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**WHO collaborative study to assess the suitability of the
1st International Standard and the 1st International Reference
Panel for antibodies to Ebola virus**

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** See Appendix 1*

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NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments **MUST** be received by **18 September 2017** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland,

attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr C Micha Nübling** at email: nueblingc@who.int.

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Summary

A WHO international collaborative study was undertaken to evaluate preparations of Ebola virus disease (EVD) convalescent plasmas for their suitability to serve as the WHO 1st International Standard (IS) and the WHO 1st International Reference Panel (IRP) for Ebola virus antibodies for use in the standardization and control of assays. The study involved participants testing the convalescent plasma sample preparations and additional monoclonal antibody samples in a blinded manner alongside the WHO International Reference Reagent (NIBSC code 15/220) using anti-EBOV assays established in their laboratories.

The candidate 1st IS for Ebola virus antibodies (study sample code 92, NIBSC 15/262) consists of ampoules containing the freeze-dried equivalent of 0.5 mL pooled convalescent plasma obtained from six Sierra Leone patients recovered from EVD. The candidate 1st IRP of anti-Ebola virus convalescent plasmas (NIBSC 16/344) consists of freeze-dried preparations of single donations of convalescent plasma obtained from four patients and one healthy blood donor. Each panel member is an ampoule containing the equivalent of 0.25mL plasma. All convalescent plasmas are confirmed PCR-negative for Ebola virus and underwent, along with the negative plasma, solvent detergent (SD) treatment prior to their development into candidate WHO biological reference materials.

In this collaborative study, 17 laboratories from 4 countries used a range of live Ebola virus neutralization assays, pseudotyped virus neutralisation assays and enzyme immunoassays to test the collaborative study samples. Surface plasmon resonance and Western blot assessments were also undertaken.

The study found that the candidate International Standard has the highest absolute titre among the convalescent plasma samples, although the geometric mean titres of all the convalescent plasmas fall within ~5-fold of each other. The potencies of three of the convalescent samples fall near the detection limit of some assays. This study also demonstrated that the agreement between laboratories for potencies relative to the candidate International Standard represents an improvement compared to the agreement in absolute titres; however, there is poor agreement between relative potencies for some assays. The results obtained from accelerated thermal degradation studies at 1 year indicate that the candidate IS is stable and suitable for long-term use.

The results of the collaborative study indicate the suitability of the candidates to serve as WHO reference materials and it is proposed that 15/262 is established as the WHO 1st IS for EBOV antibodies with an assigned potency of 1.5 IU/mL when reconstituted as directed in the instructions for use. It is also proposed that 16/344 is established as the WHO 1st IRP of anti-EBOV convalescent plasmas with panel member code 95 (NIBSC 15/280) assigned a unitage of 1.1 IU/mL when reconstituted as directed in the instructions for use. The other panel members have not been assigned a unitage.

The implementation and use by laboratories of the proposed WHO reference materials for EBOV antibodies will facilitate the characterization of the factors that contribute to assay variability and standardization of results across assays and laboratories.

Introduction

The WHO Expert Committee on Biological Standardization (ECBS) is the scientific body that establishes WHO biological reference standards which serve to define an internationally agreed unit to allow comparison of biological measurements worldwide. WHO International Standards (IS) for biological substances 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 [3]. Another class of WHO biological reference standard is the International Reference Reagent (IRR). WHO IRRs do not carry the designation of ISs, but are nevertheless of great value in the standardization of assays applied to biological products and diagnostic materials.

In October 2015, the WHO ECBS established a preparation of plasma (NIBSC product code 15/220) obtained from a patient recovered from Ebola virus disease (EVD) to serve as the WHO 1st IRR for Ebola virus (EBOV) antibodies with an assigned potency of 1 unit per mL. NIBSC 15/220 was assessed in a WHO international collaborative study and is known in that study as EBOV Ab Sample Code 79 [1].

15/220 has been serving as the interim WHO biological reference standard for EBOV antibodies

(https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=15/220)

while further work was undertaken to develop EVD convalescent plasma samples for use as WHO IS and as members of an IRP for EBOV antibodies. This report describes the preparation and international collaborative study evaluation of candidate convalescent plasma preparations to serve as the WHO 1st IS and members of the 1st IRP for EBOV antibodies for use in the calibration and control of assays. The study involved testing eight blinded EBOV antibody samples alongside the WHO IRR (15/220) in assay(s) that are in routine use in participants' laboratories.

Aims of study

The aims [2] of this WHO international collaborative study are to

- assess the suitability of the candidate to serve as the 1st International Standard for EBOV antibodies with an assigned unitage in IU per ampoule for use in the harmonization of EBOV antibody assays. There is no international conventional reference measurement procedure for EBOV antibodies and the IU is not traceable to the International System of Units (SI) of quantity.
- identify additional candidate EBOV antibody preparations for inclusion in a WHO International Reference Panel for EBOV antibodies.
- characterise each candidate preparation in terms of reactivity/specificity.
- assess each candidate'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 candidate is suitable to serve as an International Standard for the variety of different samples and assay types.
- advise WHO ECBS on the establishment of the candidate found to be suitable to serve as the 1st International Standard.

