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Establishment of 1st WHO International Standard for anti-MERS-CoV antibody

Giada Mattiuzzo^{1#}, Emma Bentley¹, Sang Hwan Seo², Nam Hyuk Cho³, Jae-Ouk Kim², Samuel Richardson¹, Mark Hassall¹, Eleanor Atkinson⁴, Jason Hockley⁴, Yeon-Sook Kim⁵, Celine Gurry⁶, Raúl Gómez Román⁶, Johan Holst⁶, Paul Kristiansen⁶, Keith Grehan⁷, Nigel Temperton⁷, Ruth Harvey¹, Manki Song², Mark Page¹ and the collaborative study participants*

¹Division of Virology, National Institute for Biological Standards and Control, UK; ²International Vaccine Institute, South Korea; ³ Department of Biomedical Sciences, Seoul National University College of Medicine, South Korea; ⁴Statistics group, National Institute for Biological Standards and Control, UK; ⁵Department of Internal Medicine, Chungnam National University School of Medicine, South Korea; ⁶Coalition for Epidemic Preparedness Innovations, Norway; ⁷Medway School of Pharmacy, University of Kent, UK.

#Study coordinator: TEL: +44 1707641283, E-mail: Giada.mattiuzzo@nibsc.org

* see appendix 1

NOTE:

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Dr Ivana Knezevic, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland. Email: knezevici@who.int.

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Summary

Middle East Respiratory syndrome coronavirus (MERS-CoV) is a zoonotic disease causing sporadic outbreaks since 2012 mainly in the Middle East region, but cases have been reported in 27 countries worldwide, usually associated with returning travelers. MERS-CoV has been identified by the WHO R&D Blueprint as one of the top ten priority pathogens for outbreak potential. Vaccines and treatments are in development and reliable assays are needed for their evaluation. The availability of an International Standard (IS) for antibodies would facilitate the standardization of MERS serological methods and facilitate the development of vaccine and therapeutics, and improve our understanding of virus epidemiology. A previous study published in 2019 compared serological methods for MERS-CoV and showed that a pool of plasma from convalescent individuals was able to harmonise the results for MERS antibodies from different laboratories and assays. In this collaborative study, a pool of sera from two MERS recovered patients, was evaluated by 11 laboratories across 22 methods for its suitability as International Standard for anti-MERS-CoV antibody.

The candidate preparation, sample S55, NIBSC code 19/178, was assessed as part of a blinded samples panel, which included four serum samples from individual MERS-recovered patients, candidate working reagent, a pool of sera from two MERS-recovered patients and two anti-MERS IgG preparations derived from trans-chromosomal bovines immunised with either Spike protein or irradiated virus. MERS-CoV negative controls were also produced as additional study materials. Expressing the MERS antibody titres of the samples as relative to the candidate IS harmonises the results from different laboratories with a reduction of the inter-laboratory variability. Furthermore, the potencies of the samples expressed in International Unit per millilitre (IU/mL) for binding antibodies and for neutralising antibodies is similar supporting the proposal that sample S55, NIBSC code 19/178, can serve as an IS for different methods and an unitage of 250 IU/ampoule has been arbitrarily assigned.

Introduction

Middle East Respiratory syndrome coronavirus (MERS-CoV) was first described in Saudi Arabia in 2012 [1]. This is a zoonotic infection and the primary transmission route to humans is through handling of dromedary camels [2,3]; however, human-to-human transmission has occurred, mainly in healthcare settings [4,5]. Symptoms include fever, cough, shortness of breath and in most cases pneumonia; as of 13th July 2020, there have been 2494 reported cases of MERS-CoV and 858 associated deaths with a fatality rate of approximately 35% [6]. The WHO Research and Development Blueprint listed MERS-CoV as one of the top priority pathogens with the potential of causing a public health emergency [7,8]. As of 13th July 2020, there are 3 vaccine candidates in phase I clinical trials, 44 vaccine candidates at pre-clinical stage, 15 monoclonal antibodies in evaluation as therapeutics as well as a few anti-viral drugs [9-11]. Serological assays have been developed to support the evaluation of the efficacy of vaccines and treatments, as well as to confirm past infection and for sero-surveillance; these include commercial and in-house assays. To assess the performance and consistency of serological assays for MERS-CoV antibodies, a multi-centre international collaborative study took place in 2018 [12]. The conclusion from that study was that the correct diagnosis of MERS-CoV was achieved in most cases when laboratories used a testing algorithm including more than one test.

Also, the use of a reference reagent greatly improved the agreement between assays, allowing more consistent and therefore more meaningful comparisons between results [12].

In this report, we have evaluated a candidate preparation to serve as the 1st WHO International Standard for anti-MERS-CoV antibodies and a Reference Panel. The Collaborative study was organised by NIBSC in collaboration with the World Health Organization (WHO), and has been facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI), which outreach to scientists in MERS-CoV-affected countries, and subsequently sponsored the sourcing and formulation of the candidate material.

International Standards (IS) are recognised as the highest order of reference materials for biological substances and they are assigned potencies in International Units (IU). International Standards are used to quantify the amount of biological activity present in a sample in terms of the IU, allowing assays from different laboratories to be compared. This makes it possible to better define parameters such as the analytical sensitivity of tests or clinical parameters such as protective levels of antibody. The availability of an IS for anti-MERS-CoV antibodies would facilitate the standardisation of MERS serological assays used for detection of MERS-CoV infection and in vaccinology studies to measure antibodies elicited by human vaccination.

The aims of this study are to:

- Assess the suitability of different antibody preparations to serve as the International Standard with an assigned unitage per mL for use in the harmonisation of MERS-CoV serological assays.
- Characterise the antibody preparations in terms of reactivity/specificity in different assay systems.
- Assess each preparation's potency i.e. readout in a range of typical assays performed in different laboratories.
- Assess commutability i.e. to establish the extent to which each preparation is suitable to serve as a standard for the variety of different samples and assay types.
- Recommend to the WHO ECBS, the antibody preparation(s) found to be suitable to serve as the standard and propose an assigned unit.

Materials and Methods

Ethical statement

Collection of convalescent sera from PCR-confirmed MERS-CoV-infected patients in the study was coordinated by Dr Manki Song (International Vaccine Institute, South Korea) and Prof Yeon-Sook Kim (Chungnam National University Hospital, South Korea). All patient donors gave informed consent for the use of their serum, and samples were anonymised. The National Institute for Biological Standards and Control (NIBSC) Human Material Advisory Committee (project 16/005MP) approved this project.

Candidate 1st WHO International Standard for anti-MERS-CoV antibody

The candidate International Standard for anti-MERS-CoV antibody (NIBSC code 19/178) is a freeze-dried preparation of a pool of sera from 2 recovered MERS patients from South Korea. Material was collected more than one year post-discharge from hospital, in 5 visits within a 4 month period. Anti-MERS-CoV antibody titre between the visits was tested by ELISA and neutralisation assay and no variation was observed. Donations from each patient were processed at NIBSC using a solvent-detergent treatment to minimise the risk of the presence of enveloped viruses [13] and tested for known blood-borne markers (HIV ab, HBsAg and HCV RNA) and found negative. Approximately 1200 of 2.5mL glass DIN ampoules containing 0.25 mL pooled sera were lyophilised between 17th-21st October 2019. Freezing was performed in CS100 freeze drier to -50°C for 4 hours. Primary drying was performed at -35°C for 30 hours at 100 µbar vacuum followed by a ramp to 25°C over 10 hours then 30 hours secondary drying at 25°C and 30 µbar vacuum. Vials were back filled with dry nitrogen to atmospheric pressure and sealed.

Sera from convalescent MERS-CoV-infected individuals

The source material donated from IVI was collected from 4 MERS recovered patients, more than one year post-discharge from hospital, anonymised to CNUH-3,-8,-9,-10. Donors were selected by the presence of high levels of anti-MERS-CoV antibodies. Pooling of the sera was decided based on the titre obtained from ELISA at IVI, MERS-CoV neutralising antibody titre from Prof Nam Hyuk Cho (Seoul National University) and pseudotyped virus neutralising antibody titre at NIBSC. The two highest titre sera (CNUH-3 and CNUH-10) were selected as source material for the candidate International Standard (19/178). Sera from the remaining two individuals (CNUH-8 and CNUH-9) were pooled to produce a candidate Working Reagent (NIBSC code 19/180) formulated as the freeze-dried equivalent of 0.25 mL in glass ampoules in the same way as the candidate International Standard as the freeze-dried equivalent of 0.25 mL.

For the collaborative study, serum from each of the four MERS recovered individuals was also provided as a liquid preparation, frozen and filled in 0.1 mL aliquots in screw cap tubes, to assess harmonisation of the results by the International Standard.

Purified polyclonal trans-chromosomal (Tc) bovine anti-MERS-CoV immunoglobulins

The source materials were donated by Dr Eddie J. Sullivan, SAB Biotherapeutics, Inc. USA, and comprises human anti-MERS-CoV IgG antibodies purified from plasma collected from trans-chromosomal (Tc) cattle [14, 15] immunised with purified Al-Hasa strain MERS-CoV spike protein nanoparticles (SAB-301) or cobalt irradiated Jordan strain MERS-CoV (SAB-300) [16]. Briefly, plasma was fractionated with caprylic acid in combination with a filtration step using a depth filter to remove non-IgG proteins; further purified by using human IgG light chain kappa specific affinity chromatography; a second purification using bovine IgG heavy chain specific affinity chromatography was undertaken to remove any residual bovine IgG. Upon receipt at NIBSC, the purified Tc bovine IgG samples were diluted to a target protein concentration of 2.5 mg/mL in sterile PBS/Ca²⁺/Mg²⁺ supplemented with 5% human serum albumin and aliquoted into 0.1 mL volumes and stored at -20°C. The concentration was chosen to provide a sufficient number of ampoules for the collaborative study without compromising its activity.

Additional samples included in the collaborative study

MERS-CoV negative human plasma was sourced from NIBSC. The plasma was from single donation packs from the UK; all packs were tested for blood borne markers (HIV, HCV, HBsAg and syphilis) and found to be negative.

Coded study samples

Table 1 lists the collaborative study samples, provided to the participants coded and blinded. Each participant received three sets of 11 samples per method in use in their laboratory, plus one spare. Samples were labelled “MERS CS Sample Sx”, where x was one of the following numbers 1, 2, 3, 5, 8, 13, 21, 34, 55, 89 or 144. Samples were shipped on dry ice under reference CS658 or CS675 as explained in the study design below.

