, as requested by the client. The mixing speed and time will be then confirmed in the Engineering runs and following trials.

4.2.1.2 Mixing study

Determination of stirring speed

Stirring speed was determined to ensure an appropriate mixing pattern of the fluid at a minimum and a maximum stirrer velocity aiming at a visible movement of the fluid surface, as well as on a non-vortex-forming / non-splashing appearance to avoid potential negative impact on the solution.

As shown in Figure 3, four aliquots of 250L (up to 1000 L) of surrogate solution was transferred from the pooling tank (RTR343) to the storage tank (RTR344) placed in RP04 position in grade C solution preparation area (room 823). For each aliquot step, the minimum and maximum mixing velocity was then determined and are listed in Table 10.

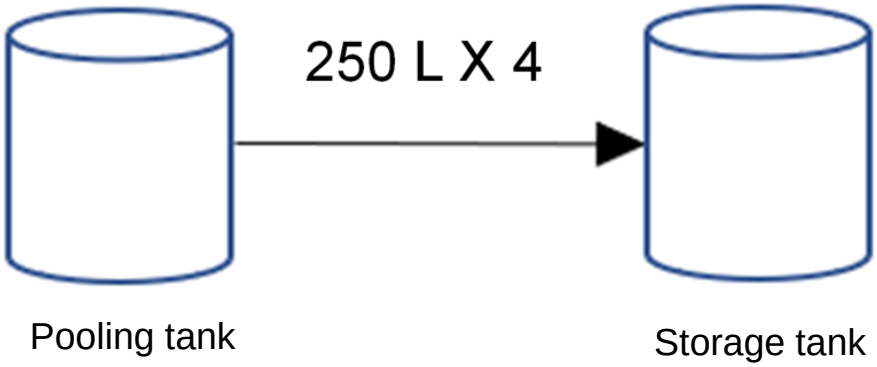


Figure 3: Stirring speed determination

Table 10 Determination of stirring speed

Parameter	Results	pCPP (Y/N)
First aliquot		
Initial weight of storage tank	0 [tare]	No
Weight of pooling tank after aliquoted transfer	757,6 Kg	No
Weight of storage tank after aliquoted transfer	252,4 Kg	Yes
Max mixing (RPM) in storage tank after every aliquot transfer	250 rpm	Yes
Visual check of no splasher/foaming at max mixing (RPM) in storage tank after every aliquot transfer	Conform	No
Min mixing (RPM) in storage tank after every aliquot transfer	100 rpm	Yes
Visual check of fluid movement at min mixing (RPM) in storage tank after every aliquot transfer	Conform	No
Second aliquot		
Initial weight of storage tank	252,4 Kg	No
Weight of pooling tank after aliquoted transfer	504,6 Kg	No
Weight of storage tank after aliquoted transfer	505,0 Kg	Yes
Max mixing (RPM) in storage tank after every aliquot transfer	250 rpm	Yes

Parameter	Results	pCPP (Y/N)
Visual check of no splasher/foaming at max mixing (RPM) in storage tank after every aliquot transfer	Conform	No
Min mixing (RPM) in storage tank after every aliquot transfer	100 rpm	Yes
Visual check of fluid movement at min mixing (RPM) in storage tank after every aliquot transfer	Conform	No
Third aliquot		
Initial weight of storage tank	505,0 Kg	No
Weight of pooling tank after aliquoted transfer	252,4 Kg	No
Weight of storage tank after aliquoted transfer	757,4 Kg	Yes
Max mixing (RPM) in storage tank after every aliquot transfer	250 rpm	Yes
Visual check of no splasher/foaming at max mixing (RPM) in storage tank after every aliquot transfer	Conform	No
Min mixing (RPM) in storage tank after every aliquot transfer	100 rpm	Yes
Visual check of fluid movement at min mixing (RPM) in storage tank after every aliquot transfer	Conform	No
Fourth aliquot		
Initial weight of storage tank	757,4 Kg	No
Weight of pooling tank after aliquoted transfer	0 Kg	No
Weight of storage tank after aliquoted transfer	1010,6 Kg	Yes
Max mixing (RPM) in storage tank after every aliquot transfer	250 rpm	Yes
Visual check of no splasher/foaming at max mixing (RPM) in storage tank after every aliquot transfer	Conform	No
Min mixing (RPM) in storage tank after every aliquot transfer	150 rpm	Yes
Visual check of fluid movement at min mixing (RPM) in storage tank after every aliquot transfer	Conform	No

In order to ensure the correct homogenization of the solution at the maximum batch size, it was experienced the need to increase the minimum mixing speed. Therefore, the identified range for the mixing speed is 150- 250 rpm.

Determination of mixing time

After determination of stirring speed, bulk solution homogeneity was verified by using the pre-determined stirring speed over an appropriate time interval to evaluate the appropriate mixing time.

A tracer solution was prepared for the purpose of this study (schematically shown in figure 4 below). In particular, NaCl tracer solution addition to surrogate solution (MabThera buffer) was used to evaluate the homogeneity in accordance with memo (“Avastin and Mabthera: Usage of surrogate for mixing trial” dated 10th December 2021) provided by Roche.
Conductivity test to perform a mixing study was performed.

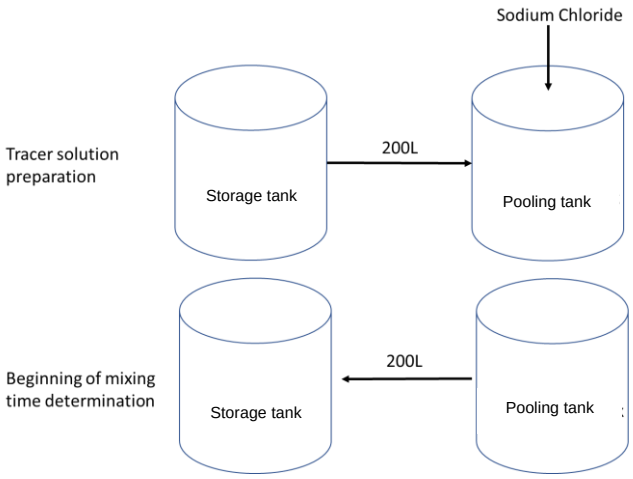


Figure 4 Preparation of tracer solution

200L (202, 0 Kg) of surrogate solution were transferred from the storage tank back to the pooling tank in order to prepare the tracer solution. 8762,9 g of sodium chloride (target 8766,0 g) were added to 202,0 Kg of surrogate solution in the pooling tank and mixed (at 100 rpm) until it will be not visible detectable in the solution. The quantity of sodium chloride was determined by Roche in order to have a conductivity of 750 mM in the tracer solution which is appropriate to generate significant measurable differences in conductivity values with respect to Mabthera buffer (conductivity = 150mM).
One sample to perform conductivity (as information only) was collected from the 808,2 Kg of solution remained in storage tank. The result of the sample (CT-500) is 19,703 mS/cm

Then the tracer solution (approximately 200L) was transferred back in the storage tank to mimic approximately the MabThera dilution ratio. Conductivity test to perform a mixing study was performed.

Samples were collected, before mixing (time point = 0 minutes), from the top, middle and bottom of the storage tank and tested for conductivity.

The solution was then mixed according to the minimum mixing speed determined during the previous one steps (determination of stirring speed for the fourth aliquot) which was equal to 150 rpm.

Samples from the top, middle, and bottom of the solution were collected at different time points (before mixing, 15 and 30 minutes) to test the conductivity. Additional samples were collected also at an additional time point (45 minutes) and these samples were however tested even if the previous sample test results were compliant. The samples were analysed using the instrument sist.521 at room temperature (20-23°C) with a measurement uncertainty equal to 1,22%.

Table 11 Results of conductivity -mixing trial

Timepoints	STEP	Result [mS/cm]
T0 (before mixing)	C-T0-T	30,35
	C-T0-T	30,75
	C-T0-T	29,48
	C-TO-M	32,61
	C-TO-M	32,34
	C-TO-M	31,91
	C-TO-B	34,05
	C-TO-B	34,79
	C-TO-B	34,35
T1 mixing after 15minutes	C-T1-T	32,86
	C-T1-T	32,70
	C-T1-T	32,62
	C-T1-M	32,49
	C-T1-M	32,61
	C-T1-M	32,78
	C-T1-B	32,78
	C-T1-B	32,93
	C-T1-B	33,07
T2 mixing after 30minutes	C-T2-T	32,70
	C-T2-T	32,68
	C-T2-T	32,53
	C-T2-M	32,97
	C-T2-M	32,51
	C-T2-M	32,63
	C-T2-B	32,12
	C-T2-B	32,81
	C-T2-B	32,34
T3 mixing after 45minutes	C-T3-T	32,48
	C-T3-T	32,52
	C-T3-T	32,56
	C-T3-M	32,11

Timepoints	STEP	Result [mS/cm]
	C-T3-M	32,22
	C-T3-M	32,26
	C-T3-B	32,86
	C-T3-B	32,57
	C-T3-B	32,47

Differently from what was reported in the protocol (acceptance criteria for conductivity = variability of conductivity between top, middle and bottom samples within 31.18 mS/cm ± 3%), it was discussed and agreed with the client that the values from the top, middle and bottom have to be within the mean value (calculated from the experimental data) ± 3% as acceptance criteria, in order to correctly assess the homogeneity. Indeed, the homogeneity of solution (top, middle and bottom) was the goal of the study, without referring to the target value of 31.18 mS/cm, that was originally provided by Roche as an indication based on experimental data obtained during the laboratory test.

In the table below are reported the results of the mean value ± 3% calculated for every time point.

Table 12 Mean +/-3% for every time point

	T0	T1	T2	T3
Mean [mS/cm]	32,29	32,76	32,59	32,45
Max [mS/cm]	33,26	33,74	33,57	33,42
Min [mS/cm]	31,32	31,78	31,61	31,48

At time zero (T0), results did not meet the acceptance criteria. However, this results is consistent with the fact that sampling at time 0 was performed before starting the mixing. The results of the test, as shown in Figure 5 below, suggest that even after 15 minutes of mixing (T1) at the minimum speed (150 rpm) of the solution at maximum batch size (1000 L), the homogeneity is guaranteed.

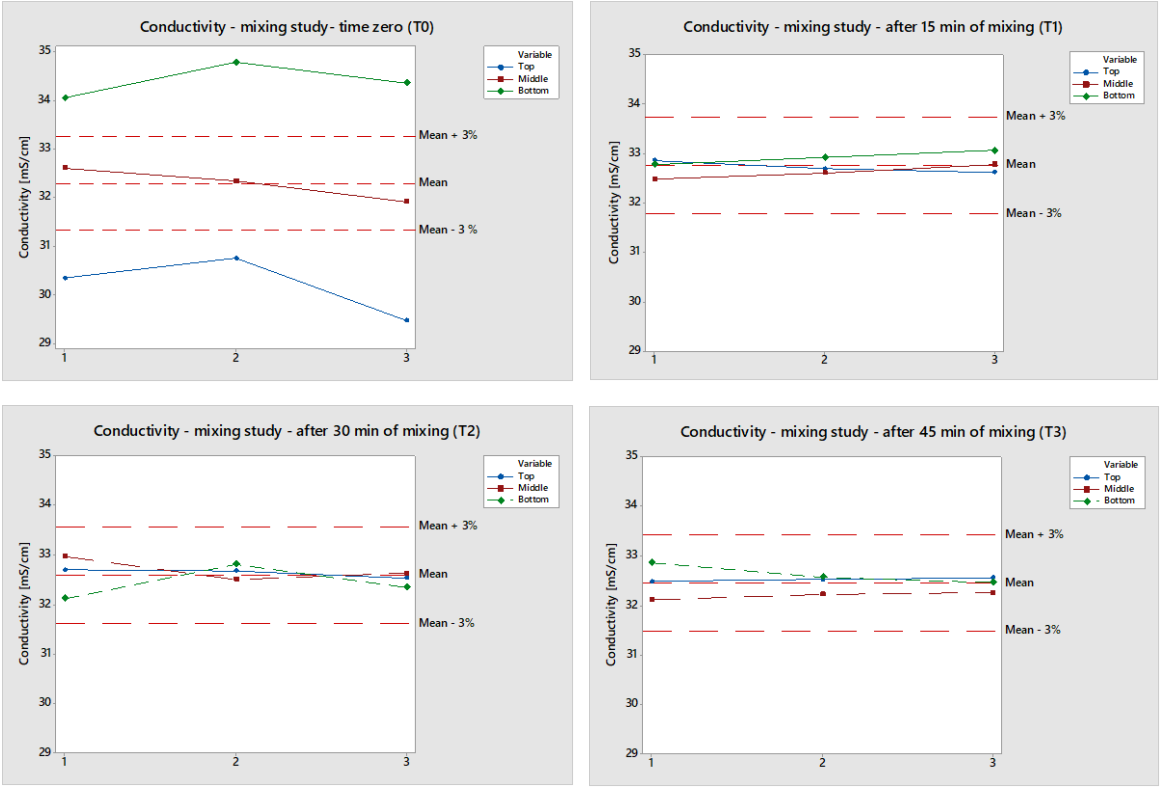


Figure 5 Results of mixing study

At the end of the activities in the solution preparation room, samples for subvisible and visible particles were also collected. These samples were needed to have a control value for the tubing stress fatigues check sampling at the beginning, middle and end of filling steps.

Results of visible and subvisible particles analysis are reported in Attachment 1. In particular, visible particles and subvisible particles of dimension ≥ 10µm and subvisible particles of dimension ≥ 25 µm meet the acceptance criteria, Subvisible particles ≥ 2µm and subvisible particles ≥ 5 µm were present but no action has to be taken considering that they had to be reported for information only without a threshold to fulfill.

The main parameters recorded during the mixing study are listed in table below.

Table 13 Determination of mixing time

Parameter	Results	pCPP (Y/N)
Initial weight storage tank after tracer solution addition	1018,0 Kg	No
Start of mixing (date dd.mm.yyyy, time hh:mm)	09/02/22 16:12 pm	No

Parameter	Results	pCPP (Y/N)
End of mixing (date dd.mm.yyyy, time hh:mm)	09/02/22 17:30 pm	No
Mixing time	15-45 minutes	Yes
Mixing rate (RPM) in storage tank	150 rpm	Yes
Sampling time points (time hh:mm)	16:27 pm 16:57 pm 17:30 pm	No

4.2.2 Part B

The assembly supplied in the solution preparation room for the installation was:
N°1 (one) assembly “Y” (273635) allows the connection of the SS tank to the extension tube. Considering that for this trial, a steam-thru connection was not available and it was replaced by a tri-clamp provided by Stain Gobain, the Y assembly was installed after SIP of tanks and then connected to the disposable assemblies. For the engineering and following trials, the Y will have the steam-thru and they will be installed before SIP of tanks and then connected to the disposable assemblies

The stainless-steel tank (RTR343) was placed in one position RP03 in grade C solution preparation area (room 823), under LAF.
Moreover, stainless steel J-tube was installed inside the tank before performing the CIP/SIP operations. It was used to limit the possible foam formation during the transfer of the surrogate solution into the tank.

4.2.2.1 Buffer preparation

In the part B Trial, the stainless-steel tank was placed in grade C solution preparation area (room 823), under LAF.

The setup in the solution preparation room is shown in Figure 6 below

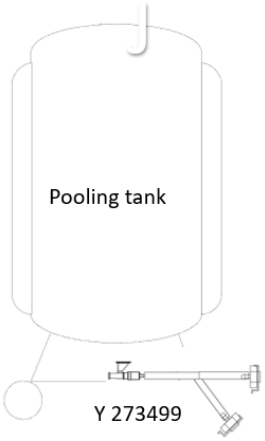


Figure 6 Assemblies setup in solution preparation room (part B)

To prepare the surrogate solution, the excipients were dispensed by dispensing department.
In dispensing, in grade D area under laminar airflow, according to specific Patheon SOP 0010, the following excipients were weighted and partitioned:

- Sodium chloride (weighted salt): target ± 0,5% = 9,000 Kg ± 0,045 Kg
- Sodium Citrate Dihydrate (weighted salt): target ± 0,5% = 7,400 Kg ± 0,037 Kg
- PS80: target ± 0,5% = 0,703 Kg ± 0,004 Kg

Then, the dispensing, according to Patheon SOP 0009, provided the excipients to the sterile area 6 department (room 823) where the surrogate solution was prepared.