- advise WHO ECBS on the establishment of the WHO 1st International Reference Panel of Anti-EBOV Convalescent Plasma.

Materials and Methods

Source materials

Prior to shipment to the UK, all convalescent plasmas were tested and found negative for EBOV RNA. Prior to receipt at NIBSC, the convalescent plasmas were held at Public Health England (PHE), Colindale, UK until confirmed by PCR that no EBOV RNA could be detected in the materials. After receipt at NIBSC, plasma donations and the negative plasma samples were SD-treated as described previously [1].

Candidate 1st WHO International Standard for antibodies to EBOV

The candidate material is a freeze-dried preparation of a pool of plasmas obtained from six Sierra Leone (SL) patients recovered from EVD (provided by Dr Calum Semple, University of Liverpool, UK, through the auspices of the Ebola Convalescent Plasma (CP) Consortium) (Appendix 2). The treatment histories of the Sierra Leone patients are not known. Approximately 1000 ampoules containing the freeze-dried equivalent of 0.5 mL pooled plasma were prepared at NIBSC.

Candidate WHO International Reference Panel Samples for EBOV antibodies

The candidate panel members are freeze-dried preparations of EVD convalescent plasma samples obtained from four patients and a negative control plasma. One convalescent plasma donation was obtained by the National Institute for Infectious Diseases Lazzaro Spallanzani, Italy (INMI) (provided by Dr. Antonino Di Caro). The INMI patient had received Favipiravir, 3 treatments of convalescent plasma from 2 donors, ZMAb and Melanocortin (TCS 10). Convalescent plasmas obtained by Oslo University (NOR), the American Red Cross (ARC) and the UK National Health Service Blood and Transplant (NHSBT) have been described elsewhere [1]. Briefly, the Ebola-specific therapeutic treatments given to the NOR patient include ZMAb, Favipiravir and TKM-100802. The ARC patient received 2 aliquots of convalescent plasma 24 hours apart, as well as 7 nightly infusions of TKM-Ebola. The ARC donor did not receive monoclonal antibody treatments. The NHSBT patient received Brincidifovir and convalescent plasma treatments.

Approximately 200 to 300 ampoules containing the freeze-dried equivalent of plasma were prepared for each panel member. Note that while the NOR, ARC and NHSBT source materials listed above are the same as those described in the 2015 ECBS report [1], they have been prepared independently for assessment in this collaborative study. For example, the NOR, ARC and NHSBT samples for this collaborative study have undergone separate SD treatments with respect to Sample 43, 79 and 28 from the 2015 study. Furthermore, these samples have been freeze-dried for this collaborative study, whereas the corresponding samples in the earlier study are liquid presentations.

Monoclonal antibodies

Purified monoclonal antibodies (MAbs) (donated by Prof A. Townsend, University of Oxford, UK) were cloned from B cells of volunteers participating in the Oxford, UK vaccine trial who were primed with the monovalent formulation of the chimpanzee adenovirus 3 (ChAd3)-vectored vaccine encoding the surface glycoprotein of Ebola virus (GSK/NIH vaccine candidate) [3]. Two MAbs have been formulated in sterile PBS-Ca²⁺-Mg²⁺

supplemented with 5% human serum albumin. One-hundred vials of each MAb in 100 μ L liquid aliquots were prepared.

Coded study samples

Table 1 lists the collaborative study samples. The samples were shipped under NIBSC dispatch reference CS570.

Study protocol

The final version of the study protocol is given in Appendix 3. In brief, participants were requested to test the samples using their established method(s) for the detection of antibodies to EBOV. Participants were asked to perform 3 independent assays on different days. An Excel reporting sheet was provided with suggested dilutions for assaying each study sample. For each assay, participants were requested to make 2 independent series of dilutions of the study samples, and assay all samples concurrently if feasible. Participants were requested to record in the reporting sheet all essential information including the raw data from each assay. Participants were requested to return results within 6 weeks of receipt of materials.

Participants

Twenty laboratories took receipt of study samples and seventeen laboratories returned results. The participants were from France (1), Germany (2), UK (4) and USA (10). All laboratories are referred to by code number allocated at random and not representing the order of listing in Appendix 1. Participating organisations include government research, public health, medical counter-measure and regulatory organisations; university and research organisations; developers of biologics, assays and reagents and providers of laboratory services for vaccine trials. Three laboratories reported that they have BSL4 containment facilities for handling Ebolavirus.

Assay methods

Assays used by 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 10a, 10b. The assay methods fall into 3 general categories: neutralization of live Ebola virus (Neut); neutralization of Ebola pseudotypes (PsN); and enzyme immunoassays (EIA). Surface plasmon resonance (SPR) and Western blot assessments were also undertaken in the study.

Statistical methods

For Neut and PsN assays, median endpoint titres or reduction neutralization titres (RNT) 50 were calculated when more than half of the reported results indicated a positive response for that sample. Relative potencies were reported against sample 92 (the candidate WHO IS, NIBSC code 15/262) and sample 15/220 (the current WHO IRR).