Participants

Twenty laboratories accepted to participate in the study. Due to the COVID19 pandemic, only 11 laboratories completed the study and they are listed in Appendix 1. The participants were from 7 countries: Australia (1), P.R. China (1), South Korea (1), Austria (1), Germany (2), United Kingdom (3) and U.S.A. (2). All laboratories are referred to by a code number randomly allocated and not reflected in the order presented in Appendix 1. Participating organisations in the study included three vaccine developers, four national control/reference laboratories, a kit manufacturer, a non-profit vaccine research organisation and two academic laboratories

Study design

Results presented in this report were obtained in two studies: a feasibility study under NIBSC dispatch reference CS658 and the WHO Collaborative study, CS675. Protocols are given in Appendix 2 and 3 respectively. The feasibility study CS658 was launched in November 2019 and participants returned results by February 2020. It involved three vaccine developers, two national control laboratories and IVI, as highlighted with an asterisk in Appendix 1. The study was conducted with an accelerated timeframe for the evaluation of a Working Standard to be used by CEPI-sponsored vaccine developers for the development and calibration of serological assays. The WHO Collaborative study took place between March-June 2020. In both cases, the participants were requested to test the same study samples using their established method(s) for the detection of antibodies against MERS-CoV. Participants were asked to perform three independent assays. For each assay, participants were requested to make at least two independent serial dilutions of the study samples. Participants were requested to record in the provided Excel reporting sheet all essential information including the raw data from each assay.

Assay methods

Assays used by the participants are summarised in Table 2. Where laboratories performed multiple assay methods, laboratory codes are followed by a letter indicating the different methods e.g. lab 1a, 1b. The methods used detected binding antibodies (ELISA, immunofluorescence assay) and functional antibodies (neutralisation assays). All the ELISAs

were specific for human IgG. The testing comprised commercial kits and in-house methods. Additionally, in one method MERS-CoV infected cells were used as a target in an indirect immunofluorescence assay. The neutralisation assays included MERS-CoV (EMC2012 isolate) and pseudo-neutralisation assays based on a HIV-based vector with a luciferase reporter gene. The spike protein from three different isolates was used in the pseudotype-neutralisation assay.

Stability study of candidate International Standard

Stability of the lyophilised ampoules of the candidate International Standard 19/178 was assessed in an accelerated degradation study. Fifteen ampoules of 19/178 were stored at the following temperatures -20, +4, +20, +37 and +45°C. Three ampoules for each temperature were retrieved at the following time points: 2 weeks, one month, 3 months and 6 months. The final 3 ampoules will be retrieved at the one year time point on 6th November 2020. The potency of the preparations was assessed in a pseudotyped virus-based neutralisation assay. The stability of the candidate material was assessed using the Arrhenius model for accelerated degradation studies with potencies expressed relative to the samples stored at -20°C [17].

MERS-CoV-spike pseudotyped lentivirus-based neutralisation assay

The potency of neutralising anti-MERS-CoV antibodies in the samples was assessed using lentiviral particles expressing MERS-CoV spike protein on their surface and containing firefly luciferase as marker gene for infection, as described by Grehan [18]. Briefly, 10,000 Huh7 cells per well in a 96-well plate were seeded the day before the neutralisation assay in 0.1 mL/well Dulbecco's modified essential medium, supplemented with 10%(v/v) foetal calf serum and penicillin/streptomycin. The following day, samples were 2-fold serially diluted in culture medium starting at 1/20. The MERS-CoV spike pseudotyped lentivirus was added to each well at a concentration of 150 TCID₅₀/well and incubated for 1 hour at 37°C. The antibody-pseudotyped lentivirus complex was then added to the cells, and incubated for 72 hours. Infection was detected by adding the firefly luciferase substrate (Promega BrightGlo) and luminescence was recorded by GloMax Navigator.

Statistical methods

For the neutralisation assays, the geometric mean (GM) of the potency of each sample was calculated from the endpoint titres or 50% reduction neutralisation titres (NT₅₀) provided by the participants.

Quantitative ELISA data were analysed by fitting a sigmoid curve model with log₁₀-transformed responses. Due to testing the samples at fewer dilutions than other laboratories, analysis of data from laboratories 1a, 1b, 10a, 10b and 11a was performed using a parallel line model.

Calculations were performed using the European Directorate for the Quality of Medicines & Healthcare (EDQM) software CombiStats™ [19]. Model fit was assessed visually, and non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference samples under consideration. The samples were concluded to be non-parallel when the slope ratio was outside of the range 0.80 – 1.25. Relative potency estimates from all valid assays were combined to generate an unweighted geometric mean (GM) for each laboratory and assay type, with these laboratory means being used to calculate overall unweighted geometric means for each analyte.

Variability between laboratories has been expressed using geometric coefficients of variation ($GCV = [10^s - 1] \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates). Further assessment of agreement in geometric mean results for each pair of laboratories was performed by calculating Lin's concordance correlation coefficient with log transformed data, although these values are only based on a small number of samples. Calculations were performed using the R package DescTools [20].

Results

Collaborative study data received

The results presented in this report have been collected through two collaborative studies, a feasibility study under NIBSC dispatch reference CS658, and the WHO Collaborative Study, NIBSC dispatch reference CS675. In October 2019, seven participants joined the feasibility study CS658. One participant withdrew due to COVID-19 commitments. The remaining participants returned results by February 2020. Between November and December 2019, 13 laboratories accepted to join the WHO Collaborative study for the establishment of the 1st WHO International Standard for anti-MERS-CoV antibody, NIBSC reference CS675. Three laboratories withdrew in early February 2020 before the collaborative study samples were shipped due to COVID-19 commitments. One laboratory could not receive the study samples, due to lockdown restriction. Two laboratories received the study samples at the end of April 2020 and could not return results on time. A further two participants could not perform experiments due to restriction in place during lockdown. Overall, from both studies 11 laboratories returned data sets from 22 methods (Table 2). These methods comprised ten ELISAs, nine neutralisation assays, two immunofluorescence assays and one immunofocus assay.

All the ELISAs were specific for human IgG. Five laboratories used commercial ELISA kits, lab 1a used the quantitative Alpha Diagnostic kit targeting nucleoprotein (Np) while labs 1b, 9a, 10a, 11a used the semi-quantitative EUROIMMUN anti-MERS coronavirus ELISA (IgG, human). Five laboratories tested the samples in their in-house quantitative ELISA methods, targeting the whole spike protein (lab 2 and 5), subunit 1 of the spike protein (S1, labs 3b and 4a) or receptor binding domain (RBD, Lab 10b). Two laboratories used MERS-CoV infected cells as target in an indirect immunofluorescence assay; lab 7 used an in-house quantitative method, while lab 9b used a commercial indirect immunofluorescence test (IIFT) targeting IgG and IgM. As the results for IgM were all negative, only data for the IgG are included. Lab 6 used a qualitative immunofocus assay, with a recombinant virus harbouring MERS-CoV Spike protein infected cells as a target.

Three laboratories returned data from neutralisation assays using MERS-CoV (labs 3a, 4e, 11b). Two laboratories performed a plaque reduction neutralisation assay (4e and 11b), while lab 3a reported the data on complete neutralisation by detection of cytopathic effect. In all cases the EMC2012 isolate was used. Samples were also tested for neutralisation in MERS-CoV spike pseudotyped virus based assays, using a HIV vector incorporating a luciferase reporter gene by six participants. The spike proteins used for pseudotyping of the lentiviral particles were from three different isolates (EMC2012, England 1, KOR/KNIH/002).

All laboratories returned results from three independent assays, with the exception of lab 7, which only performed the test twice, due to time constraints.

Scoring study samples as reactive for MERS-CoV antibodies

The collaborative study samples (Table 1) included eight expected positive samples (S1, S2, S3, S5, S8, S13, S34 and S55) and three negative samples (S21, S89, S144). Sample S2 contains purified polyclonal human IgG from a transchromosomal (tc) bovine immunised with MERS-CoV spike protein, therefore reactivity towards other MERS-CoV proteins is not expected. Three samples, the candidate WHO IS (S55), the candidate Working Reagent (S8) and the negative plasma (S144) were lyophilised and required reconstitution with 0.25 mL of denionised water. The remaining samples were liquid preparations, shipped frozen.

Table 3 summarises the qualitative outcome (positive/negative) in testing the samples by each method. Five laboratories reported a false positive for sample S21 and S144, human negative plasma: 3 out of 3 independent experiments by lab 2, 4a and 6, 2 out of 3 by lab 3b, and 1 out of 3 by lab 11a. These were methods measuring binding antibodies, and the results were possibly caused by the assay cut-off being set too low. Lab 1c was the only lab performing a neutralisation assay reporting S21 as a low positive in one out of 3 independent assays. Lab 1a, using the Alpha Diagnostics anti-MERS-CoV NP ELISA kit, reported the tc bovine samples S2 and S13 as negative. While this is expected for S2, as the tc bovine was immunised only with the spike protein and not NP, we cannot provide an explanation for the false negative result for S13: polyclonal IgG from tc bovine immunised with killed MERS-CoV. As this method is the only assay targeting anti-NP antibody, we cannot conclude whether S13 does not contain anti-MERS-CoV NP antibody, or the commercial kit is incompatible with the tc bovine samples. Lab 11 scored the candidate Working Reagent, S8, and the candidate WHO IS, S55 as negative in both the commercial ELISA and in-house neutralisation assay. This result is quite surprising as S55 is a pool of samples S3 and S34, both of which scored positively in the same assay; similarly, S8 is a pool of S1 and S5, both of which also scored positively in the same assay. Furthermore, both samples were clearly positive in the same commercial kit run by labs 1b, 9a and 10a. As these were the only lyophilised products, together with the negative control S144, we suggest that the issue may have been due to reconstitution of the samples.

Binding antibody immunoassays

The geometric mean of the results as reported by the participants are summarized in Table 4, and also shown in graphical form in Figure 1A. Ten laboratories performed immunoassays for the detection of MERS-CoV binding antibodies; the methods were five commercial ELISAs, five in-house ELISAs, two immunofluorescence assays and one immunofocus assay. The EUROIMMUN kit used by labs 1b, 9a, 10a, 11a produced semi-quantitative results. In Table 4 those data are reported as the inverse of the last sample dilution which scored above the cut-off of the assay. Results from lab 3b, 10b and the immunofluorescence assays performed from lab 7 and 9b were reported in the same manner. Labs 1a, 4a, and 5 report the potency relatively to an internal control as an arbitrary unit. Lab 2 used an internal control as well, but their data are expressed in ng/mL. Lab 7 performed the experiments twice, but didn't reach an endpoint for most of the samples in their first experiment; therefore, the data reported in Table 4 are from one independent experiment. Lab 6, performing an immunofocus assay, returned results as

qualitative without an endpoint dilution series, therefore their results could not be included in Table 4.