Polysorbate 80 stock solution

Polysorbate 80 was prepared using a 10 L glass bottle, according to recipe reported in Table 6. The dispensing added 703 g in the glass bottle that was then delivered to sterile area 6 where WFI (703 g) was added to obtain the final concentration. The PS80 solution was mixed using a magnetic stirring bar, applying a stirring speed not causing splashes or foam of the fluid, until completely dissolved. Visual checks confirmed the homogeneity of PS80 solution.

Table 14 Recipe for PS80 stock solution – to be prepared in 10L glass bottle

Component	Quantity (grams)
PS80	703 g ± 0.5 %
WFI to be added to get final weight	q.s
Total PS80 stock solution formulation	8200 g ± 1%

The main parameters recorded during the preparation of the PS80 stock solution are listed in the table below.

Table 15 PS-80 Solution preparation

Parameter	Results	pCPP (Y/N)
Start of PS 80 solution preparation (date dd.mm.yyyy, time hh:mm)	11/02/22 08:57	No
PS 80 solution start of mixing (time hh:mm) and initial mixing speed	11/02/22 10:24 300 rpm	No
PS 80 solution mixing speed modification and start of mixing at the new mixing speed	11/02/22 10:39 400 rpm	No
PS 80 solution end of mixing (time hh:mm)	11/02/22 11:28	No
Mixing time	1 h and 4 minutes	No
End of PS 80 solution preparation (date dd.mm.yyyy, time hh:mm)	11/02/22 11:28	No

All the excipients were pooled in the stainless-steel tank for the compounding of the surrogate solution.

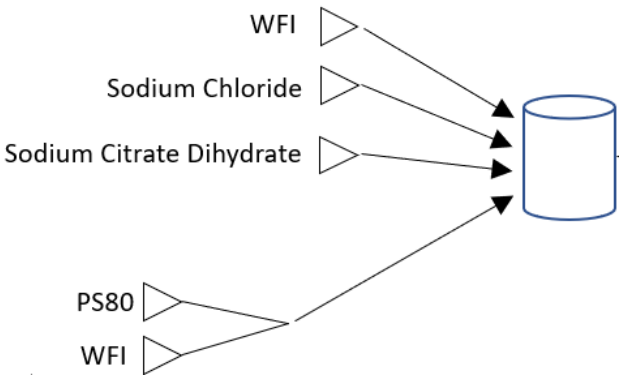


Figure 7 Surrogate preparation in stainless-steel pooling tank

Water for injection (WFI) and Nitrogen (N_2) were supplied by Patheon to the Sterile area 6 department through dedicated utilities.

After having filled the stainless-steel pooling tank with MabThera surrogate solution (1010,20 Kg), the solution was mixed for excipients dissolution and homogeneity. The main parameters recorded, during the preparation, are listed in Table 17 Surrogate solution preparation

Table 16 Final surrogate formulation in the SS tank (1010 kg)

Components addition	Quantity (grams)
WFI	891800 g ± 1 %
Sodium Chloride (weighted salt)	9000 g ± 1 %
Sodium Citrate Dihydrate (weighted salt)	7400 g ± 1 %
Polysorbate 80 stock solution	8200 g ± 1 %
WFI to rinse PS80 stock solution bottle	2100 g ± 1 %
WFI to be added to get the final weight	q.s.
Total surrogate formulation	1010000 g ± 1%

Table 17 Surrogate solution preparation

Parameter	Results	pCPP (Y/N)
Initial weight of pooling tank	0 (Tare)	No
Start of Surrogate solution preparation (date dd.mm.yyyy, time hh:mm)	11/02/22 09:36	No
Start of mixing in pooling tank (date dd.mm.yyyy, time hh:mm)	11/02/22 10:05	No
Mixing rate (RPM)	100 rpm	Yes
Quantity of sodium chloride to be compounded	8995,3 g	No
Quantity of sodium citrate dihydrate to be compounded	7398,5 g	No
Quantity of PS80 solution to be compounded	8200,4 g	No

Parameter	Results	pCPP (Y/N)
Mixing rate (RPM) after excipients addition	200 rpm	Yes
End of Surrogate solution preparation (date dd.mm.yyyy, time hh:mm)	11/02/22 12:42	No
Final weight of pooling tank	1010,20 Kg	Yes
Mixing temperature	20°C (19-23°C)	Yes
Mixing time	2 h and 37 minutes	Yes
End of mixing in pooling tank (date dd.mm.yyyy, time hh:mm)	11/02/22 12:42	Yes

Once, the surrogate solution was confirmed, by visual check, appropriate and all excipients appeared dissolved, samples to test the osmolality, visible and subvisible particles were taken.

Result of osmolarity is 355 mOsm/Kg which is inside the acceptance range (324-396 mOsm/kg), confirming the good preparation of the buffer. Results of visible and subvisible particles analysis , are reported in attachment 2. In particular, visible particles and subvisible particles of dimension ≥ 10µm and subvisible particles of dimension ≥ 25 µm meet the acceptance criteria, Subvisible particles ≥ 2µm and subvisible particles ≥ 5 µm were present but no action has to be taken considering that they had to be reported only for information, without a threshold to fulfill.

4.3 PREPARATION OF PACKAGING COMPONENTS

Primary Packaging components were provided to the Sterile Department by Dispensing department according to the Bill of Materials and were treated as described below.

Glass vials

Vials for the surrogate trial were supplied and manufactured by Schott and have the following characteristics: 42.5 mm and 50 mL (PTH Code 241584) for the 500 mg/vial dosage (part A) and 22 mm and 10 mL (PTH Code 241582) for the 100 mg/vial (part B). The same vials will be used for the manufacturing of MabThera 500 mg/vial and 100 mg/vial respectively.
The vials were brought to the 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 carrousel where 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). After exiting the tunnel, the vials passed through an opening to the filling machine.

Stoppers

Stoppers were supplied by Daikyo Seiko in (4000 stoppers/bag), ready to sterilize by autoclave (PTH code: 273438). Stoppers will be used for MabThera manufacturing of both 100mg/vial and 500mg/vial. Stoppers were loaded in the filling machine through the rapid transfer port (RTP) port of the isolator. Considering that no microbiological analysis was foreseen during the manufacturing of the two trials, stoppers were not sterilized.

Seals/Caps

Seals were applied to final product vials using a Machine ALU400 SA5017 (CPL012). The Seals were supplied by Datwayler (6500 pcs/bag). Seals with a red cap (PTH Code: 273433) were used for manufacturing of 100 mg/vial dosage and seals with a grey cap were used for 500 mg/vial dosage (PTH Code: 273434). They are supplied in a ready-to-use bag and are not pre-treated by the vendor and Patheon prior to the crimping operations.

4.4 OPERATIONS IN FILLING ROOM

Objectives:

- Simulate the set-up operations
- Tubing fatigue evaluation during filling
- Evaluate the suitability of the disposable assemblies in terms of dimensions and connections
- Ensure filling machine format parts are installed and adjusted appropriately
- Assess the appropriate filling parameters for the manufacturing of 500mg/mL (Part A) and 100mg/mL (Part B) dosages
- On the field training of manufacturing personnel

The assemblies that were supplied in the filling room for the installation are:

- N°1 (one) extension tube (273502) is a tube that has sterile connections and was used to connect the Y tube of the storage tank to the assembly for sterilizing filtration (273504).
- N°1 (one) assembly for sterilizing filtration (273504) that incorporates two (2) filters KVGLG10HH1 0.22µm, allowing the solution to be sterilized through redundant filtration. The assembly's inlet was sterile connected to item 273502 and its outlet was sterile connected to the filling bag. This assembly was placed on a stainless-steel trolley for support purposes.
- N°1 (one) filling bag (273510 for part A) that was installed inside the isolator of the filling machine through the RTP port. Its inlet was sterile connected to the sterilizing assembly, while its outlet had 10 filling needles.

- N°1 (one) filling bag (273508 for part B) that was installed inside the isolator of the filling machine through the RTP port. Its inlet was sterile connected to the sterilizing assembly, while its outlet had 10 filling needles.

The tank was then moved from the solution preparation room to the filling room, close to the filling machine, through an electric transpallet.
The setup of the assemblies in the filling room is shown in the figure below: (a) for the 500mg/vial dose strength and (b) for the 100mg/vial dose strength below.

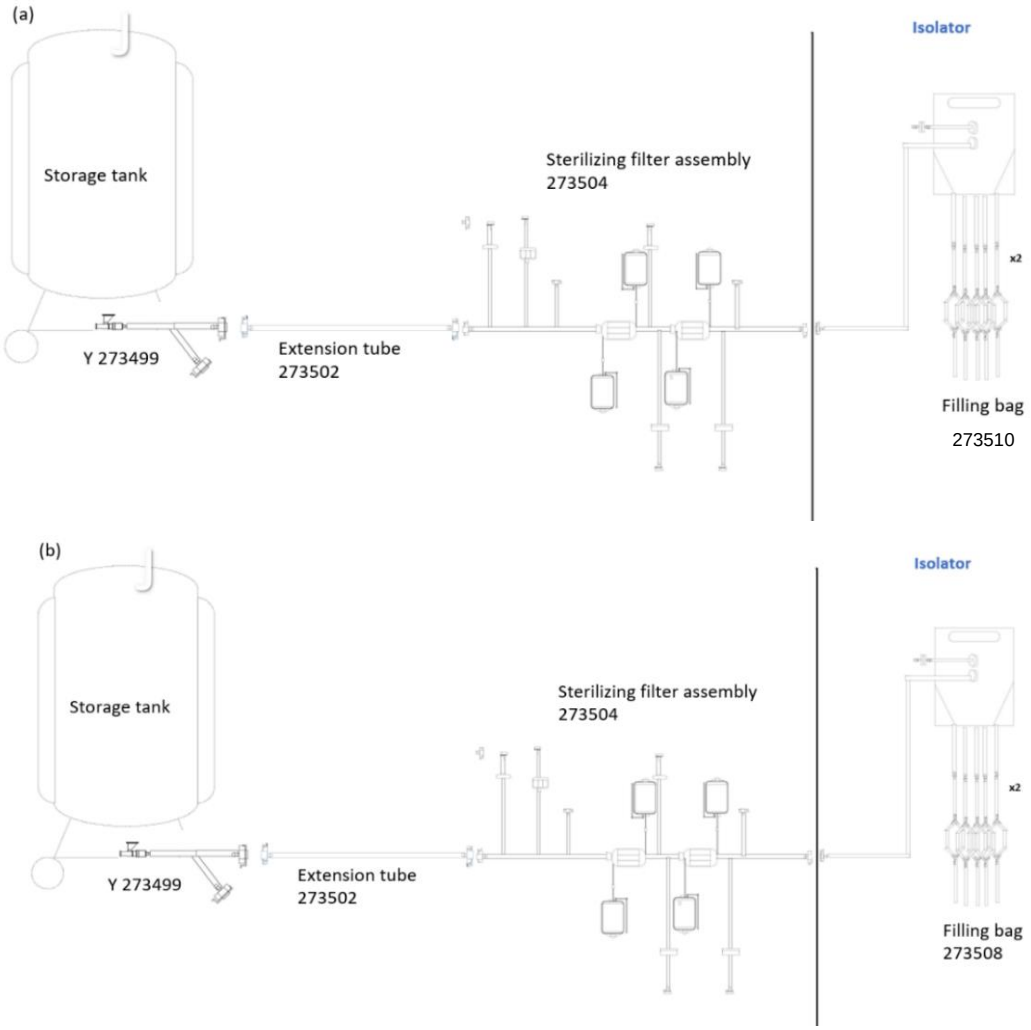


Figure 8 Set up in filling room. (a) for dose strength 500mg/vial and (b) for dose strength for 100mg/vial

4.4.1 Simulation 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 used for the filter integrity test and related parameters were the following ones:

- bubble point value at 3450 mbar (value at 15-25 °C for KVGLG10HH1 filter, as per Millipore recommendation);
- maximum pressure: 7000 mbar
- gas used: nitrogen

4.4.1.1 Part A

In Table 18 and in Table 19 are reported the results of bubble point test performed on the two sterilizing filters, before the use, during the surrogate trial part A.

Table 18 First sterilization filter - Integrity test results -pre use

Filter function	First sterilization filter
Filter code	KVGLG10HH1
Filter serial number	C1NB69132Z0057
Type of integrity test	Bubble point
Wetting agent	WFI
Gas agent	Nitrogen
Minimum bubble point	3450 mbar
Bubble point measured before use	4000 mbar

Table 19 Second sterilization filter - Integrity test results- pre use

Filter function	First sterilization filter
Filter code	KVGLG10HH1
Filter serial number	C1NB69132Z0060
Type of integrity test	Bubble point
Wetting agent	WFI
Gas agent	Nitrogen
Minimum bubble point	3450 mbar
Bubble point measured before use	3750 mbar

The first test on the sterilizing filters was interrupted to ensure the safety of the operators because the barrier did not let the gas pass (details about investigation are reported in dedicate paragraph 5.0) . Considering that no microbiological analysis was in the scope of the trial, the tubes upstream the barrier were cut and the bubble point test on sterilizing filters re-done. The sterilizing filters resulted conforming (bubble point measured ≥minimum bubble point as per datasheet).

4.4.1.2 Part B

InTable 20below are reported the results of bubble point test performed on the two sterilizing filters, before the use, during the surrogate trial part B.

Table 20 First sterilization filter - Integrity test results -pre use

Filter function	First sterilization filter
Filter code	KVGLG10HH1
Filter serial number	C1NB69132Z0072
Type of integrity test	Bubble point
Wetting agent	WFI
Gas agent	Nitrogen
Minimum bubble point	3450 mbar
Bubble point measured before use	**3750 mbar**

Table 21 Second sterilization filter - Integrity test results- pre use

Filter function	First sterilization filter
Filter code	KVGLG10HH1
Filter serial number	C1NB69132Z0064
Type of integrity test	Bubble point
Wetting agent	WFI
Gas agent	Nitrogen
Minimum bubble point	3450 mbar
Bubble point measured before use	**4050 mbar**

The first test on the sterilizing filters was interrupted to ensure the safety of the operators because the barrier did not let the gas pass (details about investigation are reported in dedicate paragraph 5.0). Considering that no microbiological analysis was in the scope of the trial, the tubes upstream the barrier were cut and the bubble point test on sterilizing filters re-done. The sterilizing filters resulted conforming (bubble point measured ≥minimum bubble point as per datasheet).

4.4.2 Simulation of Post-use Filter Integrity test

After the filtration, nevertheless the microbiological integrity was not in scope of the present protocol, the filter integrity test (FIT) of filters included in the disposable assembly 273504 were performed offline in order to train operators to handle it.

The following steps were performed for each filter:

- Disconnection of the filter from the disposable assembly 273504
- Flush of the filter with wetting agent (water, IPA or IPA/water according to the filters datasheet)
- 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.

4.4.2.1 Part A

The parameters documented after the use during the manufacturing of the trial A are reported in table below.

Table 22: Parameters documented on the sterilizing filters

Parameter	Criteria	Results	pCPP
Post-Use Sterilizing Filter Integrity test (Filter I)	Conform ≥3450 mbar	3450 mbar	Yes
Post-use Sterilizing Filters Integrity test (Filter II)	Conform ≥3450 mbar	3450 mbar	Yes
Barrier Filters Integrity test (Barrier I)	Conform ≥1280 mbar	1400 mbar	No
Barrier Filters Integrity test (Barrier II)	Conform ≥1280 mbar	1400 mbar	No
WFI filter Integrity test	Conform ≥3320 mbar	3700 mbar	No
Nitrogen filters Integrity test (Nitrogen I)	Conform ≥1170 mbar	1500 mbar	No
Nitrogen filters Integrity test (Nitrogen II)	Conform ≥1170 mbar	1600 mbar	No

Additionally, also the vent filter KEGBG050HH10 of the filling bag was tested after the use. The result was conforming (bubble point equal to 1650 mbar).

4.4.2.2 Part B

The parameters documented after the use during the manufacturing of the trial B are reported in table below.

