Jun 14, 2020

MERS-CoV Spike Glycoprotein (GP) – ELISA Jenner Clinical SOP Template

Mustapha Bittaye[1], Sarah Gilbert[1], Katie Ewer[1], Teresa Lambe[1]

[1]The Jenner Institute, University of Oxford

1 *Works for me* dx.doi.org/10.17504/protocols.io.bgr6jv9e

Coronavirus Method Development Community

Mustapha Bittaye

ABSTRACT

This Standard Operating Procedure (SOP) describes the techniques used to measure the titres of total human immunoglobulin G (IgG) responses against a full length recombinant clamped MERS-CoV spike glycoprotein (GP) in human plasma and serum samples. The measured antibody titres will serve to indicate the humoral immune response to a candidate ChAdOx1 MERS vaccine encoding the full length spike GP of the MERS-CoV in human subjects.

This assay must be performed in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice and the Human Tissue Act. Informed consent must be in place for samples to be tested in this assay.

All staff employed by University of Oxford who work on outbreak pathogen vaccine trials including clinicians or visiting scientists working in the lab, must follow this protocol.

ATTACHMENTS

MERS-CoV_Spike_GP_ELISA_Jenner_Clinical_SOP_template_2018_v2.pdf

DOI

dx.doi.org/10.17504/protocols.io.bgr6jv9e

PROTOCOL CITATION

Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe 2020. MERS-CoV Spike Glycoprotein (GP) – ELISA Jenner Clinical SOP Template. **protocols.io** dx.doi.org/10.17504/protocols.io.bgr6jv9e

KEYWORDS

IgG, immunoglobulin G, MERS-CoV, COVID-19, Coronavirus, 2019-nCoV

CREATED

May 22, 2020

LAST MODIFIED

Jul 20, 2020

OWNERSHIP HISTORY

May 22, 2020 Julia Rossmanith protocols.io

Jun 08, 2020 Mustapha Bittaye

PROTOCOL INTEGER ID

37406

GUIDELINES

This assay must be performed in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice and the Human Tissue Act. Informed consent must be in place for samples to be tested in this assay.

INTRODUCTION

One of the primary readouts for assessing the protective humoral immunity against this candidate vaccine is the measurement of total IgG responses to MERS-CoV spike GP in the blood of vaccinated volunteers. This will be done as described in this SOP using an in-house ELISA that includes a standard curve derived from a pool of the volunteers' sera containing highly concentrated anti-MERS IgG.

SCOPE

This SOP applies to all studies sponsored by the University of Oxford that are conducted by the Jenner Institute where measurement of total IgG against the candidate ChAdOx1 MERS vaccine is required, for which ELISA is the primary readout.

DEFINITIONS/ABBREVIATIONS

ELISA = Enzyme-Linked ImmunoSorbent Assay.
MERS-CoV = Middle East Respiratory Syndrome Coronavirus.
ChAdOx1 MERS = replication-deficient simian adenovirus vector ChAdOx1, expressing a codon-optimised coding sequence for the full-length spike GP of the MERS-CoV isolate Camel/Qatar_2_2014 (GenBank, accession number KJ650098.1), including a 32 amino acid N-terminal tissue plasminogen activator leader sequence.

RELATED DOCUMENTS

J284 Standardised ELISA set-up
ELISA analysis template.xls
ELISA Record sheet.pdf

REFERENCES

ICH Harmonised Tripartite Guideline for Good Clinical Practice
MHRA Guidelines 'Good Clinical Practice for Clinical Laboratories

TRAINING

Record the level of training required by users

Training type	**Required (tick at least one box)**
Read and Understand	YES
Attend face to face training based on SOP	YES
Competency assessment	YES
List other specific training types if required	

Read and understand *training means that the user will be sufficiently trained to perform the activity described after reading the SOP and having any questions answered by the author or other trained individual.*
Attend face to face training based on SOP *training means that the user must read and understand the SOP, but must also attend face to face training. This enables additional discussion to be undertaken and clarification where necessary. The trainer is able to emphasise specific requirements that may differ from standard practice. This may often be performed as a group session.*

Citation: Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe (06/14/2020). MERS-CoV Spike Glycoprotein (GP) Ã¢ÂÂ ELISA Jenner Clinical SOP Template. https://dx.doi.org/10.17504/protocols.io.bgr6jv9e

Competency assessment *requires the user to read and understand the SOP and also to receive face to face training (usually 1:1 or small groups). Specific practical aspects of the procedure will be covered and the individual must be able to demonstrate competency in all aspects prior to being signed off by the trainer:*