Enzyme immunoassay data were analysed using a parallel line or sigmoid curve model with untransformed or log transformed responses. Calculations were performed using the EDQM software CombiStats Version 5.0 [4]. 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 and no estimates are calculated in such cases. For each sample, relative potency estimates from all valid assays within laboratories were combined to generate an unweighted geometric mean (GM) for each laboratory, with these laboratory

means being used to calculate overall unweighted geometric means for each sample. Variability between assays within laboratories and between laboratories are expressed using geometric coefficients of variation ($GCV = [10^s - 1] \times 100\%$ where s is the standard deviation of the \log_{10} transformed GM estimates).

Stability study

Ampoules of the candidate IS 15/262 have been placed at -20°C , $+4^\circ\text{C}$, 37°C , 45°C and 56°C and will be retrieved at specific time points for assessment. To date, accelerated degradation data has been obtained for the 1 year time-point. The degradation samples were assayed concurrently using an in-house neutralization assay based on pseudotyped lentiviral vector particles [5]. The stability of the candidate IS was assessed using the Arrhenius model for accelerated degradation studies with potencies expressed relative to the samples stored at -20°C [6, 7].

To limit the number of ampoules consumed in stability testing of individual panel members, the predicted stability of the candidate IRP is inferred from the stability of the candidate IS.

Results and data analysis

Production of candidate WHO 1st IS and 1st International Reference Panel members for EBOV Ab

In January and March 2016, NIBSC filled and freeze-dried the candidate reference materials using documented procedures. The product summaries for the candidate IS (NIBSC code 15/262) and IRP members (NIBSC codes 15/280, 15/284, 15/286, 15/288) are shown in Table 3. To limit the number of ampoules consumed in quality control testing, the negative plasma sample (15/288) was used as a surrogate for determining the dry weight, moisture and oxygen content of the candidate IRP as a whole. 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 [2]. The residual oxygen content of the candidate IS and negative panel member fell within the NIBSC working limit of 1.1%. Currently there are approximately 1000 ampoules of 15/262 and 100 ampoules of each of the reference panel members available for issue.

Prior to their dispatch to the participating laboratories, the reactivities of the freeze-dried candidates and MAb preparations were confirmed at NIBSC using the in-house pseudotype neutralization assay [5].

Collaborative study data received

Seventeen laboratories returned data sets for 26 assay methods (Table 2). Two labs (15b, 16a) returned data for live virus neutralization assays performed under BSL4 containment. Seven laboratories reported results for pseudotype neutralisation assays using lentiviral vectors (LVV) (labs 2, 4, 9, 11) or vesicular stomatitis virus vectors (VSV) (10b, 12, 13). Ten labs returned data for fifteen EIA-based methods (1a, 1b, 1c, 1d, 3, 5, 6a, 6b, 6c, 7, 8, 10a, 14, 15a, 16b). Laboratory 6 also provided Western blot analyses of the study samples. Laboratory 17 provided results for their SPR method.

Where applicable, all laboratories used buffer-based diluents to prepare sample dilutions. Examples of diluents used are “buffer”, “commercial” or “kit” sample buffer, “transport

medium”, DMEM, DMEM + 1% FBS, PBS + 3% or 5% non-fat milk + 0.1 or 0.05% Tween; PBS + 2% FCS or 1% milk, EME + 10% FBS, “cell culture medium”, SF medium.

Laboratory 1 indicated that their EIA methods are not amenable to parallel line analysis. For method 1a, study samples were diluted 1:4 in commercial sample diluent prior to testing. As this is a screening assay, samples were not tested in duplicate and no serial dilutions were performed. For method 1b, study samples were diluted 1:50 in commercial sample diluent. No serial dilutions were performed and samples were tested in duplicate. Results listed as ‘ND’ are samples that were ‘reactive’ by method 1a, but have a ‘titre’ too low to be detected by method 1b. For method 1c, study samples were tested ‘neat’ and not in duplicate. For method 1d, routine plasma samples are usually tested at 1:200 dilution with in-house transport medium. For the collaborative study, serial dilutions of samples in duplicate were performed prior to testing.

Laboratory 2 experienced some pipetting problems due to viscosity of some samples. Concentration-dependent inhibition was noted for some samples.

Laboratory 6 returned an overall result of negative for sample 39. As for the previous study, the NP antigens do not differentiate between exposure to SUDV and EBOV as the EBOV convalescent plasmas have reactivities against SUDV NP in both ELISA and Western blot analysis. The Western blot analysis of the samples for antibodies against EBOV GP-1 isoform, EBOV GP-1, 2 isoform, EBOV NP, SUDV GP and SUDV NP is shown in Appendix 4.

Laboratory 10 provided some details of their experience with testing the samples by ELISA (method 10a) and rVSV EBOV GP PsN (method 10b) (See Appendix 5). For the rVSV PsN it should be noted that although the assay does begin at a 1:10 dilution of sample, the PRNT60 results start at the 1:20 dilution and exclude the initial 1:10 dilution due to a “plaque explosion phenomenon” that has been seen in the assay where there is an excessive number of plaques in the first dilution only.