Table 23: Parameters documented on the sterilizing filters

Parameter	Criteria	Results	pCPP
Post-Use Sterilizing Filter Integrity test (Filter I)	Conform ≥3450 mbar	3600 mbar	Yes
Post-use Sterilizing Filters Integrity test (Filter II)	Conform ≥3450 mbar	3600 mbar	Yes
Barrier Filters Integrity test (Barrier I)	Conform ≥1280 mbar	1500 mbar	No
Barrier Filters Integrity test (Barrier II)	Conform ≥1280 mbar	1500 mbar	No
WFI filter Integrity test	Conform ≥3320 mbar	3700 mbar	No
Nitrogen filters Integrity test (Nitrogen I)	Conform ≥1170 mbar	Not -conforming	No
Nitrogen filters Integrity test (Nitrogen II)	Conform ≥1170 mbar	Not-conforming	No

The post use test of the two nitrogen filters of the assembly resulted non-conforming which will be exploited in the dedicated paragraph 5.0

4.4.3 Sterilizing filtration and filling operations

4.4.3.1 Part A

The filling operation was performed using the **Xtrema SL1082** filling/stoppering machine (INF021). Inside the isolator, the filling bag was connected to a weight cell that controls the gate valve placed on the disposable assembly connected to the bottom of the storage tank. This system allows the filling bag to be automatically refilled during the whole filtration step and so the filtration is intermittent. The thresholds for the automatical refilling of the filling bag are: minimum as the 20% of the nominal working volume and maximum as the 80% of the nominal working volume.
During the installation of the recipe, it was highlighted that considering the volume of the 50 mL vial with respect to the mechanical setup of the filling machine, for the filling of the 50mL only 6 peristaltic pump can be used to fill the vials. The four additional needles present on the filling bag were then pinched. Three scales are used by the machine for this format.

The storage tank was pressurized with nitrogen to guarantee the transfer of surrogate solution from the tank to the filling machine, through the filter assembly.

In particular, during trial part A, the storage tank was pressurized with a nominal pressure 0.7 barg (with a range ±0.1 barg). During the trial, it was observed the presence of foam inside the filling bag. In order to minimize the foam, the suggestion of the technician supplier of the machine was to decrease the pressure in the storage tank in order to let the solution enter in the filling bag with less pressure. Therefore, this action was implemented during surrogate trial B.

At the beginning of the filling operation, the filling recipe was identified based on the fill weight data reported in Table below. The target fill volume of MabThera Drug Product 500mg/vial dosage strength in 50 mL glass vial is 51,109 mL, corresponding to a target fill weight of 51,620 g considering a MabThera surrogate solution density of 1.01g/mL.

Table 24: Fill Volume and Fill Weight limits

Dosage	Limits	Fill volume (ml)	Fill weight (g)	Accuracy (%)
500 mg/vial	Upper Action Limit	52,099	52,620	102%
	Upper Alert Limit	51,366	51,880	100.5%
	Target	**51,109**	**51,620**	**100%**
	Lower Alert Limit	50,851	51,360	99,5%
	Lower Action Limit	50,396	50,900	99%
	Density of surrogate solution = 1,01 g/mL at room temperature			

During the filling, some scraps were observed due to tare weight (weight of the empty vials). Indeed, on the machine, the vials are weighted empty (before the filling) in order to acquire the net weight. After the filling station, the vials are then re-weighted to have the gross weight and the software calculates the net. If the weight of the tare (empty vials) is out of range, the vials are rejected without having the solution filled in them. Since the tare limits were not set appropriately at the beginning of the trial, some adjustments were then needed. To adjust these limits, the recipe has to be closed and re-open with some stops of the machine.

During the filling, 100% fill-dose IPC was automatically executed and stops of the line was recorded, giving a representative understanding of the format parts fitting, assembly suitability and general operations, the main purpose of the trial.
The filling machine was operated at maximum filling speed (100 vials/min) that was identified at the beginning of filling operations and set for the rest of the trial.

Stoppers were manually loaded into the stopper bowl of the Xtrema SL1082 (INF021). Stoppers are then conveyed through a vibrating system to a stainless-steel rail and placed on top of the filled vials for the stoppering. 100% automatic controls are in place to detect stopper presence on vials.

Finally, the parameters documented during the sterilizing filtration and filling operations are listed in the Table 25 below.

Table 25: Parameters documented during sterilizing filtration and filling Operations

Parameter	Results	pCPP
Filtration ΔP on filters	ΔP ≤ 1 barg	Yes
Filtration time	≤15 hours	Yes
Temperature during filtration and filling	20,7°C < T < 21,5°C	Yes
Max Filling machine speed	100 vials/min	Yes
Start of filling (date dd.mm.yyyy, hh:mm)	10/02/22 15:02 pm	NO
End of filling (date dd.mm.yyyy, hh:mm)	11/02/22 01:40 am	NO
Pressure value in storage tank during filling	0,7± 0,1 barg	NO
IPC vials weight	Record – see data below	Yes

For the surrogate exercise, the following vials were manufactured during the filling of the 500 mg/50mL dosage

Table 26 Scrap analysis

Cause of scraps	Good	Scrap
Vials for purge	0	30
Vials for calibration I	139	41
Vials for calibration II	26	34
Vials of production – not filled	0	144
Vials of production - filled	16828	142
End of productions	0	127

A deeper analysis was performed on the two following aspects:

- vials of production – not filled

Table 27 Scrap analysis - vials of production -not filled

Cause of scrap	Qty
Filling interrupted	20
Error in scale 1	1
Error in scale 2	1
Error in scale 3	1
Tare weight out of range scale 1	40
Tare weight out of range scale 2	34
Tare weight out of range scale 3	47
Total	**144**

- vials of production - filled

Table 28 Scrap analysis - vials of production - filled

Cause of scrap	Qty
Net out of limit, scale 1, needle 1	22
Net out of limit, scale 1, needle 4	10
Net out of limit scale 2, needle 2	22
Net out of limit scale 2, needle 5	11
Net out of limit scale 3, needle 3	22
Net out of limit scale 3, needle 6	12
Missing stopper	42
good scrapped	1
Total	**142**

Considering the results of Table 28, the scrap is 1% whereas the good is 99% of the produced vials.

In the following graphs are reported the trend of the 100% filling weights IPC during production mode (excluded purge, calibration and end of production mode) divided per every needles.

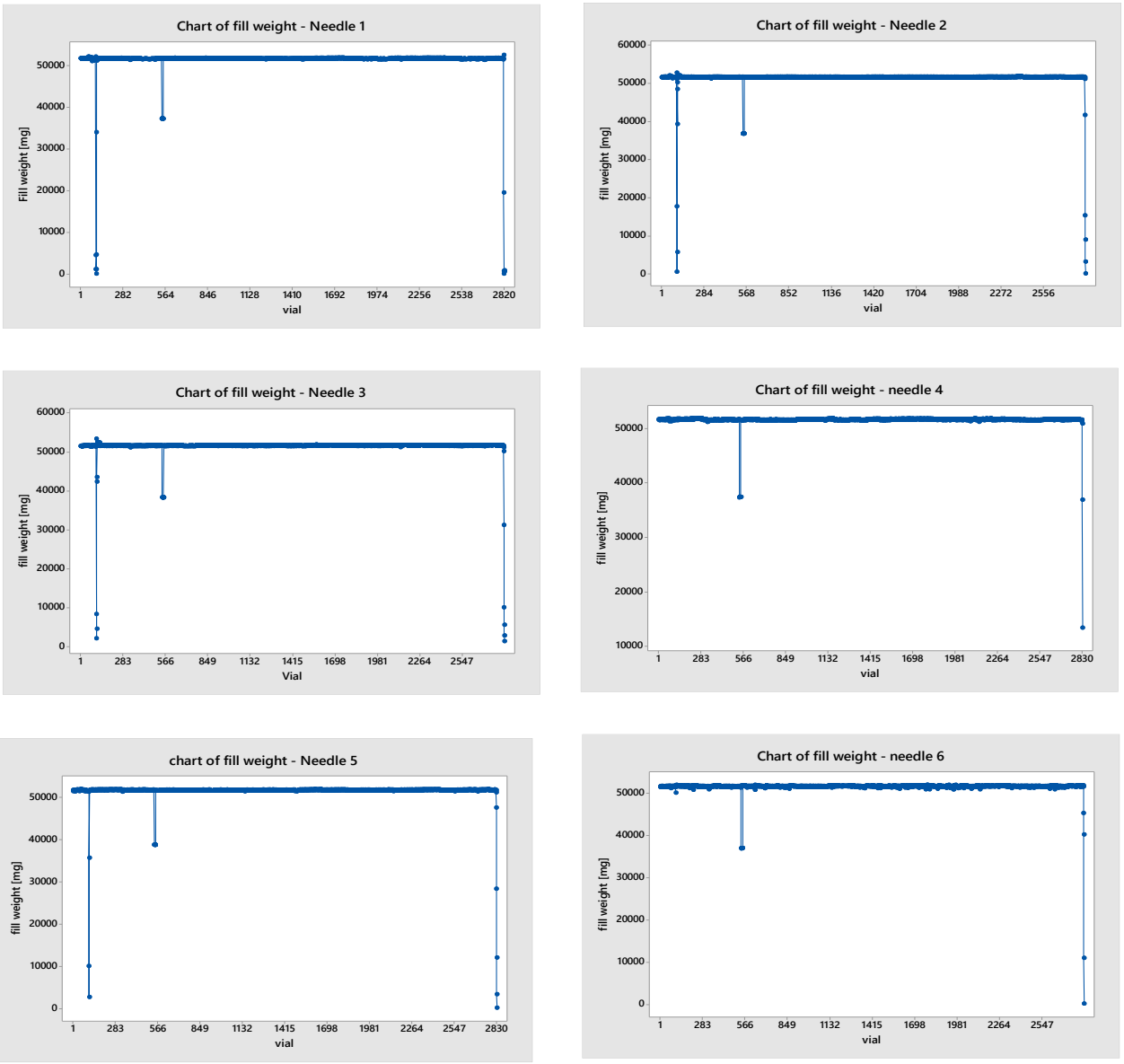


Figure 9 Trend of 100% filling weight IPC for every needle

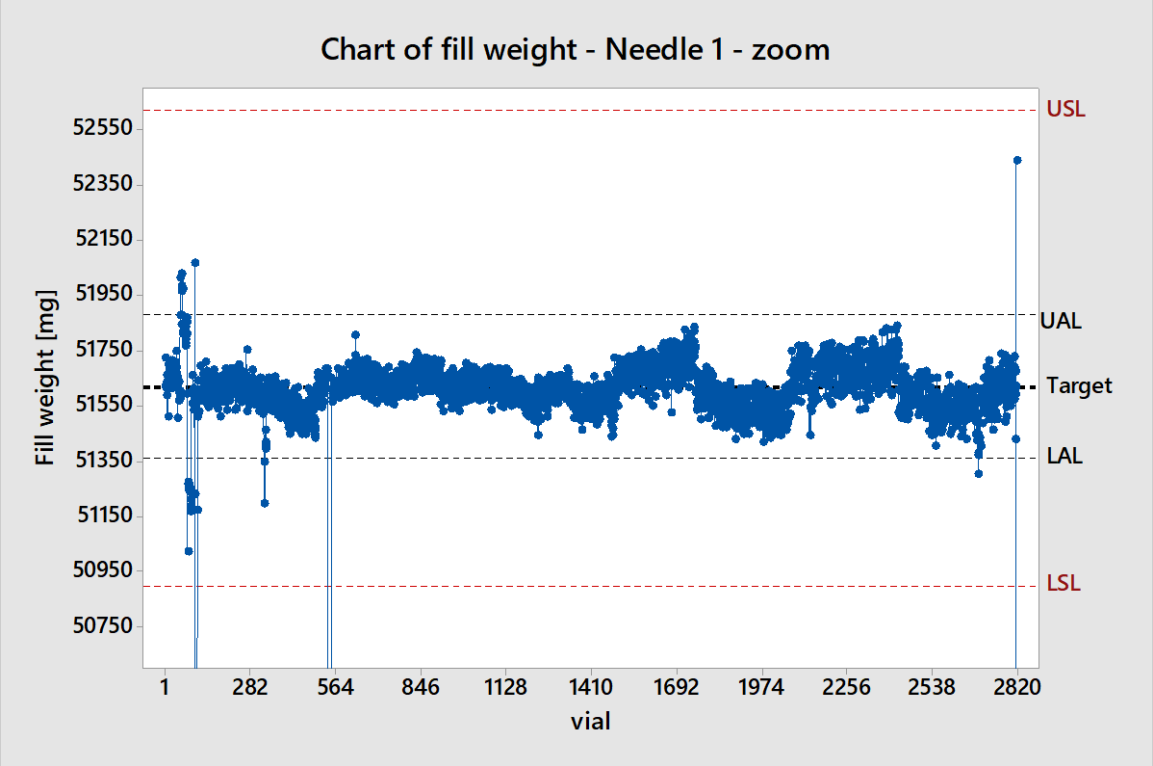
From the trend of the filling weights for each needle, some outliers can be identified approximately at the same filling cycle for every needle. Three points of discontinuity were identified and was performed an analysis to understand if they can be linked to the specific conditions that are not representative of the production.

The first point is on 10/02/22 starting from around 16:36 and the audit trail was inspected. A lot of stoppage of the line was present and it is compatible with the fact that at this point, the set up of the recipe was ongoing by the IMA technician and so they can be excluded from the analysis.

The second point is on 10/01/22 starting from around 18:17 (points around #566 in the graph). These are the first vials filled after the stoppage and set of the new recipe with the adjusted limits

for the tare. At the start after stoppage, this type of effect can happen and so they can be excluded from the analysis.
The third point is on 11/02/22 starting from 01:39, just before the end of production. Also these values can be considered outliers and are not compatible with the specific issue due to the filling step and so they can be excluded from the analysis. Indeed, end of production mode started immediately at 1:40 11/02/22.

Considering the info listed above, the trend of the filling weights for every needle was zoomed in order to have a detailed picture of the trend.



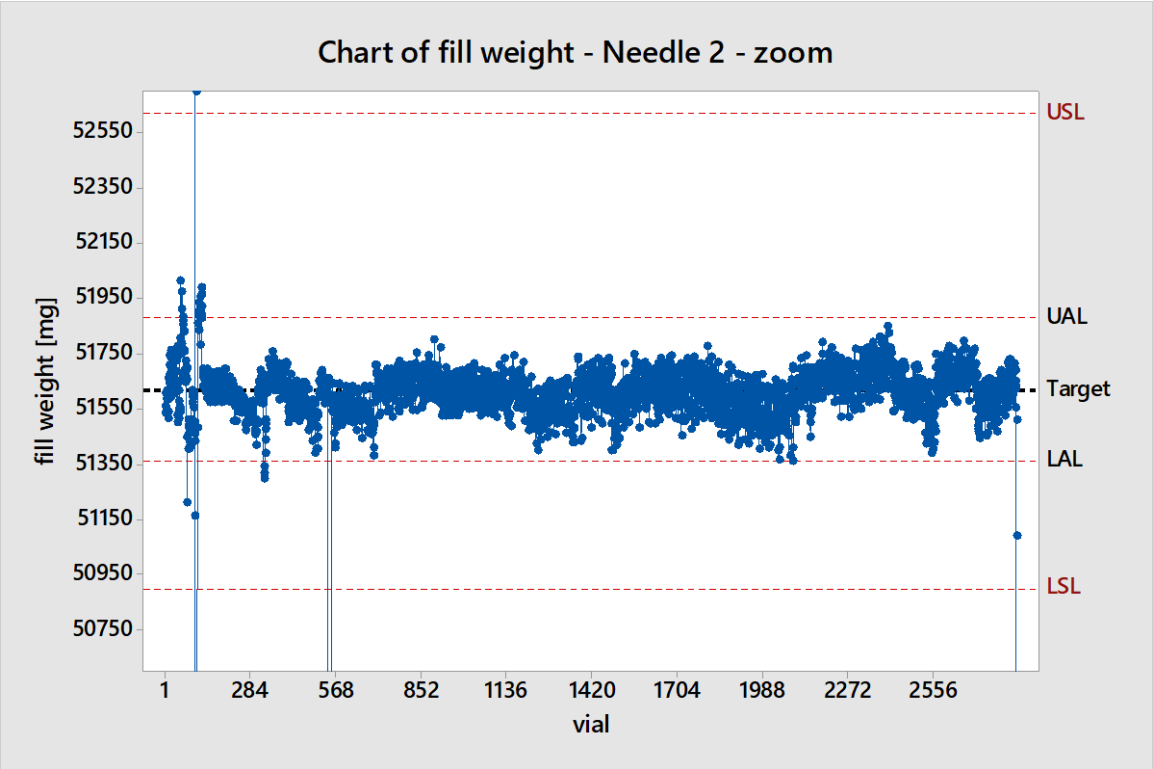


Chart of fill weight - Needle 2 - zoom
fill weight [mg]
52550
52350
52150
51950
51750
51550
51350
51150
50950
50750
USL
UAL
Target
LAL
LSL
1
284
568
852
1136
1420
1704
1988
2272
2556
vial