MATERIALS

NAME	CATALOG #	VENDOR
TWEEN® 20	P7949	Sigma Aldrich
Blocker™ Casein in PBS	37528	Thermo Fisher
Clear Flat-Bottom Immuno Nonsterile 96-Well Plates, 400μL, MaxiSorp	442404	Thermo Fisher
Dulbecco's PBS (without calcium magnesium)	D8537	Sigma Aldrich
Anti-Human IgG (γ-chain specific)–Alkaline Phosphatase antibody produced in goat	A3187	Sigma Aldrich
Pierce™ Diethanolamine Substrate Buffer (5X)	34064	Thermo Fisher Scientific
4-Nitrophenyl phosphate disodium salt hexahydrate	N2765	Sigma Aldrich
Fisherbrand™ Polypropylene Microcentrifuge Tubes	11558232 (FB74031)	Fisher Scientific
Corning™ Costar™ Sterile Disposable Reagent Reservoirs	10320551	Fisher Scientific
Water (sterile-filtered BioReagent suitable for cell culture)	W3500	Sigma Aldrich
DPBS powder no calcium no magnesium	21600069	Thermo Fisher Scientific

MATERIALS TEXT

Additional reagent required:

- MERS-CoV clamped spike GP (0.5 mg/mL)

Equipment:

- Fridge (+4°C)
- Freezer (-20°C and -80°C)
- Vortexer
- Eppendorf Racks
- Pipettes including 8- or 12-well multi-channel and automatic multi-channel
- Pipetteboy and stripettes
- Bio-tek ELx800 Microplate Reader with Gen5 ELISA software
- Timer
- Sufficient tips for pipettes. 0.1-10 µl, 2-20 µl, 20-200 µl, 100-1000 µl
- Safety Glasses
- PBS-Tween Immunowash

Buffers and solutions:

- **DPBS** Use for coating plates with MERS-CoV Spike Glycoprotein.
- **DPBS/T (10 L)** (DPBS with [M]**0.05 % (v/v) Tween**) for washing plates. Dissolve **one DPBS powder tub** in **10 L deionised water** (15.0 MΩ setting). Add **5 mL Tween-20** . Shake and return to the ELISA plate wash station.
- **Blocking and Dilution buffer** Blocker Casein in PBS.
- **Anti-human IgG Alkaline phosphatase** 1:1000 dilution in casein. **6 µl** in a final volume of 6 ml (**6 mL** per plate).
- **Developer** Dilute [M]**5 X Diethanolamine (DEA) substrate buffer** to [M]**1 X** in sterile-filtered, Bioreagent

Citation: Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe (06/14/2020). MERS-CoV Spike Glycoprotein (GP) Ã¢ÂÂ ELISA Jenner Clinical SOP Template. https://dx.doi.org/10.17504/protocols.io.bgr6jv9e

Water. Add **one 20 mg pNPP tablet** per 20 ml. 10 ml is required per plate (minimum that can be made up is 20 ml). Make up **just before use**, wrap in foil and vortex well.

SAFETY WARNINGS

All staff employed by University of Oxford who work on outbreak pathogen vaccine trials including clinicians or visiting scientists working in the lab, must follow this protocol.

Senior immunologist is responsible for training new members of staff to be competent with this SOP and ensuring that sample receipt is conducted according to this SOP:

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE STARTING

Prepare PBS/T as described in Section 'Materials'.

Day 1 – Coating ELISA plates on the bench

1 Print off a new ELISA record sheet for each experiment (accessed from: X:\KEwer\7. ELISA\Templates and protocols).

2 Number the experiment with the next experiment number and fill this in with the required information throughout the experiment.

3 Calculate the number of Nunc Immuno ELISA plates required (One plate: 23 samples and an internal control in triplicate in addition to the standard curve in duplicate).

4 Thaw an aliquot of MERS-CoV clamped spike GP (typically **0.5 mg/ml stock solution** kept at **-80 °C** and prepare as shown in the table below. Calculate accordingly if stock protein concentration differed).

Number of plates to coat	Volume of Spike GP (0.5 mg/ml stock solution)	Volume of DPBS
1	10 µl	4.99 ml
2	20 µl	9.98 ml
3	30 µl	14.97 ml
4	40 µl	19.98 ml
5	50 µl	24.95 ml
6	60 µl	29.94 ml

5 

Coat plates with **50 µl/well** of MERS-CoV Spike clamped GP at [M]**1.0 µg/ml** in DPBS).

6 

Cover plates in clingfilm and store overnight for **18:00:00** at **4 °C** . Note time of coating and record on the experiment record sheet.

Day 2 – Blocking plates

7 Thaw required serum samples (up to 23 samples per plate) at **Room temperature** for at least **01:00:00** .

Citation: Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe (06/14/2020). MERS-CoV Spike Glycoprotein (GP) Ã¢ÂÂ ELISA Jenner Clinical SOP Template. https://dx.doi.org/10.17504/protocols.io.bgr6jv9e

8 Flick off coating solution into the sink.

Wear eye protection.