In general there is a good similarity in the ranking of the samples, but the value of the reported potencies are up to 200-fold different between the methods. This is not surprising as the laboratories expressed their data with different outputs (*e.g.* arbitrary unit, ng, inverse of the dilution factor). The raw data reported by the participants were analysed by parallel line analysis at NIBSC and reported as relative to the candidate WHO IS, sample S55 with an arbitrary assigned unitage of 1000 International Unit per millilitre (IU/mL). Expressing the data in IU/mL increases harmonisation of the data, with a difference in sample potencies of less than 5-fold (Table 5 and Figure 1B). The results from lab 7 and 11a are not reported in Table 5 and Figure 1B. Lab 7 reported only one set of data as final titres and not a dilution series, therefore it was not possible to submit the data for parallel line analysis. Lab 11 scored the candidate WHO IS, sample S55 as negative so this therefore could not be used as the calibrant.

Neutralisation assays

Table 6 shows the geometric means of the neutralisation assay results as provided by the participants. The majority of the participants returned the titres as 50% neutralisation titre (NT₅₀) except lab 3a which provided the results as 100% viral neutralisation titre. The same data are also shown in Figure 2A. All laboratories correctly identified the negative samples (S21, S89 and S144); lab 1c scored the sample S21 positive (NT₅₀=23) in only one of three independent assays and therefore the geometric mean titre is reported as negative in Table 6 and Figure 2A. In all cases, the tc bovine polyclonal IgG samples were the most potent antibody preparations. Similar to the results for the ELISA, lab 11 scored the lyophilised samples, Working Reagent S8 and the candidate WHO IS S55 as negative. Sample S55 was a low positive (PRNT₅₀=20) in only one of three independent assays, and therefore reported as negative in Table 6.

The titres obtained in the MERS-CoV neutralisation assay were lower than those produced by the pseudotyped lentivirus (PV)-based neutralisation assay (Figure 2A). All the PV neutralisation assays used the same vector (HIV-1 based) and the same reporting system (luciferase). The spike protein from 3 different isolates was used to pseudotype the lentiviral particles, but no obvious pattern was observed based on the spike sequence.

The neutralising antibody potencies for each sample between laboratories were more consistent than had been observed for the ELISAs, at less than 50-fold difference. Nevertheless, expressing the titres in IU/mL, by calibration to the candidate WHO IS, sample S55 with an assigned arbitrary value of 1000 IU/mL increases harmonisation of the data to less than 2.5-fold difference between laboratories (Table 7 and Figure 2B).

Inter-laboratory variability and data concordance

The inter-laboratory variability has been expressed as a percentage of the Geometric Coefficient of Variation (%GCV) in Table 8; the variability between laboratories is reduced when sample potencies are expressed relative to the candidate IS sample S55 for both ELISA and neutralisation assays. The geometric mean of the sample titres reported in IU/mL for the ELISA is also very similar to the potencies for the neutralisation assay when expressed as relative to the candidate IS (Table 8); this allowed calculation of the potency in IU/mL of each sample combining the data from the ELISA methods and neutralisation assay. In almost all cases, the

GM value obtained for ELISA and neutralisation assay separately sits within the 95% confidence limit of the combined potency.

Expression of the antibody titres in IU/mL improves the concordance of the assays as shown in Table 9 *versus* Table 10. Concordance coefficients can range from -1 (perfect inverse correlation) to 1 (perfect matching); thus, values closer to 1 represent a similar pattern between two labs and a value closer to 0 suggests no pattern. This analysis does not include labs which have only returned qualitative data (lab 6) or final titres with no dilution series (lab 7). Expressing the results as relative to the candidate International Standard S55 greatly improved the concordance of the assays (Table 10). The poor concordance observed for lab 1a, 11a and 11b is biased by the false negative results for the tc bovine IgG samples S2 and S13 for lab 1a and for the candidate IS, S55, and Working reagent, S8, for labs 11a and 11b. It is not an indication of the performance of the assays. Indeed, lab 11a is using the same commercial kit as lab 1b, 9a and 10a, which have a good concordance coefficient of 0.92 (1b vs 9a), 0.86 (1b vs 10a) and 0.99 (9a vs 10a). Lab 11 could not be included in the Table 10, as by scoring the calibrant S55 negative, a relative titre could not be calculated. The majority of the ELISA methods have good concordance coefficients, regardless of the antigen used as target. Furthermore there is a good level of concordance with the neutralisation assays as well. MERS-CoV neutralisation and pseudotyped virus neutralisation assays have similar concordance levels between them.

Production of the candidate WHO IS and Stability study

In October 2019, NIBSC filled and freeze-dried the candidate reference material, NIBSC code 19/178. The product summary for the candidate WHO IS, sample S55, is shown in Table 11. The % coefficient of variation (CV) of the fill mass for all products and the mean residual moisture of the candidate IS and negative panel member were within acceptable limits for a WHO IS [19]. The residual oxygen content of the candidate IS fell within the NIBSC working limit of 1.1%. Currently there are approximately 1100 ampoules of 19/178 available for distribution.

The stability of the candidate International Standard has been assessed by an accelerated thermal degradation study. Ampoules of the candidate WHO IS, NIBSC code 19/178 (sample S55) were stored at different temperatures, -20°C (baseline), +4°C, +20°C, +37°C and +45°C for 2 weeks, 1 month, 3 months and 6 months. The freeze-dried preparations were reconstituted as per instructions for use and tested concurrently in triplicate by pseudotyped-based neutralisation assay. Real time data on the degradation samples are reported as relative to the baseline -20 °C, and showed minimal loss of potency up to a month, even at elevated temperatures (Figure 4). The long-term stability of the candidate WHO IS was estimated by the Arrhenius model (Table 12). The predicted loss in potency for 19/178, when stored at -20°C, was 0.395 % per year. However, statistical assessment using the chi-squared test indicated that the datasets do not readily fit the model ($\chi^2=27.292$, $p=0.004$). The results obtained from both the real time data and the predicted stability suggested that the preparation 19/178 is adequately stable to serve as 1st WHO IS for MERS-CoV antibody, and can be shipped at ambient temperature.

Discussion and conclusion

The purpose of this study was to evaluate a pool of convalescent sera from MERS-CoV recovered patients as a candidate International Standard to harmonise the results from serological assays. Eleven laboratories took part in the study; this is lower than the recommended number of participants for a WHO collaborative study [21] due to the COVID-19 pandemic afflicting countries worldwide, which has affected the logistics of delivery of the study samples and participant laboratories' resources. Nevertheless, the candidate IS has been evaluated in 22 methods which comprise the most frequently used ELISA and neutralisation assays, and also immunofluorescence test and immunofocus assay. The candidate IS (sample S55) was fit for purpose in all assays, and was able to harmonise the inter-assay variation for both binding and neutralisation assay when the potencies of the study samples were reported as relative to the IS. For the binding antibody assays, titres of the samples provided by the participants were up to 200-fold different (Table 4 and Figure 1A) but this difference was reduced to less than 5-fold, when reported in IU/mL (Table 5 and Figure 1B). Similarly, the neutralisation assays, expressing sample potencies as relative to the candidate IS, sample S55, reduced the difference in antibody titer from 50-fold to 2.5-fold (Table 6 and 7, and Figure 2). Furthermore, the geometric mean value for each sample for ELISA and neutralisation assay when expressed in IU/mL were very close. This supports the use of the same arbitrarily chosen unitage for both types of assay (Table 8).

These results confirm the previously published analysis that reporting antibody potency relative to a common reference reagent reduces inter-laboratory variability for MERS-CoV serology tests [12].

This study was not able to determine the presence of IgM in the candidate IS. This is not surprising, as the sera were collected over 1 year post disease. Only one participant (Lab 9) performed an immunofluorescent test specifically for IgM, and reported all the samples as negative (data not shown). Consequently, the candidate standard S55 can not be used to harmonise MERS-CoV IgM assays. Likewise the proposed working reagent (S8) can not act as positive control to confirm the presence of IgM.

The use of immunised tc bovine polyclonal IgG samples as a reference reagent has been proposed in the past when sourcing convalescent plasma or serum is problematic. In the study conducted by Harvey and colleagues [12], the same tc bovine polyclonal preparations S2, S13 and S89 were used, and they performed well in the majority of assays, as in this report, and S13 (sample 16 in [11]) could harmonise the data when using clinical samples. However, in both studies, some assays failed to score the tc bovine IgG samples as positive. In this report lab 1a, using a commercial kit against MERS-CoV Np reported sample S2 and S13 as negative. This is expected for S2 which was from a tc bovine immunised with spike only. It is more difficult to determine the reason for S13, whether it is an assay incompatibility with this type of preparation or whether the sample does not contain anti-Np antibodies. As this type of preparation may be very useful in the future as a reference reagent, a more detailed investigation should be performed to understand the reason behind the lack of performance in some assays.

Proposal

It is proposed that the pool of serum, sample S55, NIBSC code 19/178, is established as the WHO International Standard for the standardisation of serological assays detecting binding and

neutralising antibodies against MERS-CoV with an arbitrary assigned unitage of 250 IU/ampoule.

The preparation is only suitable for detection of human IgG and this information will be reported in the Information for Use (Appendix 4).

Approximately 1100 ampoules (0.25mL/ ampoule) are available for distribution.

Furthermore, it is proposed that sample S8, a pool of serum samples at lower antibody titre, NIBSC code 19/180, should be made available as a Working Reagent calibrated to the 1st WHO International Standard for anti-MERS-CoV antibodies by this collaborative study. The potency calculated for the working reagent is 424 IU/mL, with 95% confidence limits of 351 to 514 (n=16, combining ELISA and neutralisation data).

Comments from participants:

Nine of the eleven participants commented on the draft report, and were all favourable to the proposal.

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Tables

Table 1. Collaborative study samples

Samples were shipped under NIBSC dispatch reference CS658 or CS675

sample	Description	formulation/vol (mL)
S1, CNUH-9	convalescent serum, human (CNUH-09)	liquid 0.1
S2, pAb Spike	Tc pAb against MERS-CoV spike protein	liquid 0.1
S3, CNUH-10	convalescent serum, human (CNUH-10)	liquid 0.1
S5, CNUH-8	convalescent serum, human (CNUH-8)	liquid 0.1
S8, WR	Working Reagent candidate	f/d 0.25
S13, pAb MERS	Tc pAb against killed MERS-CoV	liquid 0.1
S21, neg	negative plasma, human	liquid 0.1
S34, CNUH-3	convalescent serum, human (CNUH-3)	liquid 0.1
S55, WHO IS	WHO International Standard candidate	f/d 0.25
S89, pAb neg	Tc pAb naïve	liquid 0.1
S144, neg	negative plasma, human	f/d 0.25

tc: trans-chromosomal bovine; pAb: polyclonal antibodies; f/d: freeze-dried

Table 2. Laboratory codes and assay methods.