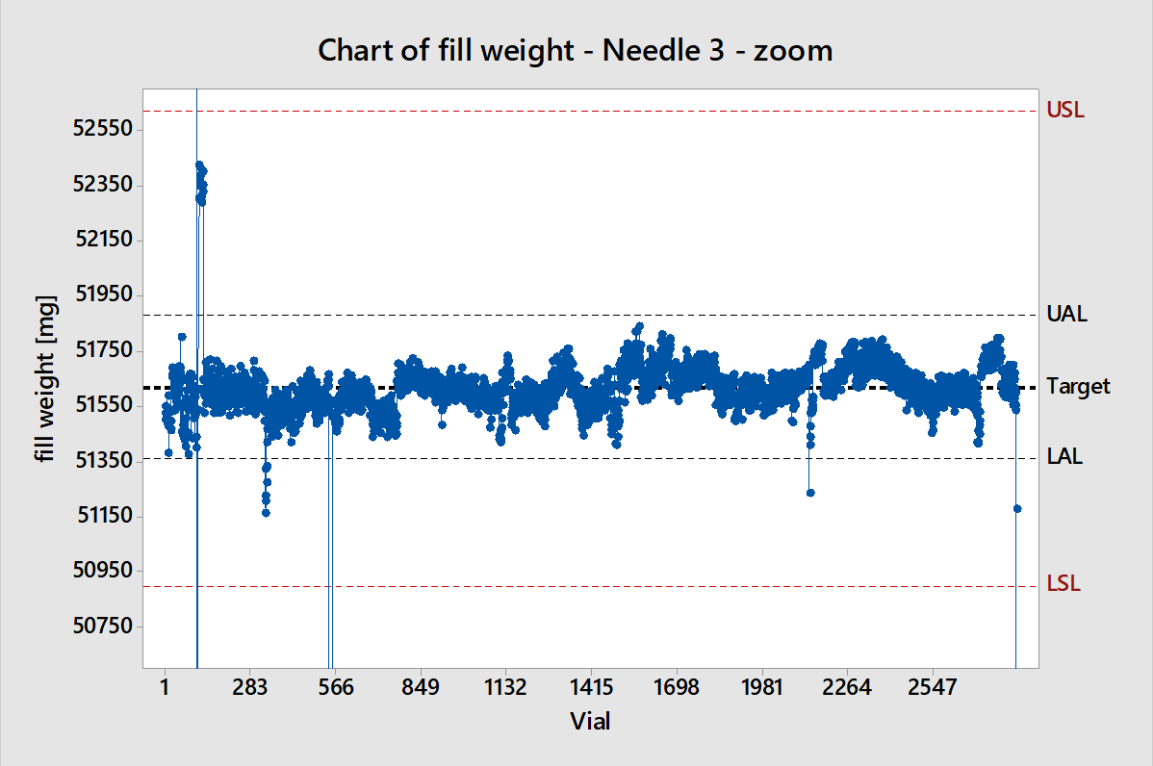


Chart of fill weight - Needle 3 - zoom
fill weight [mg]
52550
52350
52150
51950
51750
51550
51350
51150
50950
50750
USL
UAL
Target
LAL
LSL
1
283
566
849
1132
1415
1698
1981
2264
2547
Vial

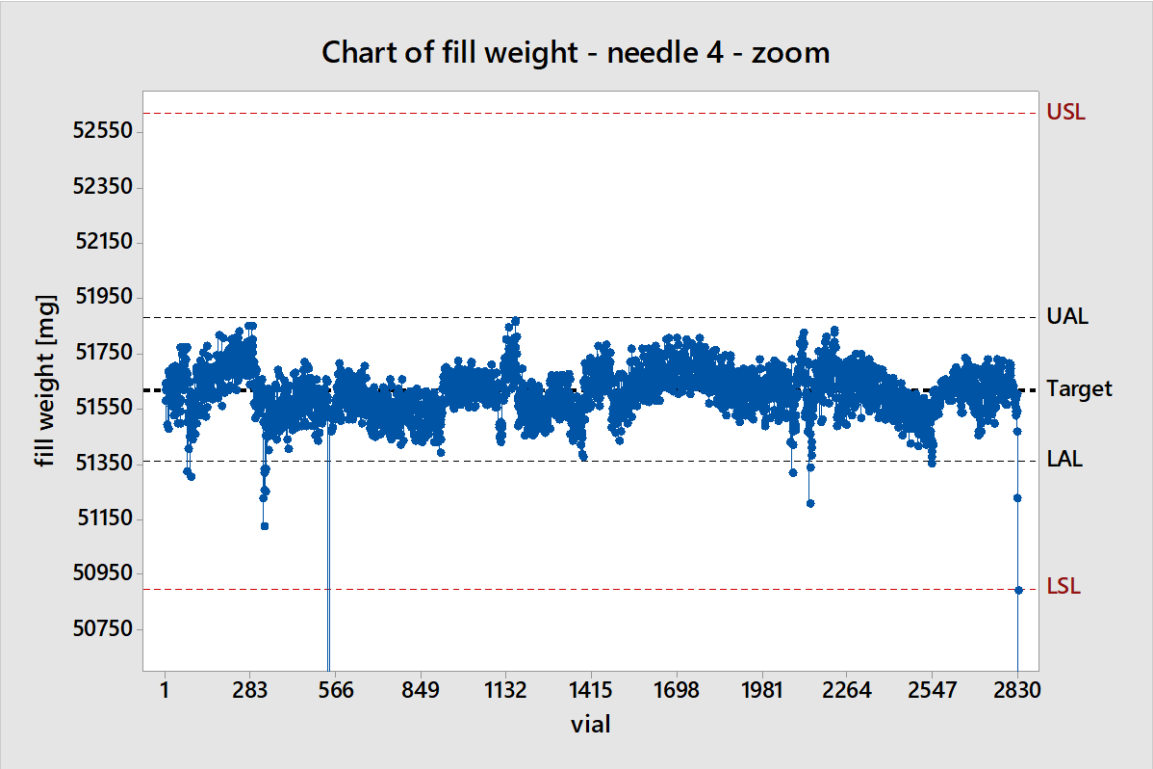


Chart of fill weight - needle 4 - zoom
fill weight [mg]
52550
52350
52150
51950
51750
51550
51350
51150
50950
50750
USL
UAL
Target
LAL
LSL
1
283
566
849
1132
1415
1698
1981
2264
2547
2830
vial

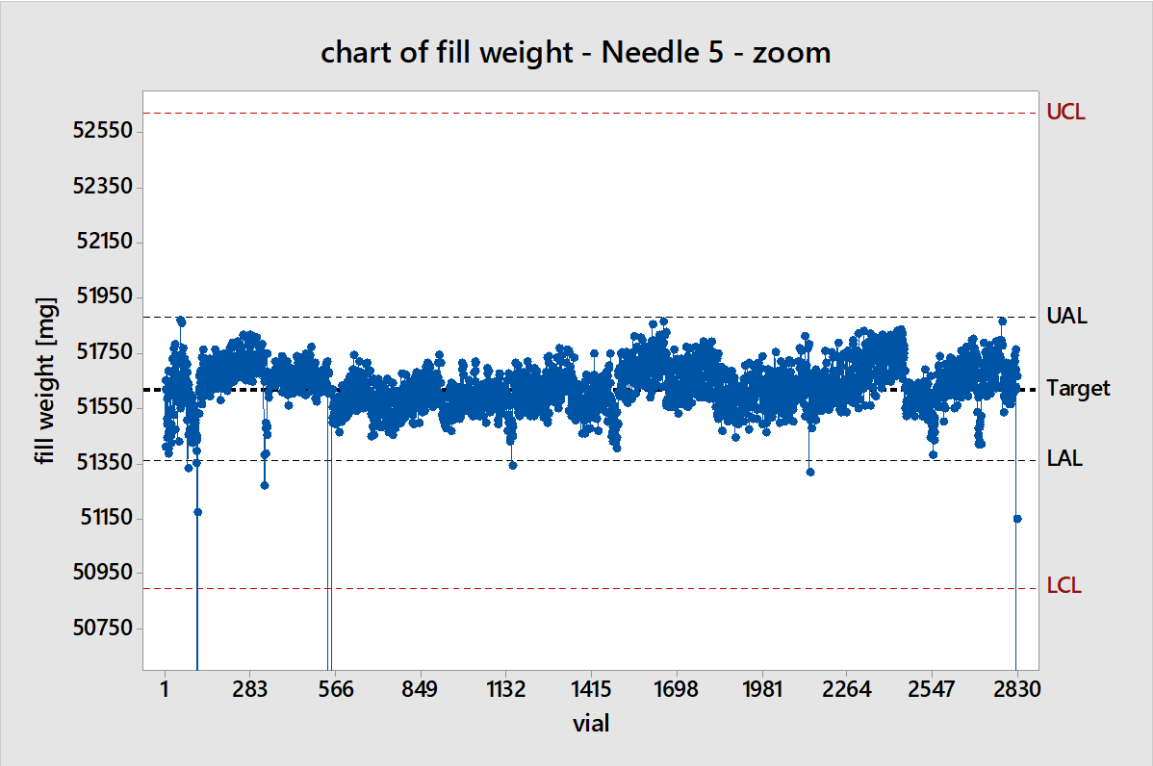


chart of fill weight - Needle 5 - zoom
fill weight [mg]
52550
52350
52150
51950
51750
51550
51350
51150
50950
50750
UCL
UAL
Target
LAL
LCL
1
283
566
849
1132
1415
1698
1981
2264
2547
2830
vial

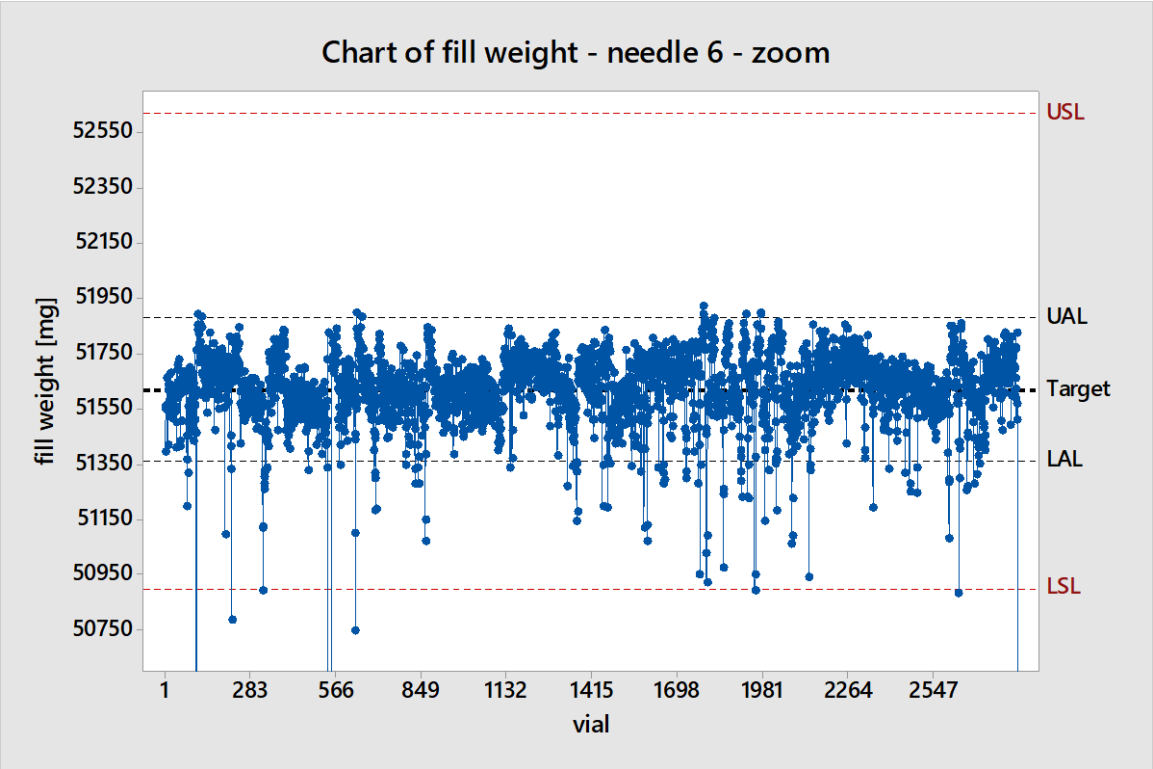


Figure 10 Trend of 100% filling weight IPC for every needle - zoom

As shown in the previous graphs, the fill weight IPCs during the filling operation were close to the desired target value.

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 11 below.

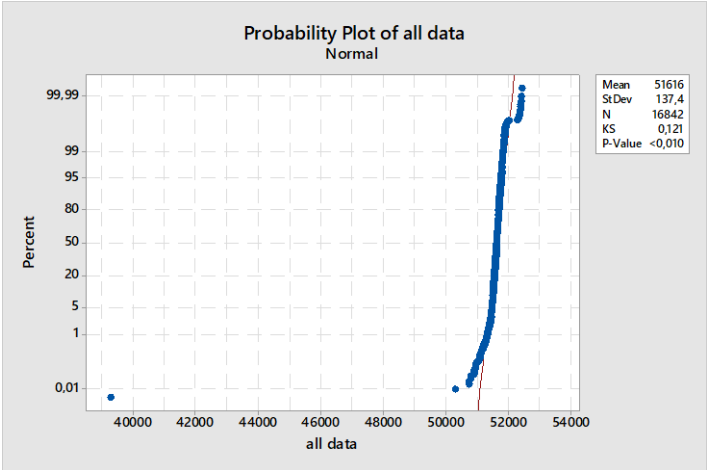


Figure 11 Normality test

As shown in Figure 11 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.

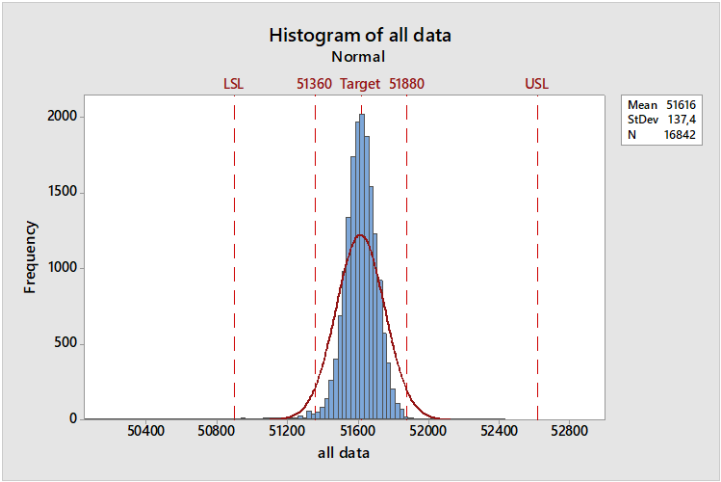


Figure 12 Histrogram

Figure 13 below shows the filling process capability. The data is not collected in subgroups because the sampling was 100% IPC (subgroup size = 1).

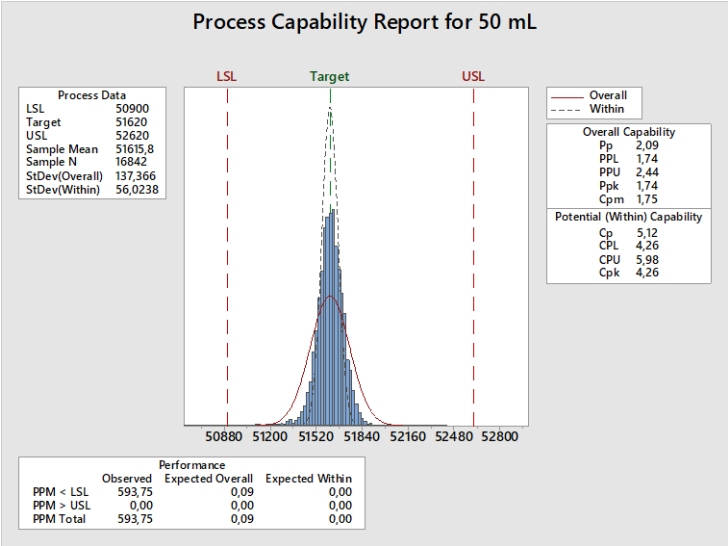


Figure 13 Process capability

As shown in figure 13, the fill weight IPCs during the filling operation showed the process in controlled status and capable considering a P_{pk} = 1.74 and C_{pk} =4,26.

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 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.