9 

Wash plates **6x** in DPBS/T using the handheld plate washer and tap dry on paper towel between washes. After the final wash, invert plates and tap firmly. Leave plates on the bench for 2 min and tap firmly on a paper towel one more time to remove residual liquid from the wells

10 

Block plates with **100 µl casein per well**.

11 

Stack plates (with an empty blank plate on top). Leave for **01:00:00** at **Room temperature** . Note time of blocking and record on the experiment sheet.

12 During blocking prepare serum samples and reference standard dilutions as in section below.

Day 2 – Sample/standard preparation

13 

Vortex the thawed samples thoroughly and dilute in casein at 1:500 (**5 µl sample** diluted in **2495 µl casein** and vortexed). All sample dilutions MUST be done using a minimum of **5 µl sample** .

14 

Add **50 µl diluted sample** to each well (each sample in triplicate). Store diluted serum **Overnight** in fridge in case repeat of samples at higher dilution is necessary.

15 

The standard curve is a two-fold dilution series with an initial dilution of 1:100 from the positive standard pool. To avoid multiple freeze-thaw of the positive standard pool, prepare aliquots of 1:100 working standards in casein to a final volume of **600 µl** (**6 µl pool sample** in **594 µl casein**) and store in **-80 °C** freezer, where it is *stable for up to a year.*

16 

All standard dilution is done using Eppendorf tubes labelled Tube1 – Tube10.

Citation: Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe (06/14/2020). MERS-CoV Spike Glycoprotein (GP) Ã¢ÂÂ ELISA Jenner Clinical SOP Template. https://dx.doi.org/10.17504/protocols.io.bgr6jv9e

To run 2 plates prepare standards as follows: Add **520 µl** of the **1:100 working standard** to the first tube (Tube1). Add **260 µl casein** to the rest of the tubes (Tube2 – Tube10). Transfer **260 µl** from **Tube1** to **Tube2** (**mix 15 times**). Transfer **260 µl** from **Tube2** into **Tube3** and continue across the rest of the tubes. Discard **260 µl** from the last tube (Tube10).

17 

Make a 1:800 dilution of the standard serum pool in casein to use for the plate internal control. (eg **175 µl casein** + **25 µl positive standard pool** per plate). Add to wells D12, E12 and F12.

18 If one plate is assayed, refreeze the remaining 1:100 working standard serum and reuse in subsequent assays. This can be freeze-thawed **up to three times** if required.

Day 2 – Plating out

19 After 1 h incubation for blocking, flick off blocking solution into the sink and tap dry.

20 Add **50 µl** of the thoroughly vortexed diluted samples (in triplicate) and the blank control (casein only) as shown in the plate layout below using an automatic pipette if available. **Without adding the standards and internal control**, cover plate with an empty blank plate and repeat step if multiple plates are prepared.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control (1:800)
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control (1:800)
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control (1:800)
St 1:100	St 1:200	St 1:400	St 1:800	St 1:1600	St 1:3200	St 1:6400	St 1:12800	St 1:25600	St 1:51200	Blank	Blank
St 1:100	St 1:200	St 1:400	St 1:800	St 1:1600	St 1:3200	St 1:6400	St 1:12800	St 1:25600	St 1:51200	Blank	Blank

Plate Layout

21 

Add **50 µl** of the 1:100 Standard as shown in the plate layout to all plates first then followed by 1:200 and so on. Add the internal control **immediately** after adding the 1:800 standard then followed by the rest of the standards.

Use an automatic pipette if available for transferring the standards

Citation: Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe (06/14/2020). MERS-CoV Spike Glycoprotein (GP) Ã¢ÂÂ ELISA Jenner Clinical SOP Template. https://dx.doi.org/10.17504/protocols.io.bgr6jv9e

Use an automatic pipette if available for transferring the standards.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control (1:800)
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control (1:800)
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control (1:800)
St 1:100	St 1:200	St 1:400	St 1:800	St 1:1600	St 1:3200	St 1:6400	St 1:12800	St 1:25600	St 1:51200	Blank	Blank
St 1:100	St 1:200	St 1:400	St 1:800	St 1:1600	St 1:3200	St 1:6400	St 1:12800	St 1:25600	St 1:51200	Blank	Blank

Plate Layout

22

Incubate plate at **Room temperature** for **02:00:00** with a blank plate on top.

Day 2 – Secondary Antibody

23

After 2 h incubation with samples, wash plates **6x** in DPBS/T using the handheld plate washer as previously described.

24

Dilute anti-human IgG antibody alkaline phosphatase 1:1000 in casein (see section 'Materials').

25

Add **50 µl detection antibody** to each well.

26

Incubate plate at **Room temperature** for **01:00:00** with a blank plate on top.