Lab	Assay method	Target	Readout
1a	ELISA (Alpha Diagnostic)	NP (IgG)	U/mL *
1b	ELISA (EUROIMMUN)	recombinant MERS structural protein (IgG)	O.D. ₄₀₅
1c	PV-neutralisation assay (HIV/Luc)	Spike (England 1)	NT ₅₀
2	ELISA (in house)	Spike (EMC2012)	O.D.
3a	Neutralisation assay (CPE)	EMC2012	VNT ₁₀₀
3b	ELISA (in house)	S1 (EMC2012)	O.D. ₄₀₅
4a	ELISA (in house)	S1 (EMC2012)	U/mL *
4b	PV-neutralisation assay (HIV/Luc)	Spike (EMC2012)	NT ₅₀
4c	PV-neutralisation assay (HIV/Luc)	Spike (KOR/KNIH/002)	NT ₅₀
4d	PV-neutralisation assay (HIV/Luc)	Spike (England 1)	NT ₅₀
4e	Neutralisation assay (PRNT)	EMC2012	NT ₅₀
5	ELISA (in house)	Spike (EMC2012)	ELISA units*
6	Chimeric virus-Immunofocus assay	S1 (England 1)	P/N
7	Immunofluorescence assay	MERS-CoV infected cells (IgG)	inverse of highest positive dilution factor
8	PV-neutralisation assay (HIV/Luc)	Spike (England 1)	NT ₅₀
9a	ELISA EUROIMMUN)	recombinant MERS structural protein (IgG)	O.D. ₄₀₅
9b	Immunofluorescence assay (EUROIMMUN)	MERS-CoV (EMC2012) infected cells (IgG/IgM)	P/N
10a	ELISA (EUROIMMUN)	recombinant MERS structural protein (IgG)	O.D. ₄₀₅
10b	ELISA (in house)	RBD (EMC2012)	O.D.
10c	PV-neutralisation assay	EMC2012	NT ₅₀
11a	ELISA (EUROIMMUN)	recombinant MERS structural protein (IgG)	O.D. ₄₀₅
11b	Neutralisation assay (PRNT)	EMC2012	PRNT ₅₀

* arbitrary unit or ELISA unit from internal standard; VNT₁₀₀ = complete neutralization of cytopathic effects; NT₅₀= 50% neutralization titre; O.D.= optical density; P/N=positive/negative (qualitative assay); PRNT₅₀= 50% plaque reduction neutralisation assay; NP=nucleoprotein; S1= subunit 1 spike protein.

Table 3. Summary of the sample scoring as positive or negative for MERS-CoV antibodies

Highlighted in red are inconsistent results

		S1, CNUH09	S2, Tc Spike	S3, CNUH10	S5, CNUH08	S8, WR	S13, Tc MERS	S21, Neg	S34, CNUH03	S55, WHO IS	S89, Tc naïve	S144, Neg
ELISA	1a	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	N/N/N	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	1b	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	2	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P
	3b	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/N/P	P/P/P	P/P/P	N/N/N	N/P/P
	4a	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	5	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	9a	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	10a	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	11a	P/P/P	P/P/P	P/P/P	P/P/P	N/N/P	P/P/P	N/N/P	P/P/P	N/N/P	N/N/N	N/N/P
IFA	6	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/?/?	P/P/P
IFT	7	P/P	P/P	P/P	P/P	P/P	P/P	N/N	P/P	P/P	N/N	N/N
	9b	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
NEUT	1c	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	3a	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	4b	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	4c	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	4d	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	4e	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	8	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	10c	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N
	11b	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	N/N/P	N/N/N	N/N/N

ELISA: enzyme-linked immunosorbent assay; IFA: immunofocus assay; IFT: immunofluorescence test; NEUT: neutralisation assay; P: positive; N:negative

Table 4. Geometric mean of three independent experiments of MERS-CoV binding antibodies, as reported by the participants

		S1, CNUH-09	S2, Tc Spike	S3, CNUH-10	S5, CNUH-08	S8, WR	S13, Tc MERS	S21, Neg	S34, CNUH-03	S55, WHO IS	S89, Tc naïve	S144, Neg	output
ELISA	1a	666	<100	1291	1341	922	<100	<100	546	853	<100	<100	unit/mL
	1b	100	1600	252	100	100	1600	<100	159	200	<100	<100	titre*
	2	8720	25446	30414	7280	7675	339387	136	12473	20655	<3	159	ng/mL
	3b	5770	148278	16506	3181	4343	161081	2596	12392	10833	<2500	4570	titre*
	4a	4162	188382	12608	3581	3866	291407	<100	7250	9482	<100	<100	unit/mL
	5	6984	135806	32277	6009	6084	254364	neg	11445	19416	neg	neg	unit
	9a	347	10079	1101	347	397	23076	<100	693	1000	<100	<100	titre*
	10a	127	6342	503	101	127	5033	<100	127	634	<100	<100	titre*
	10b	432	13971	1863	253	343	13422	<100	432	931	<100	<100	titre*
	11a	215	>1000	448	215	<10	>1000	<10	208	<10	<10	<10	titre*
IFT	7	160	>640	320	160	80	>640	<40	320	320	<40	<40	titre*
	9b	1000	10000	10000	1000	1000	100000	<10	10000	10000	<10	<10	titre*

* titer was calculated as the inverse of the highest positive dilution factor;

Table 5. Geometric mean of MERS-CoV binding antibodies expressed relative to the candidate WHO IS, sample S55, with an assigned arbitrary value of 1000 IU/mL

		S1, CNUH-09	S2, Tc Spike	S3, CNUH-10	S5, CNUH-08	S8, WR	S13, Tc MERS	S21, Neg	S34, CNUH-03	S55, WHO IS	S89, Tc naïve	S144, Neg
ELISA	1a	803	-	1366	1404	986	-	-	702	1000*	-	-
	1b	419	NP	1319	373	404	NP	-	867	1000*	-	-
	2	422	12648	1476	352	371	18771	-	652	1000*	-	-
	3b	687	10735	1424	536	611	15859	35	1275	1000*	-	41
	4a	440	19014	1269	372	396	31032	-	756	1000*	-	-
	5	360	6995	1662	310	313	13101	-	594	1000*	-	-
	9a	396	12026	1083	340	450	22140	-	626	1000*	-	-
	10a	328	10832	1010	282	452	16229	-	606	1000*	-	-
	10b	618	43383	2756	300	444	41918	-	800	1000*	-	-
IFT	9b	464	1000	1000	100	100	10000		1000	1000*	-	-

NP: not parallel; (-): negative; * assigned.

Table 6. Geometric mean of three independent experiments of MERS-CoV neutralising antibodies, as reported by the participants

Lab	S1, CNUH-09	S2, Tc Spike	S3, CNUH-10	S5, CNUH-08	S8, WR	S13, Tc MERS	S21, Neg	S34, CNUH-03	S55, WHO IS	S89, Tc naïve	S144, Neg
1c	139	3426	903	122	171	3459	<20	409	451	<20	<20
3a	80	941	274	59	47	1185	<10	118	137	<10	<10
4b	141	3200	283	79	100	3200	<10	224	224	<10	<10
4c	252	4032	566	159	141	4032	<10	566	356	<10	<10
4d	63	1600	126	35	40	1600	<10	126	112	<10	<10
4e	43	748	108	33	47	1005	<10	78	78	<10	<10
8	747	17486	2165	796	690	14735	<40	1435	1343	<40	<40
10c	879	13886	2082	771	545	24735	<100	1639	1342	<100	<100
11b	40	596	141	28	<20	503	<20	32	<20	<20	<20

Table 7. Geometric mean of MERS-CoV neutralising antibodies expressed relative to the candidate WHO IS, sample S55, with an assigned arbitrary value of 1000 IU/mL

Lab	S1, CNUH09	S2, Tc Spike	S3, CNUH10	S5, CNUH08	S8, WR	S13, Tc MERS	S21, Neg	S34, CNUH03	S55, WHO IS	S89, Tc naïve	S144, Neg
1c	307	7592	2002	270	380	7666	-	906	1000*	-	
3a	583	6858	2000	429	340	8640	-	857	1000*	-	
4b	630	14254	1260	354	445	14254	-	1000	1000*	-	
4c	707	11314	1587	445	397	11314	-	1587	1000*	-	
4d	561	14254	1122	315	354	14254	-	1122	1000*	-	
4e	550	9604	1381	427	600	12909	-	997	1000*	-	
8	556	13015	1612	592	514	10968	-	1068	1000*	-	
10c	655	10350	1552	574	406	18436	-	1221	1000*	-	

(-): negative; * assigned

Table 8. Geometric mean value and variance of the antibody titres in the study samples.

The percentage of geometric coefficient of variation (%GCV) is calculated on the sample potencies as reported by the participants using ELISA (Table 4) or neutralisation assay (Table 6). The geometric mean value (GM) and variability (%GCV) is calculated for when the binding (Table 5) and neutralising antibody titres (Table 7) are expressed as relative to the candidate WHO IS, sample S55 with an assigned potency of 1000 IU/mL. Laboratory 6, 7, 1a, 11a, 11b were excluded from the calculation. Values are highlighted in green when a reduction in the %GCV is measured. The 95% confidence limit (95% CL) for the geometric mean values are reported as lower confidence limit (LCL) and upper confidence limit (UCL).

		S1, CNUH09	S2, Tc Spike	S3, CNUH10	S5, CNUH08	S8, WR	S13, Tc MERS	S21, Neg	S34, CNUH03	S55, WHO IS	S89, Tc naïve	S144, Neg
ELISA	%GCV (reported)	424%	523%	503%	398%	394%	608%		591%	439%		
	%GCV (IU/mL)	29%	79%	36%	22%	21%	51%		29%			
	GM (IU/mL)	445	13935	1431	352	423	21036		748	1000		
	95%CL (LCL-UCL)	360-551	8144-23845	1105-1855	298-415	360-497	14384-30764		604-927	assigned		
Neut Assay	%GCV (reported)	213%	187%	219%	257%	201%	226%		278%	194%		
	%GCV (IU/mL)	30%	36%	25%	29%	23%	30%		22%			
	GM (IU/mL)	542	10592	1534	393	425	11160		1057	1000		
	95% CL (LCL-UCL)	424-693	8041-13953	1251-1882	310-499	351-514	8932-13944		877-1273	assigned		
Combined	GM (IU/mL)	488	12149	1479	370	424	15322		879	1000		
	95% CL (LCL-UCL)	420-567	9285-15898	1277-1712	327-420	351-514	11738-19999		750-1030	assigned		

Table 9. Level of concordance between laboratory methods, as reported by the participants.