4.4.3.2 PART B

The filling operation was performed using the **Xtrema SL1082** filling/stoppering machine (INF021). Inside the isolator, the filling bag was connected to a weight cell that controls the gate valve placed on the disposable assembly connected to the bottom of the storage tank. This system allows the filling bag to be automatically refilled during the whole filtration step and so the filtration is intermittent. The thresholds for the automatical refilling of the filling bag are: minimum as the 20% of the nominal working volume and maximum as the 80% of the nominal working volume.
For the 10 mL format, 8 peristaltic pumps of Xtrema filling machine were used to fill the vials. The two additional needles present on the filling bag were then pinched. For these formats 6 scale of the machine were used.

The storage tank was pressurized with nitrogen to guarantee the transfer of surrogate solution from the tank to the filling machine, through the filter assembly.
In particular, during trial part B, the storage tank was supposed to be pressurized at 0.9 barg (with a range ± 0.1 barg to be able to challenge the maximum pressure (equal to 1 barg)) to let to challenge the maximum value admissible with respect to ΔP max allowable on sterilizing filters. However, considering the information acquired during trial A, , to minimize the foam creation in the filling bag, it was decided to set the pressure in the storage tank at 0,3 barg for the first 2/3 of the trail (crimping configuration A and B), then it was increased to 0,4 barg (during crimping configuration C) and finally to 0,5 barg (during crimping configuration D). A reduction of the foam with respect to trial A was observed.

At the beginning of the filling operation, the filling recipe was identified based on the fill weight data reported in Table 29..
The target fill volume of MabThera Drug Product 100mg/vial dosage strength in 10 mL glass vial is 10.525 mL, corresponding to a target fill weight of 10.630 g considering a MabThera surrogate solution density of 1.01g/mL.

Table 29: Fill Volume and Fill Weight limits

Dosage	Limits	Fill volume (ml)	Fill weight (g)	Accuracy (%)
100 mg/vial	Upper Action Limit	10,921	11,030	104%
	Upper Alert Limit	10,634	10,740	101%
	Target	**10,525**	**10,630**	**100 %**
	Lower Alert Limit	10,416	10,520	99%
	Lower Action Limit	10,297	10,400	98%

During these steps, 100% fill-dose IPC was automatically executed and stops of the line were recorded, giving a representative understanding of the format parts fitting, assembly suitability and general operations, the main purpose of the trial.

The filling machine was set at maximum filling speed (350 vials/min). In order to minimize the foam creation during the filling, the speed was decreased to 180 vials/min which was set for the rest of the trial. After this adjustment, the foam creation was significantly reduced.

Stoppers were manually loaded into the stopper bowl of the Xtrema SL1082 (INF021). Stoppers were then conveyed through a vibrating system to a stainless-steel rail and placed on top of the filled vials for the stoppering. 100% automatic controls are in place to detect stopper presence on vials

Finally, the parameters documented during the sterilizing filtration and filling operations are listed in table below.

Table 30: Parameters documented during sterilizing filtration and filling Operations

Parameter	Results	pCPP
Filtration ΔP on filters	Record ΔP ≤ 1 barg	Yes
Filtration time	< 10 h	Yes
Temperature during filtration and filling	19°C < T < 21°C	Yes
Max Filling machine speed	350 vials/min, decreased to 180 vials/min to minimize foam cration	Yes
Start of filling (date dd.mm.yyyy, hh:mm)	14/02/22 12:29	NO
End of filling (date dd.mm.yyyy, hh:mm)	14/02/22 21:53	NO
Pressure value in storage tank during filling	[0,3 -0,5] barg	NO
IPC vials weight	Record – see data below	Yes

For the surrogate exercise, the following vials were manufactured during the filling of the 100 mg/10mL dosage

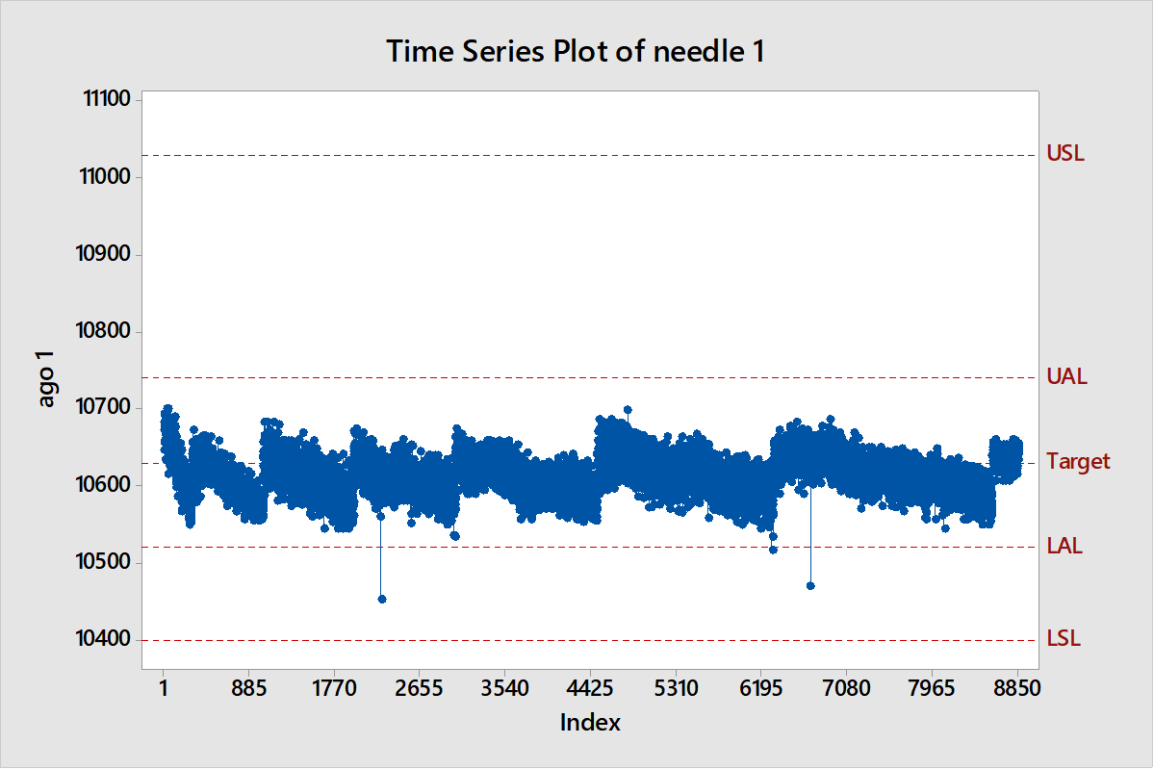
Table 31 Results of scrap

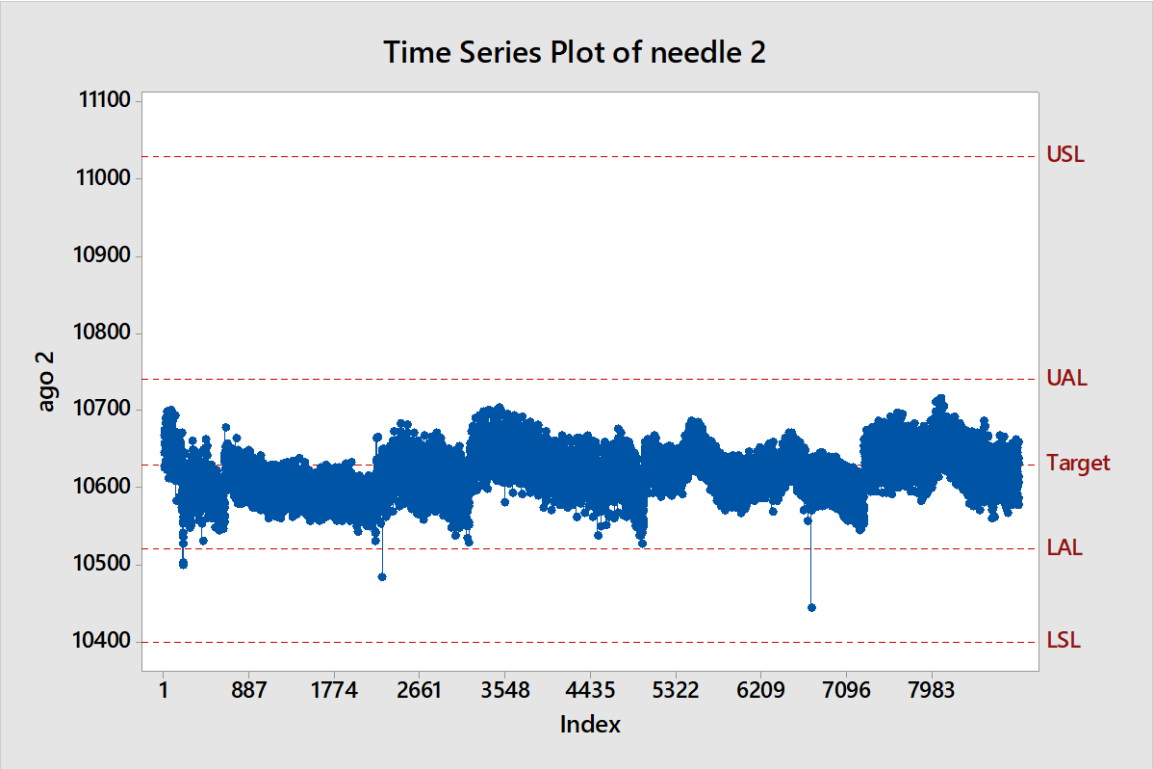
Cause of scraps	Good	Scrap
Vials for purge	0	80
Vials for calibration I	145	95
Vials of production – not filled	0	36
Vials of production - filled	70905	14

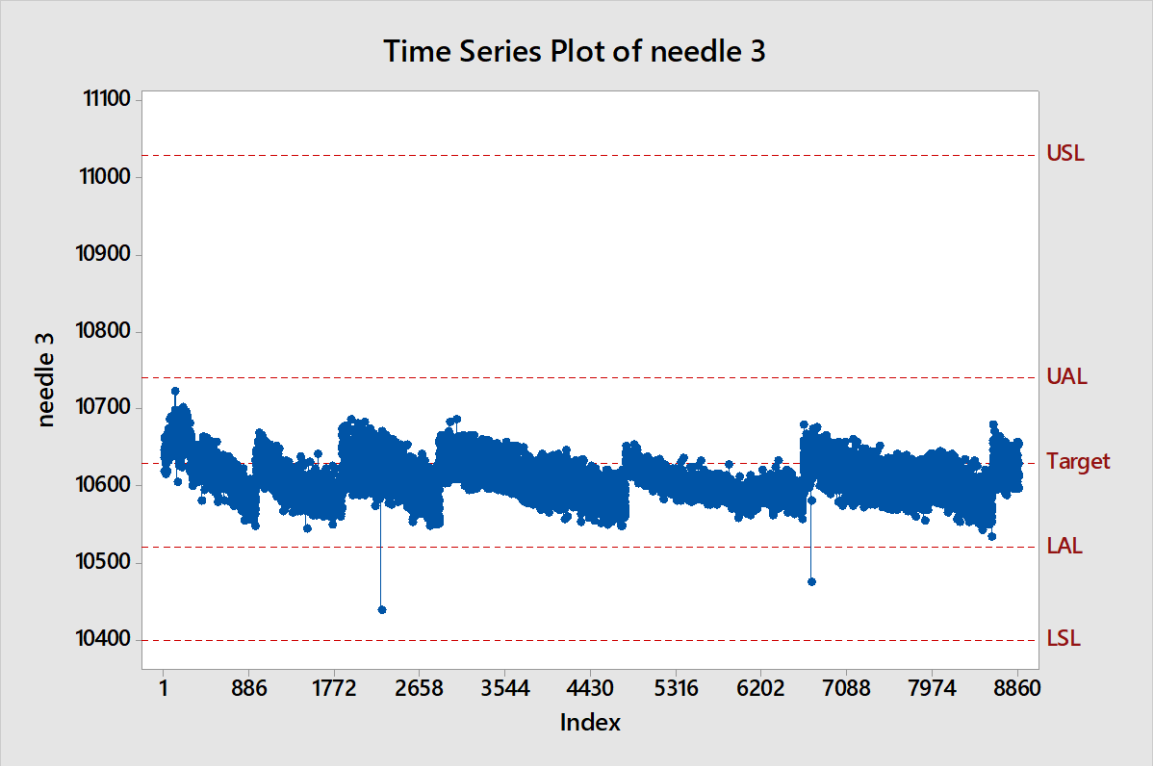
All the scrap related to vials of production – not filled are related to filling interrupted whereas for the vials of production – filled a deeper analysis was performed. 13 scarps can be related to missing stoppers whereas 1 scrap can be related to weight out of tolerance.

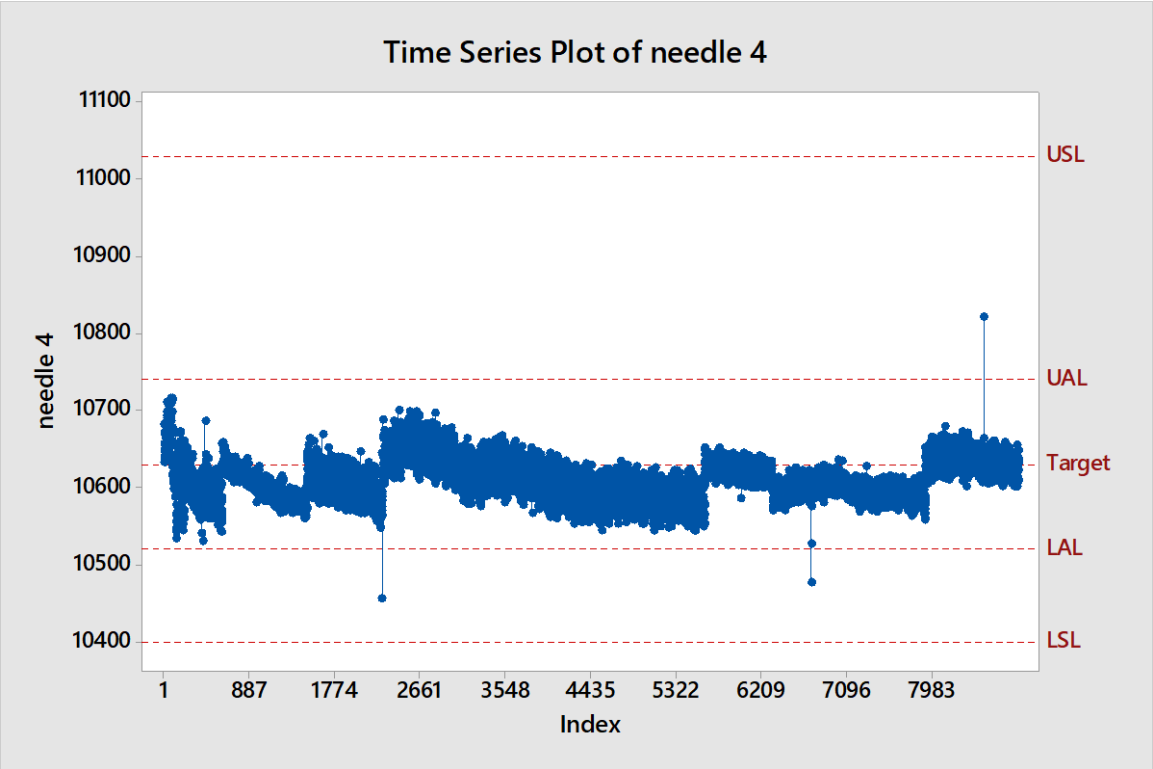
Considering the vials of production -filled, the scrap is 0,07% whereas the good is around 99,93%.