Laboratory 11 stated that for their LVV PsN, samples 38, 66 and 95 gave dilution curves that were hard to interpret in the first set of testing. They tried slightly different approaches for the second and third set of assays with no resolution of the issue.

Laboratory 12 reported samples 38, 66, and 85 as negative or marginally positive stating that FRNT (Fluorescence Reduction Neutralization Titre) results could not be determined for these samples.

In addition to the raw data readouts, laboratory 13 provided their PsVNA50 and PsVNA80 data in graphical form (Appendix 6).

Laboratory 15 provided tables and a summary of results by ELISA (method 15a) and FRNA (method 15b) (Appendix 7). Samples were not heat inactivated prior to use. During sample dilution, it was noted that the reconstituted samples were viscous after incubation. It is unclear if this will impact the results from different assays.

Laboratory 16 provided graphs comparing the endpoint titres obtained with their neutralisation assay against live EBOV/Mayinga (method 16a) and reactivities at dilution obtained in their EIA against inactivated EBOV/Makona (16b) (Appendix 8).

Laboratory 17 provided raw data readouts for SPR. (Appendix 9).

Scoring study samples as reactive for Ebola virus antibodies

Plasma sample reactivities

The scorings of the samples as positive or negative for EBOV antibodies are shown in Table 4. Having come from a healthy blood donor in the UK, sample 39 is expected to be negative. The EVD convalescent patient samples (15/220, 38, 66, 85, 92, 95) have previously been shown to be positive for antibodies to EBOV.

Participants were requested to include the cut-off value indicating sero-reactivity for each assay and to indicate whether each sample dilution tested is considered positive or negative according to their criteria. The methods that returned the expected results for reactivity of the plasma samples are 16a (Neut), 4 (LVV PsN), 10b, 13 (VSV PsN), 1a, 1c, 1d, 3, 5, 7, 8, 14, 15a and 16b (EIA) and 17 (SPR) (Table 4).

Convalescent plasma samples 38, 66 and 85 were scored negative in at least one assay using method 15b (Neut) or 12 (rcVSV-PsN). The quantitative EIA method 1b did not detect EBOV antibodies in samples 38 and 85 at the single 1/50 dilution tested. These results may be a reflection of assay sensitivity as commented by the participants above.

Laboratories 2, 9, 11 (LVV-PsN), 6a, 6b, 10a, (EIA) scored the negative sample 39 as positive in at least 1 assay, but otherwise correctly scored the convalescent plasmas as positive (Table 4).

As reported previously, the NP-based assays used by laboratory 6 did not differentiate between Sudan ebolavirus and EBOV antibodies [1]. The Western blots provided by laboratory 6 demonstrate that the convalescent plasma samples are reactive against both vaccine and non-vaccine EBOV antigens (G and NP) while the negative sample is confirmed non-reactive (Appendix 4).

MAB reactivities

The monoclonal antibody preparations (samples 93 & 94) are clonally derived from recipients of a vaccine encoding the GP of EBOV and are expected to be reactive against GP only. They have not been extensively characterised. It is therefore not known how they may perform in assays.

Laboratories that scored both MAb samples as positive are 16a (Neut); 10a, 11, 12, 13 (PsN); 1a, 1d, 4, 5, 6a, 8 and 14 (anti-GP EIA).

Methods that scored both MAb samples as negative include two anti-GP EIA (1c and 2) and an EIA based on inactivated whole virus coated onto plates (3). Also, as expected, the monoclonal antibodies were not detected using methods that did not target antibodies against the G protein (EIA methods 6b, 6c, 7).

Methods that scored MAb sample 93 overall as positive and 94 as negative are one Neut (15b), one LVV PsN (9), two EIA (15a, 16b) and the SPR (17). Laboratory 16 commented that, with the exception of sample 94, their ELISA and Neut data correlated well with each other. They concluded that sample 94 antibodies most likely recognize conformation-dependent epitopes that are sensitive to SDS and heat treatment.

Laboratory 10b was not able to obtain consistent results for samples 93 and 94 due to flat titration curves as explained in Appendix 5.

There were no cases where a laboratory reported sample 93 as negative and sample 94 positive.

Neut and PsN assays

Table 5 shows individual assay results along with the median titre estimates for Neut and PsN. The median titre values are also expressed relative to sample 92 (the candidate WHO IS) and sample 15/220 (the current WHO IRR). The EBOV variant, if known, is also given for the challenge virus or antigen used. For the convalescent plasma samples, Tables 6-8 summarise the individual laboratory estimated median titres (Table 6), the geometric mean (GM) potencies relative to sample 92 (Table 7) and sample 15/220 (Table 8). Tables 6-8 also show the overall GM and inter-laboratory geometric coefficient of variation (GCV), which measures variation across laboratories and methods.