Concordance coefficients were calculated using the potencies reported by the participants. Light green indicates an arbitrary chosen concordance coefficient between 0.75-0.89; darker green indicates an arbitrary chosen concordance coefficient between 0.9 and 1. Commercial ELISA kits include Alpha Diagnostics (Alph Diagn) and EUROIMMUN; the target antigen of the in-house ELISA methods is denoted by Spike: MERS-CoV Spike protein; S1: subunit 1 of the Spike protein; RBD: receptor binding domain; EMC2012: MERS-CoV neutralisation assay using EMC2012 isolate; PV-Neut: pseudotyped virus based neutralisation assay. IFT – immunofluorescence assay

	Lab	1a	1b	2	3b	4a	5	9a	10a	10b	11a	1c	3a	4b	4c	4d	4e	8	10c	11b	9b
ELISA	1a-Alpha Diagn		0.01	0.01	-0.02	0.00	0.01	0.03	0.04	0.12	0.08	0.01	0.01	-0.02	-0.06	-0.02	0.00	0.08	-0.07	0.02	-0.04
	1b-EuroImmun	0.01		0.13	0.15	0.18	0.13	0.54	0.84	0.55	0.35	0.82	0.93	0.95	0.79	0.89	0.80	0.37	0.37	0.69	0.21
	2-Spike	0.01	0.13		0.76	0.92	0.98	0.31	0.21	0.31	0.02	0.18	0.11	0.17	0.18	0.13	0.10	0.35	0.37	0.10	0.52
	3b-S1	-0.02	0.15	0.76		0.98	0.95	0.39	0.25	0.38	0.02	0.23	0.14	0.20	0.23	0.16	0.14	0.45	0.48	0.11	0.67
	4a-S1	0.00	0.18	0.92	0.98		0.93	0.45	0.29	0.43	0.03	0.25	0.15	0.23	0.26	0.18	0.14	0.50	0.52	0.13	0.68
	5-Spike	0.01	0.13	0.98	0.95	0.93		0.33	0.21	0.32	0.03	0.19	0.12	0.17	0.19	0.13	0.10	0.36	0.39	0.10	0.59
	9a-EuroImmun	0.03	0.54	0.31	0.39	0.45	0.33		0.78	0.97	0.27	0.78	0.44	0.67	0.80	0.48	0.38	0.90	0.91	0.33	0.58
	10a-EuroImmun	0.04	0.84	0.21	0.25	0.29	0.21	0.78		0.82	0.59	0.92	0.74	0.93	0.89	0.75	0.63	0.58	0.58	0.60	0.34
	10b-RBD	0.12	0.55	0.31	0.38	0.43	0.32	0.97	0.82		0.37	0.80	0.47	0.69	0.81	0.49	0.38	0.88	0.88	0.36	0.53
	11a-EuroImmun	0.08	0.35	0.02	0.02	0.03	0.03	0.27	0.59	0.37		0.54	0.29	0.36	0.38	0.09	0.09	0.09	0.07	0.12	0.05
Neut	1c-PV neut	0.01	0.82	0.18	0.23	0.25	0.19	0.78	0.92	0.80	0.54		0.70	0.91	0.96	0.68	0.56	0.59	0.58	0.49	0.35
	3a-EMC2012	0.01	0.93	0.11	0.14	0.15	0.12	0.44	0.74	0.47	0.29	0.70		0.85	0.67	0.95	0.91	0.31	0.31	0.81	0.19
	4b-PV neut	-0.02	0.95	0.17	0.20	0.23	0.17	0.67	0.93	0.69	0.36	0.91	0.85		0.91	0.87	0.74	0.49	0.49	0.62	0.27
	4c-PV neut	-0.06	0.79	0.18	0.23	0.26	0.19	0.80	0.89	0.81	0.38	0.96	0.67	0.91		0.67	0.53	0.62	0.62	0.42	0.34
	4d-PV neut	-0.02	0.89	0.13	0.16	0.18	0.13	0.48	0.75	0.49	0.09	0.68	0.95	0.87	0.67		0.95	0.33	0.34	0.83	0.21
	4e-EMC2012	0.00	0.80	0.10	0.14	0.14	0.10	0.38	0.63	0.38	0.09	0.56	0.91	0.74	0.53	0.95		0.26	0.26	0.93	0.16
	8-PV neut	0.08	0.37	0.35	0.45	0.50	0.36	0.90	0.58	0.88	0.09	0.59	0.31	0.49	0.62	0.33	0.26		0.98	0.23	0.62
	10c-PV neut	-0.07	0.37	0.37	0.48	0.52	0.39	0.91	0.58	0.88	0.07	0.58	0.31	0.49	0.62	0.34	0.26	0.98		0.22	0.68
	11b-EMC2012	0.02	0.69	0.10	0.11	0.13	0.10	0.33	0.60	0.36	0.12	0.49	0.81	0.62	0.42	0.83	0.93	0.23	0.22		0.14
	9b-IFT	-0.04	0.21	0.52	0.67	0.68	0.59	0.58	0.34	0.53	0.05	0.35	0.19	0.27	0.34	0.21	0.16	0.62	0.68	0.14	

Table 10. Level of concordance between laboratory methods, expressed as relative to the candidate WHO IS.

Concordance coefficients were calculated using the potencies expressed in IU/mL as relative to the candidate International Standard sample S55. Light green indicates an arbitrary chosen concordance coefficient between 0.75-0.89; darker green indicates an arbitrary chosen concordance coefficient between 0.9 and 1. Commercial ELISA kits include Alpha Diagnostics (Alph Diag) and EUROIMMUN; the target antigen of the in-house ELISA methods is denoted by Spike: MERS-CoV Spike protein; S1: subunit 1 of the Spike protein; RBD: receptor binding domain; EMC2012: MERS-CoV neutralisation assay using EMC2012 isolate; PV-Neut: pseudotyped virus based neutralisation assay. IFT - immunofluorescence assay

	Lab	1a	1b	2	3b	4a	5	9a	10a	10b	1c	3a	4b	4c	4d	4e	8	10c	9b
ELISA	1a-Alpha Diag		0.02	0.09	-0.13	0.04	0.10	0.05	0.03	0.06	0.05	0.09	-0.12	-0.15	-0.14	-0.03	0.12	0.00	-0.02
	1b-Euroimmun	0.02		0.96	0.74	0.99	0.91	0.92	0.86	0.82	0.91	0.90	0.92	0.80	0.92	0.89	0.84	0.82	0.48
	2-Spike	0.09	0.96		0.96	0.99	0.98	1.00	0.99	0.94	0.95	0.95	0.98	0.95	0.98	0.97	0.97	0.97	0.71
	3b-S1	-0.13	0.74	0.96		0.82	0.86	0.96	0.94	0.90	0.96	0.94	0.98	0.98	0.97	0.99	0.97	0.99	0.61
	4a-S1	0.04	0.99	0.99	0.82		0.98	0.99	0.97	0.97	0.84	0.91	0.97	0.94	0.97	0.85	0.96	0.96	0.68
	5-Spike	0.10	0.91	0.98	0.86	0.98		0.97	0.98	0.88	0.92	0.97	0.96	0.93	0.96	0.90	0.93	0.94	0.77
	9a-Euroimmun	0.05	0.92	1.00	0.96	0.99	0.97		0.99	0.93	0.93	0.93	0.98	0.94	0.98	0.97	0.95	0.96	0.70
	10a-Euroimmun	0.03	0.86	0.99	0.94	0.97	0.98	0.99		0.91	0.95	0.93	0.97	0.93	0.97	0.97	0.95	0.95	0.73
Neut	10b-RBD	0.06	0.82	0.94	0.90	0.97	0.88	0.93	0.91		0.85	0.84	0.93	0.89	0.92	0.89	0.89	0.91	0.60
	1c-PV neut	0.05	0.91	0.95	0.96	0.84	0.92	0.93	0.95	0.85		0.97	0.94	0.94	0.95	0.97	0.94	0.92	0.76
	3a-EMC2012	0.09	0.90	0.95	0.94	0.91	0.97	0.93	0.93	0.84	0.97		0.95	0.96	0.95	0.97	0.96	0.96	0.69
	4b-PV neut	-0.12	0.92	0.98	0.98	0.97	0.96	0.98	0.97	0.93	0.94	0.95		0.98	1.00	0.99	0.98	0.98	0.65
	4c-PV neut	-0.15	0.80	0.95	0.98	0.94	0.93	0.94	0.93	0.89	0.94	0.96	0.98		0.98	0.98	0.98	0.98	0.64
	4d-PV neut	-0.14	0.92	0.98	0.97	0.97	0.96	0.98	0.97	0.92	0.95	0.95	1.00	0.98		0.98	0.97	0.98	0.69
	4e-EMC2012	-0.03	0.89	0.97	0.99	0.85	0.90	0.97	0.97	0.89	0.97	0.97	0.99	0.98	0.98		0.99	0.98	0.65
	8-PV neut	0.12	0.84	0.97	0.97	0.96	0.93	0.95	0.95	0.89	0.94	0.96	0.98	0.98	0.97	0.99		0.98	0.61
	10c-PV neut	0.00	0.82	0.97	0.99	0.96	0.94	0.96	0.95	0.91	0.92	0.96	0.98	0.98	0.98	0.98	0.98		0.65
	9b-IFT	-0.02	0.48	0.71	0.61	0.68	0.77	0.70	0.73	0.60	0.76	0.69	0.65	0.64	0.69	0.65	0.61	0.65	

Table 11. Candidate International Standard for anti-MERS-CoV antibody formulation review

Microbiological test for bacterial and mould/yeast colony count returned negative