Day 2 – Development and plate reading

27

Prepare the pNPP substrate (development buffer) as shown in the table below. Make up the substrate during the

Citation: Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe (06/14/2020). MERS-CoV Spike Glycoprotein (GP) Ã¢ÂÂ ELISA Jenner Clinical SOP Template. https://dx.doi.org/10.17504/protocols.io.bgr6jv9e

antibody incubation, at least **00:30:00** prior to development to allow adequate time for the pNPP tablet to dissolve and protect from light until use (see section 'Materials').

Number of plates to develop	Number of pNPP tablets required	Volume of 5X DEA buffer required	Volume of H20
1	1	4 ml	16 ml
2	1	4 ml	16 ml
3	2	8 ml	32 ml
4	2	8 ml	32 ml
5	3	12 ml	48 ml
6	3	12 ml	48 ml

28

After 1 h incubation with secondary antibody, wash plates **6x** in DPBS/T using the handheld plate washer as previously described.

29

Add **100 µl development buffer** to each well. Add the development buffer to the internal control column (Column 12) immediately after the Column containing standard 4 (Column 4) and then continue from Columns 5 – 11.

30 Turn the plate reader on and log in to the computer.

31 Open the Gen5 software, create new experiment using the protocol template (MERS STANDARDISED ELISA_SAMPLE ANALYSIS TEMPLATE) located in this folder (X:\Vaccine\10. MERS-CoV\1. MERS001_Oxford\12.0 Immunology\8. ELISA\Data and analysis\MERS001 STANDARDISED ELISA_SAMPLE ANALYSIS DATA).

32 Wipe dry the bottom of the plate with clean tissue paper and place the plate in the plate reader.

33

Start taking readings of the plate at OD405 after **00:10:00** and repeat readings until the control readings and curve parameters fit expected values.

- internal control OD (wells D12, E12, F12) should be around 0.9-1.1 (1300-2300 EU)
- St 1:100 wells (G1 and H1) should be 3.5-3.8
- St 1:800 wells (G4 and H4) should be close to the values in the internal control wells (D12, E12, F12).
- Blank wells should be <0.2
- Standard curve reaches 4 parameter ranges below:

Parameters	A	B	C	D	R^2
Ideal range	0.01-0.2	1-1.3	5-13	4.5-6	>0.996

Citation: Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe (06/14/2020). MERS-CoV Spike Glycoprotein (GP) Ã¢ÂÂ ELISA Jenner Clinical SOP Template. https://dx.doi.org/10.17504/protocols.io.bgr6jv9e

34 Export the plate data, standard curve and curve results to Excel. Record time of reads. Save the Gen5 Experiment and the Excel worksheet in the corresponding experiment folder. Trial specific assay data must be stored in the corresponding clinical trial folder on the X drive or for blinded studies use X:\KEwer\7.ELISA\ 14.Confidential Immunology Data.

Day 2 – Data Analysis

35 

Analyse the data in the Excel sheet “ELISA analysis template” (accessed from: X:\KEwer\7. ELISA\Templates and protocols). All **blue-shaded** fields in the worksheets MUST be completed or confirmed as correct.

36 Fill in the assay information and plate layout in the blue boxes in the “Plate layouts and raw data” page, then paste the plate layout, plate data and parameters (A,B,C,D and R^2) into the “plate layout and data” sheet.

37 Check for outliers (“high CV” on Results page) and take average of duplicates instead of triplicates if necessary. On the Raw data, highlight outlying well in yellow, calculate the average of the other two wells and type this into the yellow outlier cell.

38 The ELISA units for each sample are on the Results sheet.

39 Individual plate data and QC can be seen on the individual plate sheets.

40 The sheet will indicate if a sample dilution is too high or low. If the sheet indicates that sample dilution is “TOO LOW” at 1:100, the sample is negative and can be assigned an arbitrary value of 1.

41 If the sheet indicates that sample dilution is too low at 1:500 repeat at 1:100. For samples that are too high at 1:500 repeat them at higher dilution e.g. 1:1000, 1:2000 or more. When analysing samples that have been retested at 1:100 or higher dilutions such as 1:1000, change the dilution in the blue boxes on the individual plate pages (“plate 1”, “plate 2”, “plate 3” etc.)

42 If further dilutions from the 1:500 dilutions are not required then the tubes can be discarded.

43 Mark samples with values greater than 225 ELISA units as positive.

44 Required: Add all assay parameter data for each experiment to a designated QC workbook.

45 Required: QC all ELISA data for clinical trials prior to publication.

Citation: Mustapha Bittaye, Sarah Gilbert, Katie Ewer, Teresa Lambe (06/14/2020). MERS-CoV Spike Glycoprotein (GP) Ã¢ÂÂ ELISA Jenner Clinical SOP Template. https://dx.doi.org/10.17504/protocols.io.bgr6jv9e