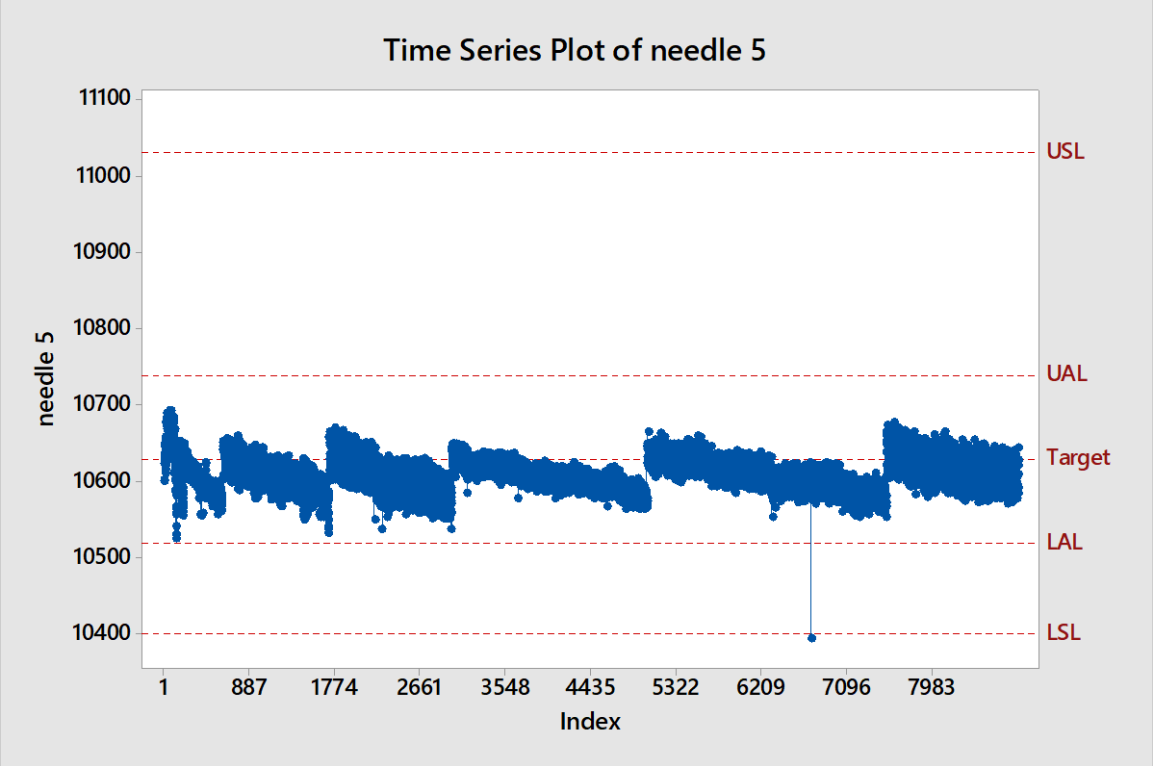
In the following graphs are reported the trend of the 100% filling weights IPC during production mode (excluded purge and calibration).

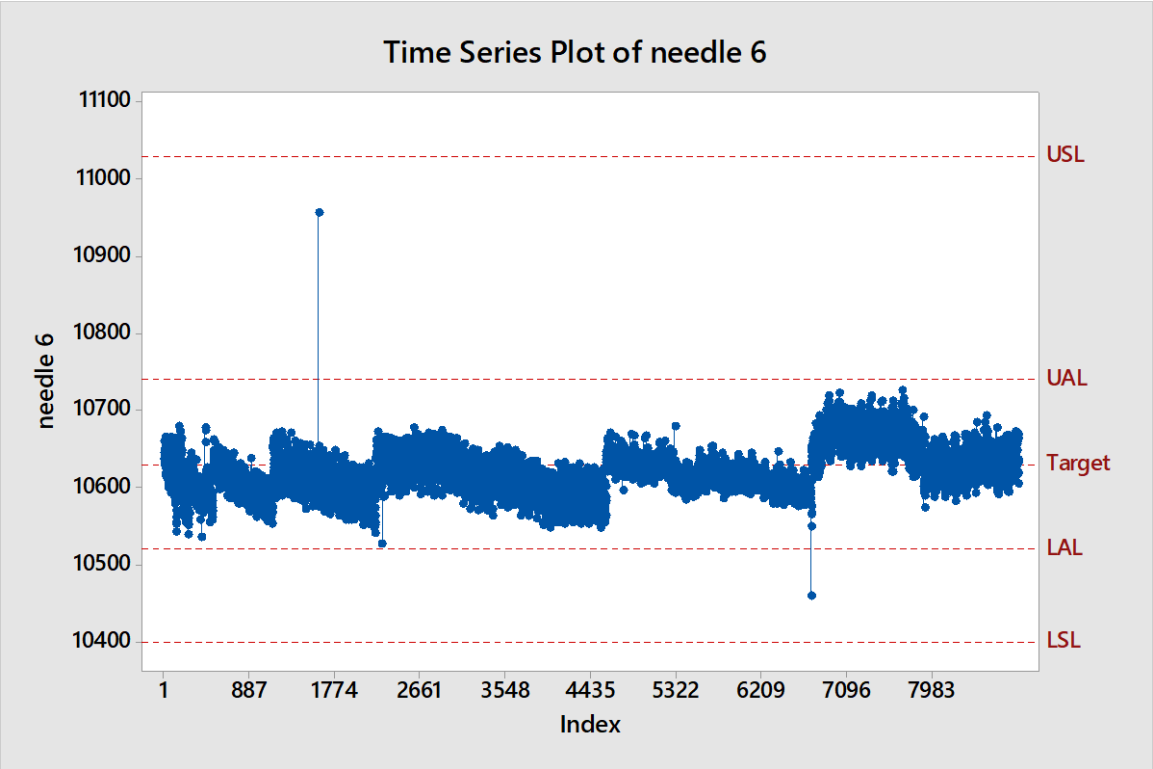


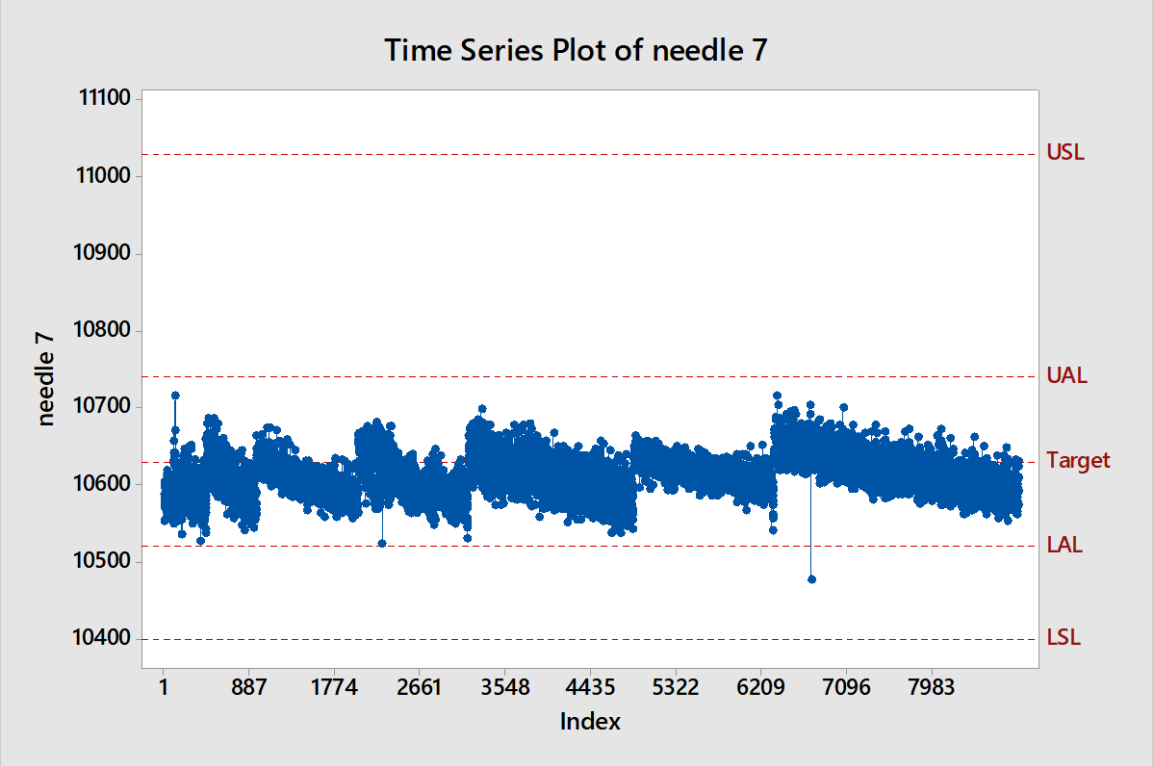

Time Series Plot of needle 2
ago 2
11100
11000
10900
10800
10700
10600
10500
10400
USL
UAL
Target
LAL
LSL
1
887
1774
2661
3548
4435
5322
6209
7096
7983
Index


Time Series Plot of needle 3
needle 3
11100
11000
10900
10800
10700
10600
10500
10400
USL
UAL
Target
LAL
LSL
1
886
1772
2658
3544
4430
5316
6202
7088
7974
8860
Index


Time Series Plot of needle 4
needle 4
11100
11000
10900
10800
10700
10600
10500
10400
USL
UAL
Target
LAL
LSL
1
887
1774
2661
3548
4435
5322
6209
7096
7983
Index


Time Series Plot of needle 5
needle 5
11100
11000
10900
10800
10700
10600
10500
10400
USL
UAL
Target
LAL
LSL
1
887
1774
2661
3548
4435
5322
6209
7096
7983
Index


Time Series Plot of needle 6
needle 6
11100
11000
10900
10800
10700
10600
10500
10400
USL
UAL
Target
LAL
LSL
1
887
1774
2661
3548
4435
5322
6209
7096
7983
Index


Time Series Plot of needle 7
needle 7
11100
11000
10900
10800
10700
10600
10500
10400
USL
UAL
Target
LAL
LSL
1
887
1774
2661
3548
4435
5322
6209
7096
7983
Index

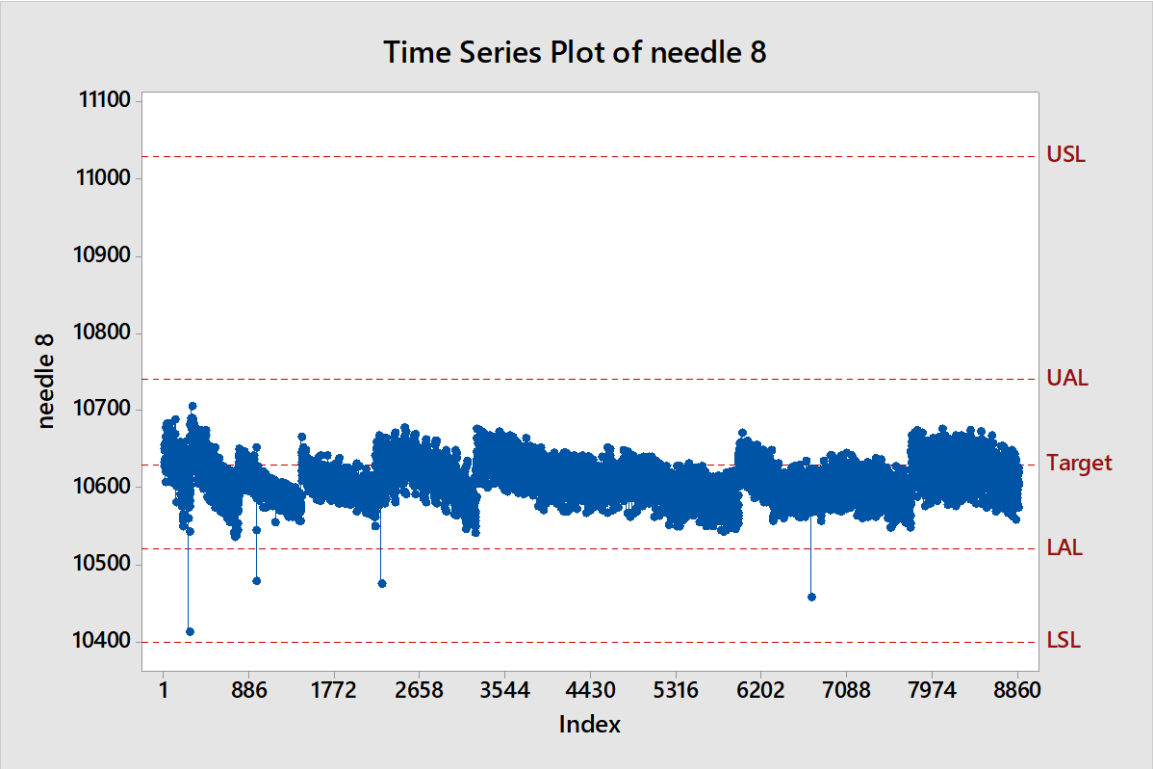


Figure 14 Trend of 100% filling weight IPC for every needle

From the trend of the filling weights for each needle, no outliers can be identified. Moreover, only one scrap is present (needle 5).

The trend for every needle is comparable and is due to the working of the filling machine. The machine checks and calculate the mean every three consecutive points and if the mean is greater than 50% of the range between the target and the alert limit, the machine performs the auto-adjust of the filling in order to be centered at the target value.

Finally, the filling performance of the machine in terms of filling weights was evaluated 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 the figures below.

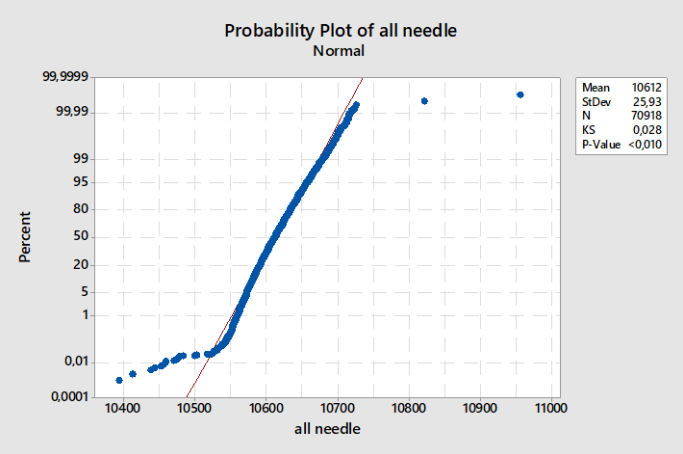


Figure 15 Normality test

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.

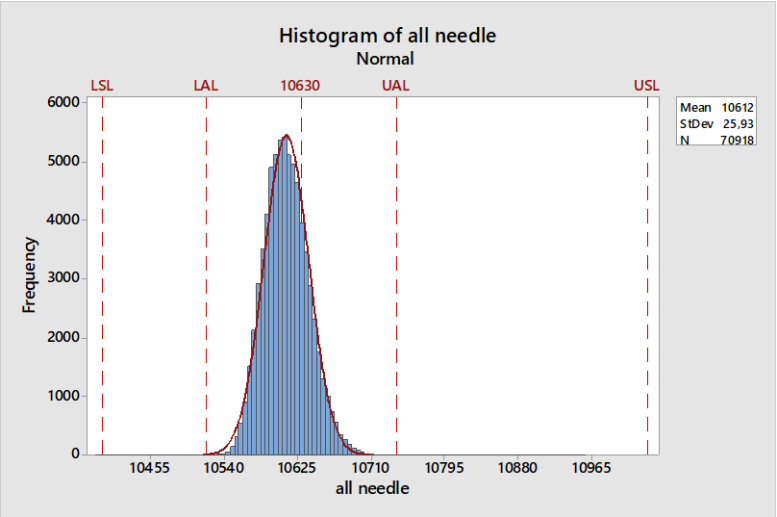


Figure 16 Histogram

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

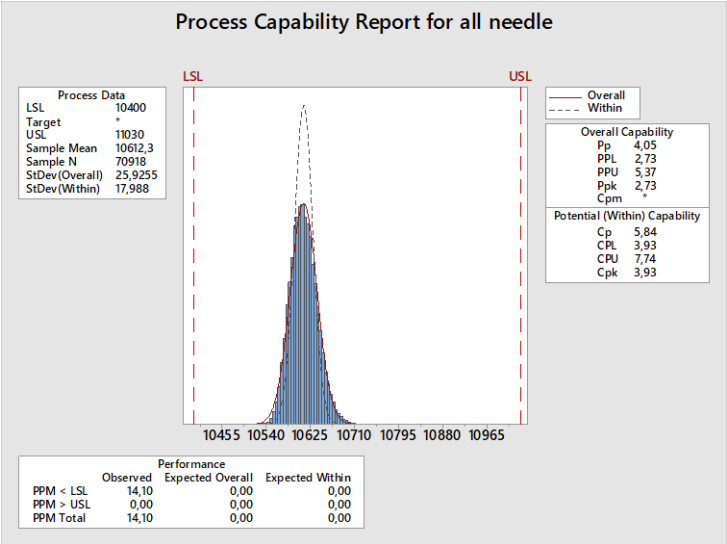


Figure 17 Process capability

As shown in figure 17, the fill weight IPCs during the filling operation showed the process in controlled status and capable considering a P_{pk} = 2,73 and C_{pk} =3,93.

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 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

4.5 CRIMPING

Objective:

- Evaluate machinability of vials and crimping operation
- Define machine parameters and operative conditions

Crimping operations were executed after vials were stoppered and using the ALU400 SA3122 (CPL012) capping machine, under unidirectional grade A airflow supply within a grade D environment.