Product name code	19/178
No. containers filled	1217
Mean fill mass (g)	0.2661 (n=76)
CV of fill mass (%)	1.5213
Mean residual moisture (%)	0.3858 (n=6)
CV of residual moisture (%)	10.26
Mean oxygen head space (%)	0.25 (n=6)
CV of oxygen space (%)	30.49

n= number of sample tested

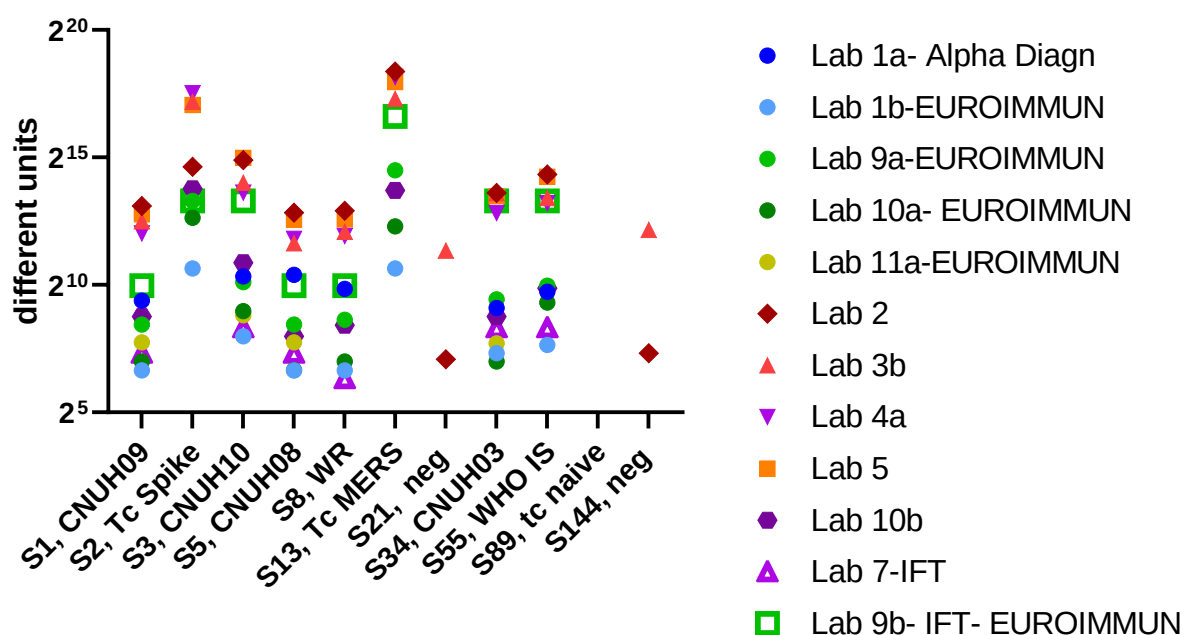
Table 12. Predicted stability of the candidate WHO IS for anti-MERS-CoV antibody, NIBSC code 19/178.

Temp (°C)	K	S.E.	% loss (per year)	95% UCL
-20	0.00396	0.00552	0.395	3.107
4	0.04937	0.03829	4.817	21.402
20	0.21103	0.08936	19.025	48.203
37	0.83803	0.1083	56.744	74.83

k: degradation rate constant; S.E.:standard error of k; UCL: upper confidence level

Figures

A.



B.

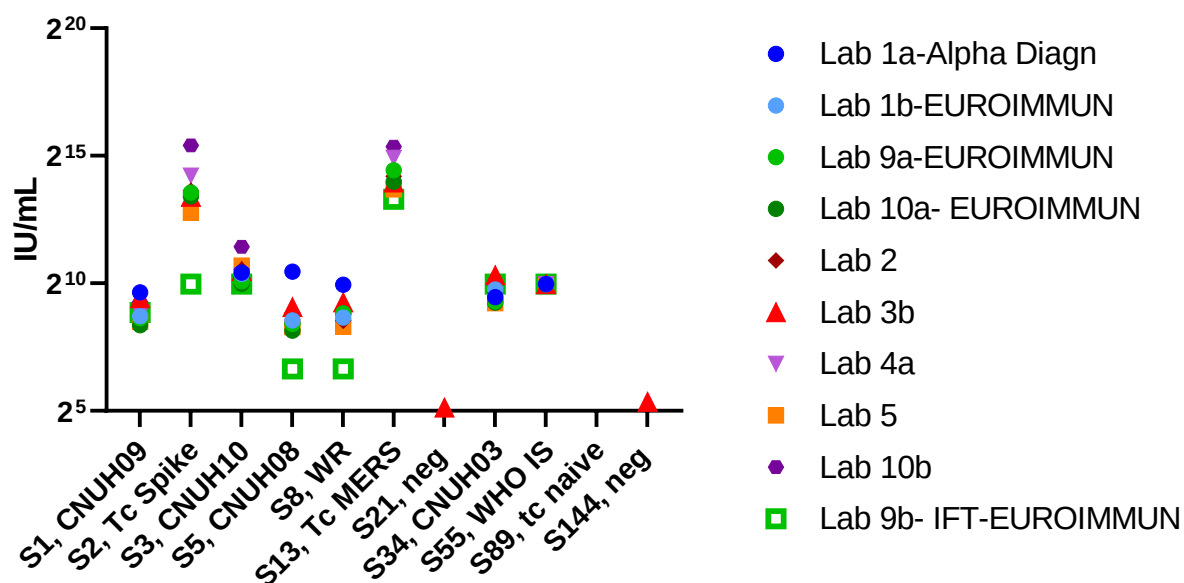
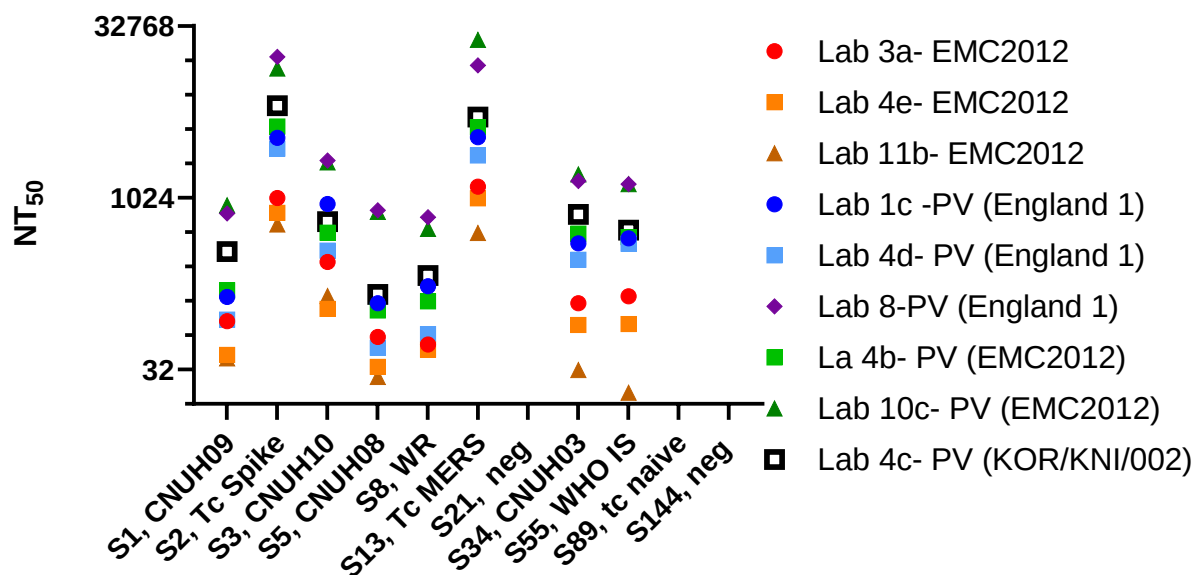


Figure 1. Harmonisation of the binding antibody titres when reported as relative to the candidate International Standard. A) The geometric mean of binding antibody titres for each sample as reported by the participants following three independent experiments (Table 4) are plotted; **B)** binding antibody titres calculated by parallel line analysis relative to the candidate

WHO IS, sample S55, assuming an arbitrary value of 1000 IU/mL. Labs 1a, 1b, 9a, 9b, 10a, 11a used commercial assays (as indicated), while labs 2, 3b, 4a, 5, 10b performed in house methods.

A.



B.

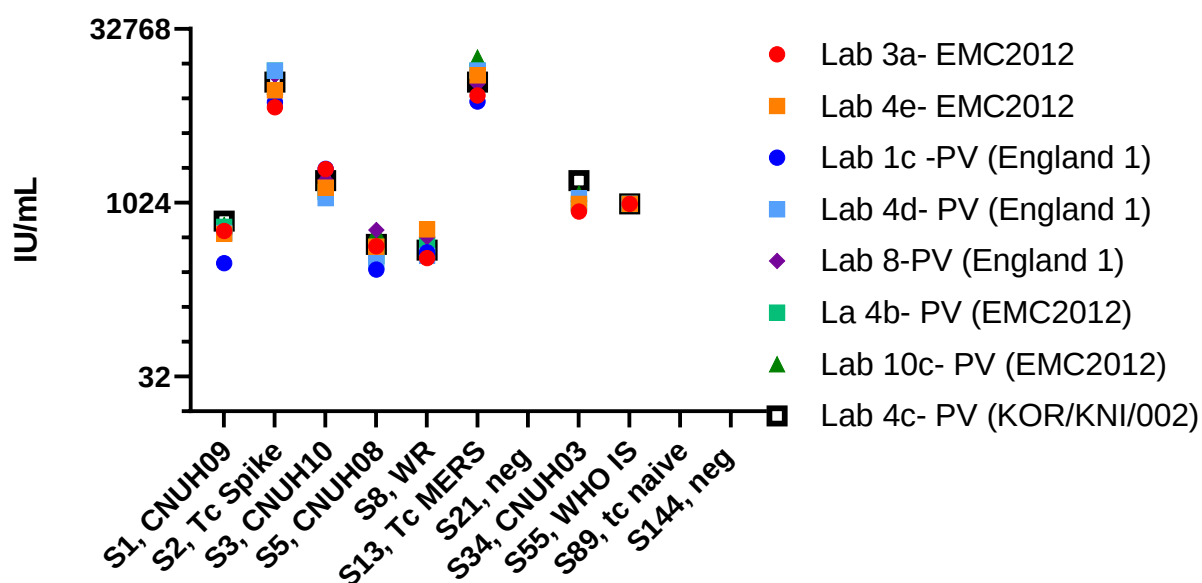


Figure 2. Harmonisation of the neutralising antibody titres when reported as relative to the candidate International Standard. A) The geometric mean of neutralising antibody titres for each sample as reported by the participants following three independent experiments (Table 5)

are plotted; **B)** neutralising antibody titres calculated by parallel line analysis relative to the candidate WHO IS, sample S55, assuming an arbitrary value of 1000 IU/mL (Table 7)