Absolute antibody titres for EBOV in convalescent plasma samples by Neut and PsN

Individual laboratory estimated median titres for the convalescent plasma samples are shown in Table 6. Within laboratories, the titres for sample 15/220 and sample 95 are consistent to within a dilution step which is expected for samples derived from the same donated plasma. There are large differences observed between laboratories with, for example, titres ranging from 160 to 5230 for sample 15/220 and 28 to 7241 for sample 66. Part of the reason for these inter-laboratory differences may be differences in assay design and dilution series used. Some laboratories adjusted their dilution range or assay conditions between assays. Other laboratories had pipetting difficulties due to sample viscosity or other technical issues.

The calculated geometric means for the convalescent plasma samples are also shown in Table 6. At a titre of 529, the candidate IS (sample 92) has the highest titre, although the GM titres of all the convalescent plasmas fall within ~5-fold of each other.

Enzyme immunoassays and surface plasmon resonance analysis

Table 9 lists the individual EIA potency estimates relative to sample 92 using parallel line analyses. Table 9 also lists the cases where samples could not be assessed due to assay invalidity or other factors. Table 10 shows individual laboratory GM potencies for samples relative to sample 92 along with intra-laboratory GCV, which measures variation within a given laboratory. Overall GM and inter-laboratory GCV are also given in Table 10 for all targeted analytes tested by EIA (i.e. antibodies against G, NP and VP40) as well as for anti-G EIA only.

Table 11 summarises the potency estimates relative to sample 15/220 for samples as described above for Table 10.

Table 12 summarises the relative potency estimates of samples tested by SPR.

Anti-EBOV potencies relative to candidate IS sample 92

Overall geometric mean potencies of convalescent plasma samples

The overall GM potency of the current WHO IRR (sample 15/220) relative to the candidate IS (sample 92) is 0.87 for neutralization-based assays (Table 7) and 0.52 for EIA (Table 10). With an overall potency of 0.81 for neutralization-based assays and 0.70 for EIA, the ARC-derived sample 95, is close to that of the related IRR (Tables 7 and 10). The other three

convalescent plasmas (samples 38, 66, 85) have overall GM potencies that are lower than that of either the ARC samples or the candidate IS (Tables 7-8 and 10-11).

The overall GM relative potencies across all assay methods are shown in Table 13. If the unitage of the WHO IRR, as established by ECBS, is maintained at 1 unit/mL, then the overall GM potency of the candidate IS has a nominal value of 1.5. In turn, the ARC-derived sample 95 has an overall GM potency of 1.1 relative to the candidate IS.

The ranking of samples in terms of relative potency across all assay methods is 92>95≥15/220. The other convalescent plasmas (samples 38, 66, 85) are ranked as having lower relative potencies, but with no clear order of ranking.

Inter-laboratory variability of convalescent plasma samples

Comparing the overall GCVs in Tables 6 and 7, the variability between laboratories is reduced when neutralisation titres are expressed relative to the candidate IS sample 92. Using sample 92 as the reference, there is better agreement between laboratories for the EIA (GCV range 41% to 92%; Table 10) than for the neutralisation-based assays (GCV range 37% to 292%; Table 7). Using the IRR sample 15/220 as a reference also improves agreement between laboratories but generally not to the same extent as that observed for sample 92 (Tables 6-8 and 10-11).

Intra-laboratory variability of convalescent plasma samples

The GCVs shown in Table 10 for EIA are an indication of intra-laboratory variability for potencies relative to the candidate IS (rather than for the absolute titres). The figures for individual laboratories and samples are based on only two or three assays however, and are sensitive to any variation in a single assay dose-response, or even a single response. Some assay methods could not be assessed for GCV as indicated in Table 9. Not all laboratories were using assays that had been optimised for parallel-line analysis and potency estimation, and any high intra-laboratory variability may not reflect the assay performance for detection of EBOV antibodies as positive or negative.

Monoclonal antibody samples

The interlaboratory agreement for the MAb samples 93 and 94 is not very satisfactory (Tables 5, 9-11). For example, the neutralisation titre estimates for sample 93 across laboratories range from 58 to 9632 (Table 5) with an overall GCV of 380% (results not shown). Also, relative potencies for sample 93 could only be determined from 4 of the 10 EIA that reported the sample as positive (Table 10). The reasons for the poor agreement between laboratories for the MAbs may include those given above for the plasma samples, but also, the variability may be a reflection of the MAb preparations themselves (e.g. dilution effects of high titred antibody sample; epitope conformation and specificity). Although MAb samples 93 and 94 may have utility as reagents, further characterisation is required in order to fully understand their reactivity in EBOV Ab assays and they are not considered further in this report.

Stability studies

Accelerated degradation studies were undertaken on the candidate 1st WHO IS 15/262. Samples of the candidate were stored at varying temperatures of -20°C, +4°C, +20°C, +37°C, +45°C and +56°C for a period of approximately 1 year. The potency of samples of the candidate IS 15/262 stored at the elevated temperatures were expressed relative to the material stored at -20°C. The results of individual assays are summarized in Appendix 10.