The aluminum/plastic seals were manually fed into the crimping machine during capping operations. Seals were applied by the automatic plunger head at the crimping station through the use of a constant pressure applied toward the edge of the glass.

A range of values regarding the height of the plunger head and the pressure of the crimping machine was identified during the surrogate trials.

The crimping machine was operated at a speed identified at the beginning of the operations.

The minimum and maximum height of plunger head values for the crimping machine and the minimum and maximum pressure for the crimping head was identified during both of the trials and a total of 4 configurations was challenged in each trial (50 mL vials for part A and 10 mL vials for part B), as reported in the Table below.

Table 32: Crimping configuration during Crimping Operations – Mabthera 50 mL

	Min Pressure = 90kPa	Max Pressure = 120 KPa
Min crimping height (plunger head) = 67,5 mm	Configuration A	Configuration C
Max crimping height (plunger head) = 68,5 mm	Configuration B	Configuration D

The target value for the pressure is 100 kPa and the target value for the plunger head is 68mm.

Table 33 Crimping configuration during Crimping Operations – Mabthera 10 mL

	Min Pressure = 80 kPa	Max Pressure = 100 KPa
Min crimping height (plunger head) = 46 mm	Configuration A	Configuration C
Max crimping height (plunger head) = 47 mm	Configuration B	Configuration D

The target value for the pressure is 90 kPa and the target value for the plunger head is 46.5 mm.

Crimped vials were then collected in the same boxes (labelled) in which the vials were first supplied.

The Container Closure Integrity test was performed on samples representative of the trial population of primary components tested using the blue dye CCI test method (according to SOP 1043).

For MabThera 500 mg/vial (part A), 200 crimped vials (and 15 for contingency) was collected for each crimping configuration to be tested for CCIT. 200 vials out of 200 tested resulted compliant (no sign of leakage visible).

For MabThera 100 mg/vial (part B) 315 crimped vials (and 15 for contingency) were collected for each crimping configuration to be tested for CCIT. 315 vials out of 315 tested resulted compliant (no sign of leakage visible).

Moreover, additional 200 vials (and 15 vials for contingency) per part A trial and 315 vials (and 15 vials for contingency) per part B trial was collected from each crimping configuration and went manually visual inspected (SOP 1930) to identify macro defects and cosmetic defects produced during filling and crimping operations (1.0 4.6).

Also, additional 3 vials + 3 vials for contingency (in part A) and additional 12 vials + 12 vials for contingency (in part B) were collected during the surrogate trials (sampling at time point = beginning, middle and end of filling). These samples were tested for subvisible particles to evaluate tubing stress fatigue, as per Roche request and must be compliant with:
Particles ≥22µm per container: report
Particles ≥5 µm per container: report
Particles ≥10 µm per container: ≤3000
Particles ≥10 µm per container: ≤300

Results are reported in attachment 5 for trial A and in attachment 6 for trial B.
In particular, for both trial A and trial B, subvisible particles of dimension ≥ 10µm and subvisible particles of dimension ≥ 25 µm meet the acceptance criteria, Subvisible particles ≥ 2µm and subvisible particles ≥ 5 µm were present but no action has to be taken considering that they had to be reported for information only without a threshold to fulfill.

Finally, additional at least 20 vials (randomly sampled across the batch at following sampling point: beginning, middle and end of filling) in part A and 20 vials in part B (randomly sampled across the batch at following sampling point: beginning, middle and end of filling) were collected to be tested for visible particles to evaluate tubing stress fatigue.
Considering that usually this analysis is performed as AQL re-check after 100% visual inspection, whereas this activity has a different scope (checking tubing stress fatigue) and not performed on vials previously visual inspected, the sampling was handled as special sampling S2 with AQL =0,65.
Results are reported in attachment 3 for trial A and in attachment 4 for trial B and meet the acceptance criteria (no visible particles present).

In total, the following quantities were collected per part A trial (500 mg/vial):

- 200 vials + 15 contingencies (for each configuration) to be tested for CCIT with Blue Dye Method

- 200 vials + 15 contingencies (for each configuration) for subsequent manual visual inspection
- 7vials + 3 vial for contingency for every time point (beginning, middle and end of filling) for tubing stress fatigue (visible particles)
- 1 vial + 1 vial for contingency for every time point (beginning, middle and end of filling) for tubing stress fatigue (subvisible particles)

and the following quantities were collected per 100 mg/10 mL trial (part B):

- 315 vials + 15 contingencies (for each configuration) to be tested for CCIT with Blue Dye Method
- 315 vials + 15 contingencies (for each configuration) for subsequent manual visual inspection
- 7 vials + 3 vial for contingency for every time point (beginning, middle and end of filling) for tubing stress fatigue (visible particles)
- 4 vials + 4 vials for contingency for every time point (beginning, middle and end of filling) for tubing stress fatigue (subvisible particles)

All samples were collected in the same boxes in which Schott supplier has first provided the vials and sent to the dedicated area.

The parameters to be documented during the crimping phase are listed in table below for 50 mL format and 10 mL format.

Table 34: Parameters documented during Crimping Operations -part A – 50 mL

Parameter	***Specification***	***pCPP***
Crimping machine speed	100 vials/min	NO
Max Crimping Pressure	120 KPa	YES
Min Crimping Pressure	90 KPa	YES
Max Crimping Height	68,5 mm	YES
Min Crimping Height	67,5 mm	YES
Start of crimping (time hh:mm)	15:06 pm 10/02/22	NO
End of crimping (time hh:mm)	01:45 am 11/02/22	NO

Table 35 : Parameters documented during Crimping Operations -part B – 10 mL

Parameter	*Specification*	*pCPP*
Crimping machine speed	340 vials/min, than 175 vials/min (*)	NO
Max Crimping Pressure	100 KPa	YES
Min Crimping Pressure	80 KPa	YES
Max Crimping Height	47 mm	YES
Min Crimping Height	46 mm	YES
Start of crimping (time hh:mm)	14/02/22 14:55	NO
End of crimping (time hh:mm)	14/02/22 21:55	NO

(*) in accordance with decreasing of filling machine speed to minimize foam creation

4.5.1 Intermediate Storage

After the crimping activities are completed, the crimped vials were stored at room temperature in the warehouse before being discarded once the technology transfer report of the surrogate trial has been approved.

4.6 MANUAL VISUAL INSPECTION

Manual Visual Inspection was performed in a not classified environment where only a portion of the vials will be inspected. Specifically, the following quantities were inspected:

- 200 + 15 for contingencies MabThera 500mg/vial for every configuration;
- 315 + 15 for contingencies for MabThera 100mg/vial for every configuration.

Vials were sampled during crimping activities as part of the sampling plan for part A and for part B and collected in the same boxes in which the vials were first shipped by the Schott supplier.

This step had the main goal to identify macro defects and cosmetic defects that occurred during filling and crimping operations.

The defects are classified as followed:
- Critical (C): Product defects that generate a hazard to the consumer
- Major (M): obvious defects which 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
- Minor (m): minor defects which have no impact on the quality of the product

The list of defects used for the present surrogate trials batch is reported in the table below (Table 36) and no particulate defects were evaluated as per protocol TT237A011.
The manual visual inspection was performed by operators which are qualified to perform a visual inspection on liquid.

4.6.1 Part A

In table below, are reported the results of the visual inspection performed on the samples collected during surrogate trial A: no defects were detected.

Table 36. Results of MVI – trail A

Description	Classification	Conf A #defects	Conf B #defects	Conf C #defects	Conf D #defects
Vial with defected glass (chipped) – Sidewall	M	0	0	0	0
Cracked vial – Sidewall, Bottom	C	0	0	0	0
Dirty external glass	m	0	0	0	0
Surface scratch on vial body (length > 1 cm, width > 2 mm)	m	0	0	0	0
Empty vial	C	0	0	0	0
Vial without stopper	C	0	0	0	0
Vial with wrong stopper	C	0	0	0	0
Vial without seal / flip-off	C	0	0	0	0
Foreign seal (mixup)	C	0	0	0	0
Vial with defected flip-off/seal (scratched, dented, dirty, damaged)	m	0	0	0	0
Seal with wrongly positioned/non-sealing seal – not crimped	C	0	0	0	0
Seal with wrongly positioned/non-sealing seal – Partially crimped	M	0	0	0	0
Partially detached flip-off (evident)	m	0	0	0	0

On completion of visual inspection, vials were collected in the polypropylene boxes, placed on pallets and stored in the Patheon warehouse at room temperature.

The following parameters was documented:

Parameter	Specification
Number of vials discarded for each defect category	0
Visual inspection process Yield %	100%

4.6.2 Part B

In table below, are reported the results of the visual inspection performed on the samples collected during surrogate trial B: 8 minor defects were found for configuraton A, 6 minor defects were found for configuraton B, 10 minor defects were found for configuration C and 9 minor defects were found for configuration D, No major and critical defects were found.

Table 37. Results of MVI – trial B

Description	Classification	Conf A #defects	Conf B #defects	Conf C #defects	Conf D #defects
Vial with defected glass (chipped) – Sidewall	M	0	0	0	0
Cracked vial – Sidewall, Bottom	C	0	0	0	0
Dirty external glass	m	0	0	0	0
Surface scratch on vial body (length > 1 cm, width > 2 mm)	m	8	6	10	9
Empty vial	C	0	0	0	0
Vial without stopper	C	0	0	0	0
Vial with wrong stopper	C	0	0	0	0
Vial without seal / flip-off	C	0	0	0	0
Foreign seal (mixup)	C	0	0	0	0
Vial with defected flip-off/seal (scratched, dented, dirty, damaged)	m	0	0	0	0
Seal with wrongly positioned/non-sealing seal – not crimped	C	0	0	0	0
Seal with wrongly positioned/non-sealing seal – Partially crimped	M	0	0	0	0
Partially detached flip-off (evident)	m	0	0	0	0

On completion of visual inspection, vials are collected in the polypropylene boxes, placed on pallets and stored in the Patheon warehouse at room temperature.

The following parameters were documented:

Parameter	Specification
Number of vials discarded for each defect category	33
Visual inspection process Yield %	97,5%

5.0 NON-CONFORMANCES

5.1 BARRIER FILTERS

Description of the event

During the execution of surrogate batches TT489 and TT490, the on line pre-use integrity test of the first sterilizing filter involved in both manufacturing trials have been interrupted. Indeed, the barrier filters included in the same filtration assembly (Patheon code 273504), did not let the nitrogen evacuate from the line (see TT237A011 attachment # 1 and TT237A011 attachment #2).The upstream lines containing the barrier filter were therefore cut to allow the flushing of product filter. The impact from sterility perspective was considered negligible since no microbiological analysis were included in the scope of the trials under consideration but the observed non-conformance was further investigated as part of process characterization.

Investigation and evaluation

As soon as the anomalies occurred, it was performed a check of the position of the barrier filters included in the sterilizing filtration assemblies (PTH code 273504) used during the manufacturing of surrogate batches TT489 and TT490, but they appeared correctly assembled. Therefore, the hypothesis of any equipment construction defect was excluded.

Consequently, It has been taken in analysis the operating steps: after the wetting of the sterilizing filters with WFI and before the pre use integrity test of the sterilizing filters (bubble point test through nitrogen, using Palltronic Flowstar IV), it was performed a draining of the line connecting the nitrogen line of the assembly to the utilities (nitrogen supplied at 6 bar).

Supplier of the filters (Millipore) and supplier of the assembly (Saint Gobain) have been questioned to support the investigation evaluation. They explained that Millipak® barrier filters can be compromised in the breathability function if they are fully wet at high pressure (eg: worst case WFI at 3 bar).

Root-cause

Based on the evaluation listed above, the root cause was identified in the drying of the line that was executed after the wetting step of sterilizing filters and previous to perform the integrity test of sterilizing filters (bubble point test) through Palltronic Flowstar IV. In fact, during the draining step, the residual WFI present in the tubing may have been conveyed to the hydrofibic part of the barrier filters, compromising their breathable capability.

Conclusion

No impact can be addressed to the surrogate batches TT489 and TT490 and to the value of related studies executed, considering that:

- it was however possible to execute the pre-use integrity test on the sterilizing filters considering that they resulted conform and in addition no microbiological analysis were foreseen in accordance with study protocol TT237A011
- the post-use integrity test of all barrier filters resulted conforming (bubble point pressure results ≥ 1280 mbar)

Moreover, one of the purposes of these technical trials was to evaluate the handling of the new disposable assemblies and the training on the field of the manufacturing the operators. Based on the non-conformance evaluation, root cause determination and and the information collected during MabThera surrogate trials (reference protocol# TT237A011), during the execution of Avastin surrogate trial (reference protocol# TT237A021), the dying of the line has not been performed. No issues on barrier filter functioning have been observed and the nitrogen properly evacuated from the line.

Suggested CAPA

Based on the outcomes and on the observations collected during surrogate batches TT489 and TT490, it can be suggested to avoid the draining of the sterilizing line with nitrogen before the pre-use filter integrity test.

This instruction has been already implemented during Avastin surrogate trials (TT491 and TT492) and moreover the instruction will be added to the related MBRs for MabThera and Avastin engineering trials.

5.2 NITROGEN FILTERS

Description of the event

At the end of the trial B (TT490), the post use integrity test of the nitrogen filters of the assembly 273504 were not -conforming (no pressure for bubble point test can be obtained). The test was executed with Palltronic Flowstar IV.

Investigation and evaluation

Once excluding any assembly construction defects by visually checking the position of the nitrogen filters included in the sterilizing filtration assemblies (PTH code 273504) used during the manufacturing of surrogate batch TT490, further evaluations have been carried out. In particular, it has been analysed the setup of sterilizing assembly focusing on the positioning of the equipment for manufacturing operations.

Indeed, the stainless steel trolley, on which the sterilizing filtration assembly (PTH code 273504) was placed, has an horizontal beam on which the sterilizing filters and product contact tubing were fixed through collars. The two tubing on which the nitrogen line is assembled freely fall along the filtration line since no vertical supports are currently foreseen on the trolley. During the pre use filter integrity test, they were placed at lower height with respect to principal tubing in which WFI flows during the wetting step.

On 21/04/22 it has been performed, in sterile area 6, an additional trial using MabThera and Avastin sterilizing filter assembly (PTH code 273504). The assembly was put on the stainlees steel trolley and the nitrogen filters were put on the vertical stainless steel beams.

After the wetting of the sterilizing filters and before the bubble point test execution with nitrogen, it was checked and confirmed the absence of water in nitrogen line of the assembly and in the staubli connection. The pre use tests of sterilizing filters were conforming. After the test on the sterilizing filters, the nitogen filters were cut form the assembly and wetted with 100% IPA. Then, the nitrogen filters were tested with Palltronic (bubble point test). The bubble point value for the first nitrogen filter was equal to 1600 mbar and for the second nitrogen filter was equal to 1550 mbar: both filters resulted conforming (filters are conforming if the measured bubble point is ≥ 1170 mbar).

Root-cause

The root cause was identified in a possible wetting of nitrogen filters caused by the conveyor of remaining WFI used for bubble point test of sterilizing filters when subjected to nitrogen pressure.

Indeed, applying a pressure on hydrophobic vent filter, wetted with WFI, could eventually damaged the filter itself.

Therefore during MabThera trials, even if the nitrogen lines were correctly pinched with pinch valve, the braided tube could have let to pass some residual WFI in the nitrogen lines, and the lower height position of the nitrogen filters with respect to principal line could have been an additional contributing factor.

Conclusion

No impact on the surrogate batch TT490 and related studies executed is expected , considering that no microbiological analysis was in scope of the trial.

Moreover, one of the scopes of the technical trial was to evaluate the handling of the new disposable assembly and train on the field of the manufacturing operators.The design of the sterilizing filter assembly was confirmed appropriate. Additionally, the occurred event highlighted the need of proceeding with the implementation of additional supports for the stainlees steel trolley .

Suggested CAPA

Based on the observations collected, the corrective action to avoid the recurrence of nitrogen filters integrity test failure could be the introduction of a proper support to keep the nitrogen filters in vertical position protecting them from any WFI dropping during the pre-use integrity test on sterilizing filters. Starting form engineering trials these support will be used.

6.0 CONCLUSION

The surrogate trial A (MabThera 500 mg) and B (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.