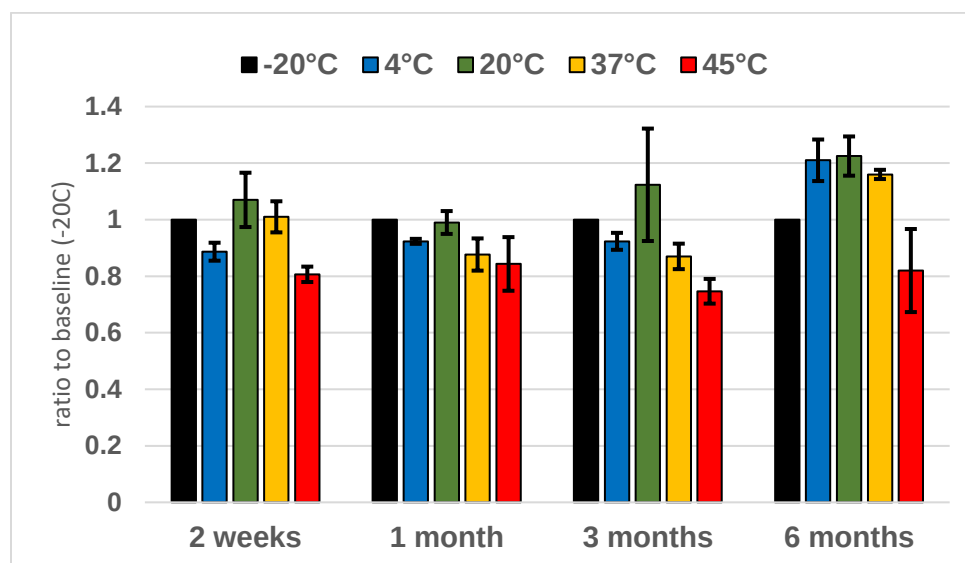


Figure 3. Thermal degradation assessment of the candidate International Standard for anti-MERS-CoV antibody. Freeze-dried ampoules of sample S55, NIBSC code 19/178 were stored at five different temperatures (-20, 4, 20, 37 and 45°C). At each time point, three vials were retrieved and reconstituted with 0.25 mL of molecular grade water. Each vial was assessed in duplicate in a pseudotyped lentivirus-based neutralisation assay. Data are reported relative to the sample kept at a storage temperature of -20°C.

Appendix 1**Collaborative study participants**

(in alphabetic order by country)

Participant	Organisation	Country
Caly Leon, Theo Karapanagiotidis	Victorian Infectious Diseases Reference Laboratory	Australia
Daniela Lupinek, Constantine Konstantoulas	Themis BioScience	Austria *
Wenling Wang, Yao Deng, Ruhan A	National Institute for Viral Disease Control and Prevention, China CDC	P.R. China
Kostanze Stiba, Katja Steinhagen	EUROIMMUN	Germany
Michael Muehlebach, Cindy Hörner, Vishakha Tiwarekar,	Paul Ehrlich Institute	Germany *
Sang Hwan Seo, Manki Song, Jae Ouk Kim	International Vaccine Institute	South Korea *
Mustapha Bittaye, Katie Ewer	Jenner Institute, University of Oxford	United Kingdom *
Emma Bentley, Samuel Richardson	National Institute for Biological Standards and Control	United Kingdom *
Nigel Temperton, Cecilia Di Genova	Viral Pseudotype Unit, University of Kent	United Kingdom
Veronique Schulten, Jean D. Boyer	Inovio Pharmaceuticals	California, U.S.A. *
Jian Zheng, Stanley Perlman	University of Iowa	Iowa, U.S.A.

*participants of the feasibility study CS658

Appendix 2 CS658 Study protocol

Protocol for the feasibility study to establish a working standard and a reference panel for anti-MERS-CoV antibodies

Background

The Coalition for Epidemic Preparedness Innovations (CEPI) together with the National Institute for Biological Standards and Control (NIBSC) are working towards producing anti-MERS-CoV antibody standards and a reference panel to support vaccine development. The availability of a communal reference preparation with an assigned unitage (unit/mL) which is assay-independent will increase harmonisation of results between laboratories worldwide and make assays from different laboratories comparable. This allows to better define parameters such as the analytical sensitivity of tests or clinical parameters such as protective levels of antibody.

Aims

The aims of this feasibility study are to

- assess the suitability of different antibody preparations to serve as the working standard with an assigned unitage per mL for use in the harmonisation of MERS serology assays.
- characterise the antibody preparations and a reference panel in terms of reactivity/specificity in different assay systems.
- assess each preparation's potency i.e. readout in a range of typical assays performed in different laboratories.
- recommend to the CEPI Programme Advisory Committee (PAC) for MERS the antibody preparation(s) found to be suitable to serve as the standard. The working standard will then be made available to vaccine developers for calibration of their assays.

Materials

Coded study samples

The study samples should be stored at -20°C or below. The study samples shall not be administered to humans.

All samples will be provided coded and blinded. The samples are labelled "MERS-CoV CS Sample Sxx" where xx is 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, or 144. The coded samples may include negative samples as well as reactive samples. Laboratories will receive at least 4 sets of study samples which should allow for 3 independent assays (plus 1 spare) by one method. Laboratories with more than one assay method will receive additional sample sets to allow 3 independent assays (plus 1 spare) per method (subject to availability).

Plasma/serum obtained from convalescent patients

The source materials are pools of serum samples obtained from patients tested positive for MERS, collected more than one year post-discharge from hospital, kindly donated by Dr Manki Song (International Vaccine Institute, Korea).

The samples are:

- four pools of convalescent sera each one from one single patient collected at multiple time points within a 4-month period with different anti-MERS antibody titres;
- two pools of two convalescent sera collected at multiple time points within a 4-month period with different anti-Lassa antibody titres;
- two pools of serum from multiple healthy UK donors.

Preparations are either freeze-dried, filled in 0.25 mL aliquots into 2.5 mL ampoules or liquid, frozen and filled in 0.1mL aliquots into screw cap tubes.

Transchromosomal (Tc) bovine anti-MERS IgG preparations

The source materials were donated by SAB Biotherapeutics, Inc. USA, and is human anti-MERS antibody purified from bovine plasma collected from transchromosomal (Tc) cattle [1] immunized with either killed MERS-CoV or recombinant MERS Spike protein. Upon receipt at NIBSC, the purified Tc Bovine IgG samples were diluted to a target protein concentration of 2.5 mg/mL in sterile PBS-Ca²⁺-Mg²⁺ supplemented with 5% human serum albumin.

Assay Methods

For testing the study samples, participants are requested to use the method(s) in routine use in their laboratory for the detection of antibodies to MERS-CoV. Laboratories may use multiple methods to test the study materials, provided that the study design (see below) is followed for each method.

Design of study

Participants are requested to:

- Perform 3 independent assays on different days for detection of antibodies against MERS-CoV.
- Reconstitute freeze-dried samples according to the Instructions for Use (IFU) supplied with the sample shipment. Use a freshly thawed/reconstituted sample for each independent assay.
- For the liquid frozen samples, use a freshly thawed aliquot for each independent assay. Each sample should be thawed at room temperature or 37°C and used immediately or placed on ice until used.
- For each independent assay, prepare and test a series of dilutions from each coded sample. If possible, at least two independent replicate series of dilutions (NOT two samplings from a single series) should be prepared and assayed. Participants are requested to dilute the samples using the sample matrix specific to their individual assay(s) (e.g. plasma, serum, buffer). **The optimal dilution range should cover at least 5 to 6 points including one point beyond the**

endpoint dilution. Adjust dilutions accordingly for subsequent assays if needed. Record in the Excel spreadsheet changes to the dilutions tested.

- Use the Excel reporting sheet to record for each dilution the raw assay readout (e.g. spectrophotometer absorbance/luminometer RLU/plaque count etc.). Our statistician will use the raw data readouts to perform the statistical analysis.
- Include the cut-off value indicating sero-reactivity for each assay and state whether each sample dilution tested is considered positive or negative according to their criteria (it is of interest for us to know whether the samples are considered 'positive' in each assay)
- Include all study samples in each assay so that the concentration of antibodies relative to one another may be calculated. Please note in the reporting sheet, if it is not practical to test all samples concurrently, indicate which samples were tested concurrently.
- Include in the results reporting sheet your internal control values. If feasible, our statistician will use the data from this study to calibrate the internal controls to the working reagent. This information will be for the participants only, who may decide to calibrate their own controls independently.
- Report qualitative data as a positive or negative reaction in the Excel reporting sheet.
- Record in the Excel reporting sheet any deviations from the assay protocol.

Results and data analysis

Participants are requested to return their results to NIBSC within 8 weeks of receipt of the study materials. If it is not practical to turn around results within 8 weeks, please inform Giada.Mattiuzzo@nibsc.org. An Excel spreadsheet is provided so that all essential information can be recorded, including details of assay methodology and the raw data obtained from each assay. The use of the reporting spreadsheet facilitates the analysis and interpretation of results. If multiple assay methods are undertaken, a separate worksheet for each method should be completed. The final version of the reporting spreadsheet will be e-mailed to each participant following shipment of study materials.

The confidentiality of each laboratory will be ensured with each participant being anonymous to the other laboratories. Analysis of the study will assess the potencies of each material relative to each other, and the sensitivity of the different assay methods.

Assay data will be analysed at NIBSC by an experienced biometrician using standard statistical techniques.

A draft study report will be sent to participants for comment. The report will include data analysis, proposed conclusions and recommendations on the selection, use and unitage of the most appropriate antibody preparation to serve as the working standard. The finalised report will be submitted to the CEPI Programme Advisory Committee (PAC) for MERS. The working

standard for MERS-CoV antibody and the reference panel will be made available immediately through NIBSC.

Participation in the feasibility study is conducted under the following conditions:

- The data obtained in the feasibility study should not be published or cited without the express permission of the NIBSC Study organizer.
- It is normal practice to acknowledge participants as contributors of data rather than co-authors in publications describing the establishment of the standard.
- Individual participant's data will be coded and reported "blind" to other participants during the preparation of the study report, and in subsequent publications.
- Participants will receive a copy of the report of the study with proposed conclusions and recommendations for comment before it is further distributed.
- Participants accept responsibility for safe handling and disposal of the materials provided.

Deadline for completed results spreadsheets is 8 weeks from receipt of study materials. If it is not practical to return results within 8 weeks, please inform Giada Mattiuzzo.

All completed results spreadsheets should be returned electronically to:

Dr Giada Mattiuzzo
Senior Scientist
Emerging Pathogens Group
Division of Virology
National Institute for Biological Standards and Control
Blanche Lane
South Mimms
Hertfordshire
EN6 3QG
UK

Tel. +44(0)1707 641283

Giada.Mattiuzzo@nibsc.org

References:

1. Matsushita, H., et al., *Triple Immunoglobulin Gene Knockout Transchromosomal Cattle: Bovine Lambda Cluster Deletion and Its Effect on Fully Human Polyclonal Antibody Production*. PLoS ONE, 2014. 9(3): p. e90383.