The long-term stability of the candidate IS 15/262 was predicted using the Arrhenius model, using all temperatures up to +56°C, which gave a statistically good fit to the data. The predicted percentage loss at 2, 4, 13, 26 and 52 weeks at the different temperatures of storage, is also shown in Appendix 10. The predictions are dependent on the estimated potencies at +37°C and above being reliable, and the apparent drop in potency not being affected by problems of reconstitution. This was seen with +45°C and +56°C and therefore the sample cannot be assigned a percentage predicted loss due to the difficulty in reconstituting the material. It is not possible to obtain reliable predictions from the data for +4°C and +20°C alone, as insufficient degradation has occurred which shows the candidate is stable at +20°C for up to 1 year. From these data, 15/262 appears to be adequately stable to serve as an IS, and is suitable for ambient transportation up to +37°C as the loss is 0.5% after 1 week (Table 14). Due to its limited batch size, the candidate IRP members were not submitted to accelerated degradation assessment.

Discussion

The candidate 1st IS for anti-EBOV (sample code 92) is a batch of ampoules containing the freeze-dried equivalent of 0.5 mL pooled convalescent plasma obtained from six Sierra Leone patients recovered from EVD.

The candidate 1st IRP of anti-EBOV convalescent plasmas consists of freeze-dried preparations of single donations of convalescent plasma obtained from four recovered patients and one negative plasma from healthy blood donor. Each panel member is an ampoule containing the equivalent of 0.25mL plasma.

All convalescent plasmas are confirmed PCR-negative for EBOV and, as well as with the negative plasma, were SD-treated prior to their development into candidate WHO biological reference materials.

Seventeen laboratories from 4 countries participated in this WHO international collaborative study to assess the suitability of the candidate materials for use in the standardization of anti-EBOV assays. Data sets were returned for 26 assay methods (Table 2). Fifteen assay methods returned the expected reactivities for the plasma samples, reporting no false negatives or false positives (Table 4). This includes the live virus Neut assay (method 16a) that was used as the reference method in the previous collaborative study [8], its complementary whole inactivated virus ELISA method (16b) and the VSV-luciferase non-replicating PsN (method 13). These 3 methods have been used in recent EVD or EBOV vaccine studies [9-17]. Nine additional EIA methods (1a, 1c, 1d, 3, 5, 7, 8, 14, 15a), one additional VSV-PsN method (10b) one LVV-PsN method (4) and the SPR method (17) also returned the expected results. Due to laboratory coding, citations for some of the methods are not available. It is known that the methods performed by laboratories 8 and 10a are very similar, using the same reference standard and controls and method 15a is a commercial assay kit. Methods 1a, 1d, 5, 8, 10a, 13, 15a, 16a and 11 were also used in the previous collaborative study [1]. In that study, these methods (which are coded differently), also reported the expected reactivities for the convalescent and negative plasma samples. As expected, the convalescent plasma samples are reactive against non-vaccine target EBOV antigens (Table 4 and Appendix 4).

As reported previously [1], many of the assays used in this collaborative study reported the SD-treated normal human plasma as a clear negative while some laboratories reported the negative plasma (sample 39 in this study) as positive (Table 4). These are 3 LVV-based PsN assays (2, 9, 11) and an in-house ELISA using commercial antigens (6a, 6b, 6c). While these results indicate that sample 39 should not be used for determining assay cut-off for the respective assays, the material may be useful in the assessment of the non-specific reactivity in certain assay methods.

Laboratory potency estimates for Neut and PsN are summarized in graphical form for titres (Figure 1) and relative potencies against sample 92 (Figure 2). A graph summarising potency estimates relative to sample 92 for EIAs is shown in Figure 3. Graphical summaries for Neut, PsN and EIA, overall, are provided of GM potencies relative to sample 92 (Figure 4) and sample 15/220 (Figure 5)

The candidate 1st IS for anti-EBOV (SL plasma pool) has a higher potency than the ARC plasma sample preparations (15/220 and sample 95). The potencies of the NOR, NHSBT and INMI convalescent samples are thought to be near the detection limit of some assays (e.g. methods 1b, 12,15b) (See Table 4 and participants' comments) and cannot be clearly ranked across laboratories against the candidate IS and ARC samples.

According to WHO guidelines, the behavior of an International Standard should resemble as closely as possible that of the biological samples in the assay systems used to test them [18, 19] i.e. it should be commutable. The commutability of a biological reference preparation is determined by many factors including methods of reference preparation such as inactivation or SD treatment and freeze-drying steps; the clinical setting (e.g. immunotherapy or testing for immunity); sample matrix (e.g. plasma, serum, urine); and epitope recognition (e.g. whole virus, vaccine antigen, EBOV variant). Aspects of commutability of the EBOV antibody samples were addressed in this and the previous study [1] by including convalescent plasma samples, vaccinee samples, anti-GP monoclonal antibody preparations and anti-GP human IgG preparations from transchromosomal bovines. Of these materials, the convalescent plasmas most closely resemble EVD clinical specimens; however, such materials are limited and difficult to obtain. As potentially sustainable sources of EBOV Ab, consideration should be given to assessing the feasibility of the other study materials to serve as biological reference materials for EBOV Ab assays.