Mixing and homogeneity study

During MabThera 500 mg surrogate trial (part A) mixing and homogeneity was performed.
The study was executed using a surrogate solution which had the same formulation and preparation of the MabThera's buffer solution, except the surrogate preparation did not foresee any pH adjustment. Indeed, given the described objectives of the trials, pH adjustment was not seen as a value-added step to be performed. MabThera's buffer solution was chosen as the surrogate solution as it mimics the physical and chemical properties of Mabthera's final bulk solution (Table 1).
MabThera buffer solution has a very similar formulation characteristic, in terms of density and viscosity, to Avastin. Therefore, Mabthera buffer was chosen as proper to perform the mixing characterization which will be applied also to Avastin. More details are reported in the memo "Avastin and Mabthera: Usage of surrogate for mixing trial" dated 10th December 2021 provided by Roche.

Moreover, the equipment (1100 L tanks) used for buffer preparation and DPS compounding for Avastin and MabThera are identical thus making the mixing and homogeneity studies applicable to both products.

The homogeneity study let to identify the minimum mixing speed (150 rpm) which guarantees homogeneity even for a batch size bigger (1000 L) than the maximum one foreseen in commercial manufacturing (˜750 L). The minimum mixing time identified during the study, which guarantees the homogeneity at maximum batch size with the minimum mixing speed, is 15 minutes.

Moreover, the mixing study identified as 250 rpm the maximum mixing speed which did not cause vortex or foam during mixing.

Finally, the conformity of the osmolality samples (taken both during trial A and trial B) confirmed the good preparation of the buffer solution.

Sterilizing filtration

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, the barrier filters did not let to evacuate the nitrogen from the line during the pre-use test of sterilizing filters. Indeed, during the wetting, the hydrophobic part of the filter could have been eventually impacted. As a corrective action, during the Avastin surrogate trail (TT237D021), the drain of the line was not executed and the barrier let the nitrogen evacuate from the line correctly.

The post use test of both nitrogen filters resulted not conforming in trial B and they could have been, most probably, damaged during the in line test on the sterilizing filters. Indeed, first they could have been improperly wetted during the wetting step of the sterilizing filters and then they could have been damaged when, already wetted with WFI, were pressurized to perform bubble point test on sterilizing filters.

As a corrective action, during the future engineering tirals, it is raccomanded to use stainless steel vertical support beams for the nitrogen filters.

Filling/stoppering

Filling accuracy was assessed overtime during the execution of both trial A and trial B and good capability of the process was observed (P_{pk}/Cpk ≥1,33).

Foam creation during filling was observed starting from trial A and it was minimized, during trial B, implementing the following actions:

- pressure in the storage tank for solution transfer was decreased from 0,7 bar (set during trial A) to 0,3 barg (set during trial B).
- machine filling speed during the second trial was decreased starting from an initial value of 350 vials/min to 180 vials/min.

The actions listed above resulted in a significant reduction of foam in vials.

Moreover, during trial A and trial B, it was observed that approximately 1 hour after the filling, the vials did not have any foam.

Visible and subvisible particles after mixing and tubing stress fatigue

Visible and subvisible particles were taken after mixing step and then at the beginning, middle and end of filling to evaluate particles generation after mixing and tubing stress fatigue.

No visible particles were found at any of the time points evaluated.

Regarding the subvisible particles, for dimension ≥ 2 µm and dimension ≥ 5 µm, no acceptance criteria was present, but they were evaluated for information only. For the dimension ≥ 10 µm and ≥ 25 µm, the acceptance criteria of 3000 particles/container and 300 particles/container respectly was met.

Moreover, considering the evaluation of the subvisible particles during the time, it must be noted that no significant worsening during the filling was present. Indeed, value at end of the filling was comparable with the value obtained at the beginning of the filling.

The results of visible and subvisible particles suggest that no tubing stress fatigue effect, during filling step at maximum batch size, was present.

Crimping

Crimping performance was evaluated through a CCI test and visual inspection. CCIT executed on samples collected during all four configurations resulted in conforming (no sign of leakage). Visual inspection on samples collected during all four configurations did not identify defects correlated to the crimping step. Thus, the maximum and minimum values identified for the height of the crimping and for the pressure of the crimping are applicable.

7.0 LIST OF ACRONYMS

Table 38: List of acronyms

Acronyms	Meaning
RTP	Rapid transfer port
WFI	Water for injection
TT	Technology transfer
CCIT	Container Closure Integrity Test
BDP	Bulk drug product

Acronyms	Meaning
pCPP	Potential Critical Process Parameter
SIP	Sterilization in place
CIP	Cleaning in place
SS	Stainless steel

8.0 LIST OF RELATED DOCUMENTS

Table 39: List of related documents

Document	Reference #	Responsible	Approval
Change Control for introduction of MabThera	191938	Laura Palmaroli	TF/Roche
Surrogate trial for Mabthera- Protocol	TT237A011	Giulia Ferri	TF/Roche
Surrogate trial for Avastin -Protocol	TT237A021	Giulia Ferri	TF/Roche
Technology Transfer Plan of Avastin and MabThera	TTP23701	Laura Palmaroli	TF/Roche
Protocol Blue dye test	PC0082 Ed.01	TF	TF
Analytical Report for MabThera Surrogate Batches	RC0428 Ed.03	TF	TF

9.0 DOCUMENT INFORMATION

Table 40: Revision History

Version	Reason for change	Author	Date
01	Issue for approval	Giulia Ferri	02/05/2022

ATTACHMENT 1 –LIQUID SAMPLES 500 –

ID. N.	Parameter	Acceptance criteria	Results	Reference
O-500	Osmolality	324-396 mosm/kg	355 mosm/kg	RC0428 Ed.03
CT - 500	Conductivity	For information only	19,70 mS/cm	RC0428 Ed.03
VP – L- 500	Visible particles Ph. Eur. 2.9.20, JP <6.06>	No visible particles	No visible particles	RC0428 Ed.03
SVP – L- 500	Subvisible particles (SAM-0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>	Particles ≥2µm per container: report Particles ≥5µm per container: report Particles ≥10µm per container: ≤3000 Particles ≥25µm per container: ≤300	9350 part./cont.(≥ 2 µm) 1450 part./cont. (≥ 5 µm) 150 part./cont. (≥ 10 µm) 0 part./cont. (≥ 25 µm)	RC0428 Ed.03
C- T0-T– 500 **C- T0-M– 500** **C- T0-B– 500**	Conductivity	values from top, middle and bottom has to be within the mean value (calculated from the experimental data) ± 3%	Sample 1 / Sample 2 / Sample 3 Top [mS/Kg] 30,35 / 30,75 / 29,48 Middle [mS/Kg] 32,61 / 32,34 / 31,91 Bottom [mS/Kg] 34,05 / 34,79 / 34,35 Mean = 32,29 mS/Kg ; Meam +3% = 33,26 mS/Kg ; Mean -3% = 31,32 mS/Kg	RC0428 Ed.03
C- T1-T– 500 **C- T1-M– 500** **C- T1-B– 500**	Conductivity	values from top, middle and bottom has to be within the mean value (calculated from the experimental data) ± 3%	Sample 1 / Sample 2 / Sample 3 Top [mS/Kg] 32,86 / 32,70 / 32,62 Middle [mS/Kg] 32,49 / 32,61 / 32,78 Bottom [mS/Kg] 32,78 / 32,93 / 33,07 Mean = 32,76 mS/Kg ; Meam +3% = 33,74 mS/Kg ; Mean -3% = 31,78 mS/Kg	RC0428 Ed.03
C- T2-T– 500 **C- T2-M– 500** **C- T2-B– 500**	Conductivity	values from top, middle and bottom has to be within the mean value (calculated from the experimental data) ± 3%	Sample 1 / Sample 2 / Sample 3 Top [mS/Kg] 32,70 / 32,68 / 32,53 Middle [mS/Kg] 32,97 / 32,51 / 32,63 Bottom [mS/Kg] 32,12 / 32,81 / 32,34 Mean= 32,59 mS/Kg ; Meam +3% = 33,57 mS/Kg ; Mean -3% = 31,61 mS/Kg	RC0428 Ed.03
C- T3-T– 500 **C- T3-M– 500** **C- T3-B– 500**	Conductivity	values from top, middle and bottom has to be within the mean value (calculated from the experimental data) ± 3%	Sample 1 / Sample 2 / Sample 3 Top [mS/Kg] 32,48 / 32,52 / 32,56 Middle [mS/Kg] 32,11 / 32,22 / 32,26 Bottom [mS/Kg] 32,86 / 32,57 / 32,47 Mean = 32,45 mS/Kg ; Meam +3% = 33,42 mS/Kg ; Mean -3% = 31,48 mS/Kg	RC0428 Ed.03

ATTACHMENT 2 – LIQUID SAMPLES 100 –

ID. N.	Parameter	Acceptance criteria	Results	Reference
O-100	Osmolality	324-396 mosm/kg	355 mosm/kg	RC0428 Ed.03
VP – L- 100	Visible particles Ph. Eur. 2.9.20, JP <6.06>	No visible particles	No visible particles	RC0428 Ed.03
SVP – L- 100	Subvisible particles (SAM-0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>	Particles ≥2µm per container: report Particles ≥5µm per container: report Particles ≥10µm per container: ≤3000 Particles ≥25µm per container: ≤300	1620 part./cont.(≥ 2 µm) 280 part./cont. (≥ 5 µm) 30 part./cont. (≥ 10 µm) 0 part./cont. (≥ 25 µm)	RC0428 Ed.03

ATTACHMENT 3 –VISIBLE PARTICLES – 500 -DURING FILLING

ID. N.	Parameter	Acceptance criteria	Results	Reference
VP-A-500	Visible particles Ph. Eur. 2.9.20, JP <6.06>	No visible particles	No visible particles	RC0428 Ed.03
VP-B-500	Visible particles Ph. Eur. 2.9.20, JP <6.06>	No visible particles	No visible particles	RC0428 Ed.03
VP-D-500	Visible particles Ph. Eur. 2.9.20, JP <6.06>	No visible particles	No visible particles	RC0428 Ed.03

ATTACHMENT 4– SAMPLING PLAN –VISIBLE PARTICLES – 100 DURING FILLING

ID. N.	Parameter	Acceptance criteria	Results	Reference
VP-A-100	Visible particles Ph. Eur. 2.9.20, JP <6.06>	No visible particles	No visible particles	RC0428 Ed.03
VP-B-100	Visible particles Ph. Eur. 2.9.20, JP <6.06>	No visible particles	No visible particles	RC0428 Ed.03
VP-D-100	Visible particles Ph. Eur. 2.9.20, JP <6.06>	No visible particles	No visible particles	RC0428 Ed.03

ATTACHMENT 5– SAMPLING PLAN – SUBVISIBLE PARTICLES (SVP) – 500 -DURING FILLING

ID. N.	Parameter	Acceptance criteria	Results	Reference
SVP-A-500	Subvisible particles (SAM-0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>	Particles ≥2µm per container: report Particles ≥5µm per container: report Particles ≥10µm per container: ≤3000 Particles ≥25µm per container: ≤300	11650 part./cont(≥ 2 µm) 3300 part./cont. (≥ 5 µm) 300 part./cont. (≥ 10 µm) 0 part./cont. (≥ 25 µm)	RC0428 Ed.03
SVP-B-500	Subvisible particles (SAM-0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>	Particles ≥2µm per container: report Particles ≥5µm per container: report Particles ≥10µm per container: ≤3000 Particles ≥25µm per container: ≤300	10700 part./cont(≥ 2 µm) 3000 part./cont. (≥ 5 µm) 400 part./cont. (≥ 10 µm) 0 part./cont. (≥ 25 µm)	RC0428 Ed.03
SVP-D-500	Subvisible particles (SAM-0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>	Particles ≥2µm per container: report Particles ≥5µm per container: report Particles ≥10µm per container: ≤3000 Particles ≥25µm per container: ≤300	8200 part./cont(≥ 2 µm) 2250 part./cont. (≥ 5 µm) 200 part./cont. (≥ 10 µm) 0 part./cont. (≥ 25 µm)	RC0428 Ed.03

ATTACHMENT 6– SAMPLING PLAN – SUBVISIBLE PARTICLES (SVP) – 100 -DURING FILLING

ID. N.	Parameter	Acceptance criteria	Results	Reference
SVP-A-100	Subvisible particles (SAM-0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>	Particles ≥2µm per container: report Particles ≥5µm per container: report Particles ≥10µm per container: ≤3000 Particles ≥25µm per container: ≤300	3010 part./cont.(≥ 2 µm) 860 part./cont. (≥ 5 µm) 120 part./cont. (≥ 10 µm) 0 part./cont. (≥ 25 µm)	RC0428 Ed.03
SVP-B-100	Subvisible particles (SAM-0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>	Particles ≥2µm per container: report Particles ≥5µm per container: report Particles ≥10µm per container: ≤3000 Particles ≥25µm per container: ≤300	4340 part./cont.(≥ 2 µm) 1350 part./cont. (≥ 5 µm) 200 part./cont. (≥ 10 µm) 0 part./cont. (≥ 25 µm)	RC0428 Ed.03
SVP-D-100	Subvisible particles (SAM-0102796 Version 2.0, USP <788>, Ph.Eur.2.9.19, JP<6.07>	Particles ≥2µm per container: report Particles ≥5µm per container: report Particles ≥10µm per container: ≤3000 Particles ≥25µm per container: ≤300	3860 part./cont.(≥ 2 µm) 1210 part./cont. (≥ 5 µm) 190 part./cont. (≥ 10 µm) 0 part./cont. (≥ 25 µm)	RC0428 Ed.03

10.0 ATTACHMENT 7– SAMPLING PLAN – CONTAINER CLOSURE INTEGRITY TEST (CCIT) - 500

ID. N.	Parameter	Acceptance criteria	Results	Reference
CCIT-A-500	Blue Dye Test	No sign of leakage should be visible	200/200 complies	RC0428 Ed.03
CCIT-B-500	Blue Dye Test	No sign of leakage should be visible	200/200 complies	RC0428 Ed.03
CCIT-C-500	Blue Dye Test	No sign of leakage should be visible	200/200 complies	RC0428 Ed.03
CCIT-D-500	Blue Dye Test	No sign of leakage should be visible	200/200 complies	RC0428 Ed.03

11.0 ATTACHMENT 8– SAMPLING PLAN – CONTAINER CLOSURE INTEGRITY TEST (CCIT) – 100

ID. N.	Parameter	Acceptance criteria	Results	Reference
CCIT-A-100	Blue Dye Test	No sign of leakage should be visible	315/315 complies	RC0428 Ed.03
CCIT-B-100	Blue Dye Test	No sign of leakage should be visible	315/315 complies	RC0428 Ed.03
CCIT-C-100	Blue Dye Test	No sign of leakage should be visible	315/315 complies	RC0428 Ed.03
CCIT-D-100	Blue Dye Test	No sign of leakage should be visible	315/315 complies	RC0428 Ed.03

12. ATTACHMENT 9 – ROCHE MEMO- AVASTIN AND MABTHERA: USAGE OF SURROAGTE FOR MIXING TRIAL

Memo

To:	Laura Palmaroli (Patheon Italia S.p.A part of Thermo Fisher Scientific)	Copies:	Michael Ampong (Roche) Vanessa Cervi (Roche)
From:	Costanza Ciaponi	Roche	
Date:	10 December 2021		

Avastin and Mabthera: Usage of surrogate for mixing trial

According to Roche RRF document (TEC-0132616), for homogeneity studies a surrogate can be used if the following conditions are satisfied:

- representative formulation characteristic
- in case of mixing of two solutions (dilution of concentrated drug substance with buffer), the density ratio of the surrogate should match or exceed the density ratio of the drug substance and the dilution buffer if this is higher than 1.08

For the mixing trial proposed, both the conditions are fulfilled. The surrogate solutions (Mabthera buffer and Mabthera buffer + 600 mM NaCl (total 750mM)) presents very similar formulation characteristics to both Mabthera formulated bulk and Avastin formulated bulk drug substance. Moreover, the surrogate solutions (Mabthera buffer and Mabthera buffer + 600 mM NaCl (total 750mM)) have density ratios very similar to those between Mabthera drug substance and formulation buffer and to those between different Avastin bulk drug substance lots as shown in the table below.

Solution	Density
Mabthera buffer	1.01 g/mL
Mabthera drug substance	1.02 g/mL
Mabthera buffer + 600 mM NaCl (total 750mM)	1.03 g/m
Avastin bulk drug substance	1.02 g/mL

Sincerely,

DocuSigned by:
Costanza Ciaponi
Signer Name: Costanza ciaponi
Signing Reason: I am the author of this document
Signing Time: 14-Dec-2021 | 3:03:34 PM PST
DA4D026D7C6A474B99B3E4D0679C5B4D

1/1

MabThera surrogate report TT237D011_clean copy020522

Final Audit Report 2022-05-05

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