Appendix 3 CS675 Study protocol

Protocol for the WHO collaborative study to establish the 1st International Standard for anti-MERS-CoV antibodies

This is multi-center International collaborative study to evaluate candidate preparations to serve as 1st WHO International Standard for anti-MERS-CoV antibodies organized by NIBSC in collaboration with the World Health Organization (WHO). The study has been facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI) which sponsored the sourcing and formulation of the candidate material.

International Standards (IS) are recognized as the highest order of reference materials for biological substances and they are assigned potencies in International Units (IU). International Standards are used to quantify the amount of biological activity present in a sample in terms of the IU, making assays from different laboratories comparable. This makes it possible to better define parameters such as the analytical sensitivity of tests or clinical parameters such as protective levels of antibody. The availability of an IS for anti-MERS-CoV antibodies would facilitate the standardisation of MERS serological assays used for detection of MERS infection and in vaccinology studies to measure antibodies elicited by vaccination in humans. The establishment of such a standard will be established according to published WHO guidelines and formally endorsed by the WHO Expert Committee on Biological Standardization (ECBS) [1].

Aims

The aims of this WHO international collaborative study are to

- assess the suitability of different antibody preparations to serve as the international standard with an assigned unitage per mL for use in the harmonization of MERS-CoV serology assays.
- characterise the antibody preparations in terms of reactivity/specificity in different assay systems.
- assess each preparation's potency i.e. readout in a range of typical assays performed in different laboratories.
- assess commutability i.e. to establish the extent to which each preparation is suitable to serve as a standard for the variety of different samples and assay types.
- recommend to the WHO ECBS, the antibody preparation(s) found to be suitable to serve as the standard and propose an assigned unit.

Materials

Coded study samples

The study samples should be stored at -20°C or below. The study samples shall not be administered to humans.

All samples will be provided coded and blinded. The samples are labelled “MERS-CoV CS Sample Sxx” where xx is 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, or 144. The coded samples may include negative samples as well as reactive samples. Laboratories will receive at least 4 sets of study samples which should allow for 3 independent assays (plus 1 spare) by one method. Laboratories with more than one assay method will receive additional sample sets to allow 3 independent assays (plus 1 spare) per method (subject to availability).

Plasma/serum obtained from convalescent patients

The source materials are pools of serum samples obtained from patients tested positive for MERS, collected more than one-year post-discharge from hospital, kindly donated by Dr Manki Song (International Vaccine Institute, Korea).

The samples are:

- four pools of convalescent sera each one from one single patient collected at multiple time points within a 4-month period with different anti-MERS antibody titres;
- two pools of two convalescent sera collected at multiple time points within a 4-month period with different anti-Lassa antibody titres;
- negative controls are two pools of serum from multiple healthy UK donors.

Preparations are either freeze-dried, filled in 0.25 mL aliquots into 2.5 mL ampoules or liquid, frozen and filled in 0.1mL aliquots into screw cap tubes.

Transchromosomal (Tc) bovine anti-MERS IgG preparations

The source materials were donated by SAB Biotherapeutics, Inc. USA, and is human anti-MERS antibody purified from bovine plasma collected from transchromosomal (Tc) cattle [2] immunized with either killed MERS-CoV or recombinant MERS Spike protein. Upon receipt at NIBSC, the purified Tc Bovine IgG samples were diluted to a target protein concentration of 1 mg/mL in sterile PBS-Ca²⁺-Mg²⁺ supplemented with 5% human serum albumin.

Assay Methods

For testing the study samples, participants are requested to use the method(s) in routine use in their laboratory for the detection of antibodies to MERS-CoV. Laboratories may use multiple methods to test the study materials, provided that the study design (see below) is followed for each method.

Design of study

Participants are requested to:

- Perform 3 independent assays on different days for detection of antibodies against MERS-CoV.

- Reconstitute freeze-dried samples according to the Instructions for Use (IFU) supplied with the sample shipment. Use a freshly thawed/reconstituted sample for each independent assay.
- For the liquid frozen samples, use a freshly thawed aliquot for each independent assay. Each sample should be thawed at room temperature or 37°C and used immediately or placed on ice until used.
- For each independent assay, prepare and test a series of dilutions from each coded sample. If possible, at least two independent replicate series of dilutions (NOT two samplings from a single series) should be prepared and assayed. Participants are requested to dilute the samples using the sample matrix specific to their individual assay(s) (e.g. plasma, serum, buffer). **The optimal dilution range should cover at least 5 to 6 points including one point beyond the endpoint dilution. Adjust dilutions accordingly for subsequent assays if needed. Record in the Excel spreadsheet changes to the dilutions tested.**
- Use the Excel reporting sheet to record for each dilution the raw assay readout (e.g. spectrophotometer absorbance/luminometer RLU/plaque count etc.). Our statistician will use the raw data readouts to perform the statistical analysis.
- Include the cut-off value indicating sero-reactivity for each assay and state whether each sample dilution tested is considered positive or negative according to their criteria (it is of interest for us to know whether the samples are considered 'positive' in each assay)
- Include all study samples in each assay so that the concentration of antibodies relative to one another may be calculated. Please note in the reporting sheet, if it is not practical to test all samples concurrently, indicate which samples were tested concurrently.
- Record in the Excel reporting sheet any deviations from the assay protocol.

Results and data analysis

An Excel spreadsheet is provided so that all essential information can be recorded, including details of assay methodology and the raw data obtained from each assay. The use of the reporting spreadsheet facilitates the analysis and interpretation of results.

The confidentiality of each laboratory will be ensured with each participant being anonymous to the other laboratories. Analysis of the study will assess the potencies of each material relative to each other, and the sensitivity of the different assay methods.

A draft study report will be sent to participants for comment. The report will include data analysis, proposed conclusions and recommendations on the selection, use and unitage of the most appropriate antibody preparation to serve as the 1st WHO IS for anti-MERS-CoV antibodies. Comments will be included in the report prior to submission to the WHO ECBS. Study participants will be notified of the outcome of the study after the annual WHO ECBS meeting.

Participation in the feasibility study is conducted under the following conditions:

- The study samples have been prepared from Materials provided by donors and therefore must be treated as proprietary. The Materials must not be used for any other purpose other than for this study;
- The Materials provided must not be shared with anyone outside of the study;
- The Materials must not be used for application in human subjects or animals in the human food chain in any manner or form;
- There must be no attempt to reverse engineer, ascertain the chemical structure of, modify, or make derivatives of, any of the Materials;
- Participants accept responsibility for safe handling and disposal of the materials provided in according to the local regulations in their organization/country.
- Data obtained through testing of the Materials must not be published or cited before the formal establishment of the standard by World Health Organization, without the express permission of the NIBSC study organiser.

NIBSC, as the Collaborative Study coordinator, notes that:

- It is normal practice to acknowledge all participants as contributors of data rather than co-authors in publications;
- Data published from participating labs will be anonymised;
- Participation of this study is at the participant's discretion and does not include remuneration costs;
- Prior to the establishment of the standard NIBSC reserves the right to disclose specific information about the use of the Material(s), without acknowledgement of the study participants;
- Participants will receive a copy of the report of the study with proposed conclusions and recommendations for comment before it is further distributed.

Deadline for completed results spreadsheets is 8 weeks from receipt of study materials. If it is not practical to return results within 8 weeks, please inform Giada Mattiuzzo.

All completed results spreadsheets should be returned electronically to:

Dr Giada Mattiuzzo
Senior Scientist
Emerging Pathogens Group
Division of Virology
National Institute for Biological Standards and Control
Blanche Lane
South Mimms
Hertfordshire
EN6 3QG
UK

Tel. +44(0)1707 641283

Giada.Mattiuzzo@nibsc.org

References:

- [1] WHO, Recommendations for the preparation, characterization and establishment of international and other biological reference standards. WHO Technical Report Series, No. 932., in Expert Committee on Biological Standardization. 2006.
- [2] Matsushita, H., et al., Triple Immunoglobulin Gene Knockout Transchromosomal Cattle: Bovine Lambda Cluster Deletion and Its Effect on Fully Human Polyclonal Antibody Production. PLoS ONE, 2014. 9(3): p. e90383.

Appendix 4 Proposed International Standard for anti-MERS-CoV antibody IFU



Medicines & Healthcare products
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WHO International Standard
1st International Standard for anti-MERS-CoV antibody
NIBSC code: 19/178
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

The WHO 1st International Standard for MERS-CoV antibody is the freeze-dried equivalent of 0.25 mL of pooled serum obtained from two individuals recovered from MERS-CoV infection. The preparation has been evaluated in a WHO International Collaborative study (1). The intended use of the International Standard is for the calibration and harmonisation of serological assays against MERS-CoV. The material has been solvent-detergent treated to minimise the risk of the presence of enveloped viruses (2).

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The assigned potency of the WHO International Standard for MERS-CoV antibody is 250 IU/ampoule. When reconstituted in 0.25 mL of distilled water, the final concentration of the preparation is 1000 IU/mL.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the freeze-dried equivalent of 0.25 mL pooled human serum.

5. STORAGE

The International Standard 19/178 should be stored at -20°C or below upon receipt.

Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufacturers instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

This material should be reconstituted in 0.25 mL sterile distilled water. Following addition of water, the ampoule may be left at ambient temperature for approximately 30 minutes until dissolved and then mixed thoroughly, avoiding the generation of excessive foam.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory



NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

- (1) Mattiuzzo et al. Establishment of the 1st WHO International Standard for anti-MERS-CoV antibody. 2020, WHO Expert Committee on Biological Standardization. WGO/BS/2020.xxxx
- (2) Dichtelmüller et al. Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion. 2009;49:1931–43.

10. ACKNOWLEDGEMENTS

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11. FURTHER INFORMATION

Further information can be obtained as follows;

This material: enquiries@nibsc.org

WHO Biological Standards:

<http://www.who.int/biologicals/en/>

JCTLM Higher order reference materials:

<http://www.bipm.org/en/committees/jc/jctlm/>

Derivation of International Units:

http://www.nibsc.org/standardisation/international_standards.aspx

Ordering standards from NIBSC:

<http://www.nibsc.org/products/ordering.aspx>

NIBSC Terms & Conditions:

http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC)
No 1272/2008: Not applicable or not classified

Physical and Chemical properties	
Physical appearance: freeze-dried	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): material of human origin.	
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion





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Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom
* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.25 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolestandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

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