This study has demonstrated that the agreement between laboratories for potencies relative to the candidate IS represents an improvement compared to the agreement in absolute titres, however, there is poor agreement between relative potencies for some assays. Commutability is not necessarily an intrinsic property of the reference material and must be assessed in the context of the assays used. As considered in the previous report, many variables exist in this study making it challenging to assess the effects of the candidates on assay variability. Factors to consider include the platform, sample matrix, the presence of complement, diluent, target cell, EBOV variant of antigen, cross-reactivity against Ebolavirus strains, assay protocol and detection readout method.

The implementation and use of sample 92 as the 1st IS for antibodies to EBOV, along with the proposed WHO International Reference Panel and the WHO IRR for EBOV antibodies, will facilitate the characterization of the factors that contribute to assay variability and standardization of results across assays and laboratories.

The results obtained from accelerated thermal degradation studies at ~ 1 year indicate that the candidate 1st IS and, by inference, the candidate 1st IRP are stable and suitable for long-term use.

Proposal

It is proposed that the Sierra Leone Anti-EBOV Convalescent Plasma Pool preparation (EBOV Ab Sample Code 92) is established as the 1st WHO International Standard for EBOV antibodies for use in the standardization of assays used in the detection and quantitation of

EBOV antibodies. The 1st IS for EBOV Ab has been assigned a unitage of 1.5 IU/mL when reconstituted in 0.5 mL water.

It is proposed that the panel of freeze-dried plasmas consisting of the ARC (Sample Code 95), NHSBT (Sample Code 66), NOR (Sample Code 38), INMI (Sample Code 85) Anti-EBOV Convalescent Plasmas and the negative sample (Sample Code 39) is established as the 1st WHO International Reference Panel for EBOV antibodies of anti-EBOV Convalescent Plasmas. It is proposed that ARC sample is assigned a unitage of 1.1 IU/mL when reconstituted in 0.25mL water. The NHSBT, NOR and INMI panel members have not been assigned a unitage.

It is proposed that the WHO IRR for anti-EBOV (NIBCS code 15/220) as established in 2015 remains available for use. Approximately 250 tubes (100uL/tube) of the current IRR 15/220 are available for distribution.

It is intended that the WHO IRR and 1st WHO International Reference Panel are used in the assessment of factors that affect assay variability.

The WHO IS and IRP for EBOV antibodies are listed under the following NIBSC product codes which may be ordered through the NIBSC on-line catalogue (<http://www.nibsc.org/products.aspx>). The proposed instructions for use are given in Appendix 11.

NIBSC 15/262: 1st WHO IS Anti-EBOV Convalescent Plasma Pool, Sierra Leone. Approximately 950 ampoules of 15/262 are available for distribution.

NIBSC 16/344: 1st WHO IRP Anti-EBOV Convalescent Plasma. Approximately 90 panels are available for distribution.

Comments from participants

There were no disagreements with the suitability of the convalescent plasma samples to serve as the WHO 1st International Standard (NIBSC code 15/262) and 1st International Reference Panel (NIBSC code 16/344) for antibodies to EBOV. One participant suggested that the interim WHO IRR (15/220) be withdrawn or not distributed in order to avoid confusion with the newly established WHO references materials. Other suggestions have been incorporated in the report.

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Tables**Table 1.** Collaborative study samples shipped under NIBSC dispatch reference CS570.

EBOV Ab Collaborative Study Sample Code	NIBSC Product Code	Sample Name and description	Preparation
79*	15/220	WHO 1 st IRR for Ebola antibodies ARC convalescent plasma 1unit/mL	100 µL frozen plasma
38	15/284	Candidate Panel Member 3: NOR Anti-EBOV Convalescent Plasma	0.25 mL plasma Freeze-dried
39	15/288	Candidate panel Member Negative Human Plasma (anti-EBOV)	0.25 mL plasma Freeze-dried
66	15/282	Candidate Panel Member 2: NHSBT Anti-EBOV Convalescent Plasma	0.25 mL plasma Freeze-dried
85	15/286	Candidate Panel Member 4: INMI Anti-EBOV Convalescent Plasma	0.25 mL plasma Freeze-dried
92	15/262	Candidate WHO 1 st International Standard Anti-EBOV Convalescent Plasma Pool Sierra Leone	0.5 mL plasma Freeze-dried
93	NA	Mab 1-P6 in buffer#	100 µL Liquid
94	Not Applicable	Mab 1-2-66-4-C12 in buffer#	100 µL Liquid
95	15/280	Candidate Panel Member 1: ARC Anti-EBOV Convalescent Plasma	0.25 mL plasma Freeze-dried

Abbreviations: * Sample Code 79 was established as the WHO 1st International Reference Reagent for Ebola antibodies (NIBSC 15/220) by ECBS in 2015 [1]. IRR = International Reference Reagent; ARC = American Red Cross; NOR = Norway; NHSBT= National Health Service Blood and Transplant; INMI = National Institute for Infectious Diseases Lazzaro Spallanzani; NA = Not Applicable; MAb = monoclonal antibody.

PBS-Ca²⁺-Mg²⁺; 5% human serum albumin.

Table 2. Laboratory codes and assay methods

Lab Code	Assay Method Description [Reference if provided]	Assay Method Category	Analyte (anti-)	unit
1a	Direct ELISA screening	EIA	EBOV GP IgG	+/- based on OD cut off
1b	Direct ELISA quantification	EIA	EBOV GP IgG	ELISA units/mL
1c	Competitive ELISA	EIA	EBOV GP IgG	OD
1d	IgG Capture ELISA	EIA	EBOV GP IgG	OD
2	Neutralization assay with pseudotyped lentiviral vector particles	LVV PsN	EBOV GP	RLU
3	Direct ELISA Whole Virion ELISA Assay	EIA	inactivated wv EBOV	OD
4	Neutralization assay LVV	LVV-PsN	EBOV/Makona GP	RLU
5	Direct ELISA	EIA	EBOV/Makona rGP IgG	OD, WHO IRR units/mL, units/mg IgG
6a	Direct ELISA	EIA	EBOV GP	OD
6b	Direct ELISA	EIA	EBOV NP	OD
6c	Direct ELISA	EIA	SUDV NP	OD
6d	Western blot analysis against EBOV GP-1 isoform, EBOV GP-1,2 isoform, EBOV NP, SUDV GP and SUDV NP			
7	Direct ELISA	EIA	EBOV VP40 and NP	OD
8	Indirect ELISA	EIA	EBOV/GP IgG	OD and ELISA units/mL
9	Neutralization assay with LVV	LVV-PsN	EBOV/Mak-C05 GP	RLU
10a	ELISA	EIA	EBOV GP IgG	OD and ELISA units/mL
10b	Neutralization of Ebola pseudotypes	VSV-PsN	EBOV GP	Plaques
11	Neutralization assay with LVV	LVV-PsN	EBOV/Mak-C15 (KJ660346) GP	RLU
12	Neutralization of Ebola pseudotypes (VSV-luciferase replication competent)	rcVSV-PsN	EBOV GP Mayinga	Fluorescence
13	Neutralization of Ebola pseudotypes (VSV-luciferase non-replicating) (PsVNA, USAMRIID) [10, 15, 17, 20]	nrVSV-PsN	EBOV/Kikwit GP-GFP	RLU
14	Direct ELISA	EIA	EBOV/Kikwit GP	OD and ELISA units/mL
15a	Direct ELISA	EIA	EBOV GP IgG	OD and ELISA units/mL
15b	Neutralisation of infectious EBOV	Neut	infectious EBOV/Mak-C05	Fluorescence
16a	Neutralisation of infectious EBOV	Neut	live EBOV/Mayinga	CPE
16b	Antibody capture ELISA	EIA	inactivated wv EBOV/Makona	OD
17	Surface Plasmon Resonance [9]	SPR	EBOV GP	Resonance Units

Abbreviations: EIA = enzyme immunoassay; Neut = neutralisation assay; PsN = pseudotype neutralisation assay; LVV = lentiviral vector; VSV = vesicular stomatitis virus vector; wv = whole virus; SUDV = Sudan ebolavirus; GP = glycoprotein; NP = nucleoprotein; rc = replication-competent; nr = non-replicating; OD = optical density; RLU = relative light units; CPE = cytopathic effect

Table 3. Production summary for the candidate International Standard and International Reference Panel members.

NIBSC code	15/262	15/280	15/282
CS Code	92	95	66
Product name	Candidate WHO 1 st IS Anti-EBOV plasma, human	Candidate Panel Member 1:	Candidate Panel Member 2:
Source material	Sierra Leone convalescent plasma pool	ARC Anti-EBOV convalescent plasma	NHSBT Anti-EBOV convalescent plasma
Presentation	Freeze-dried in 3 ml DIN ampoule		
Appearance	Robust cake	Robust cake	Robust cake
Filling machine	Bausch and Strobel AFV5090	Hamilton	Hamilton
Date of filling	29 January 2016	4 March 2016	4 March 2016
No. of ampoules filled	1203	204	192
Mean fill weight (g)	0.5220 n=84	0.2536 n=10	0.2548 n=10
CV of fill weight (%)	0.6652	0.3543	0.3467
Freeze dryer	Serail CS15	Serail CS15*	Serail CS15*
Date of completion of lyophilization	02 February 2016	4 March 2016	4 March 2016
Mean dry weight (g)	0.03718 n=6	ND	ND
CV of dry weight (%)	0.2800	NA	NA
Mean residual moisture (%)	0.1616 n=12	ND	ND
CV of residual moisture (%)	8.87	NA	NA
Mean oxygen content (%)	0.31 n=12	0.29 n=12	0.41 n=12
CV of oxygen content (%)	24.42	43.28	26.19
No. of ampoules available to WHO after quality control and collaborative study	~1000	~100	~100

Table 3. Continued.

NIBSC code	15/284	15/286	15/288
CS code	38	85	39
Product name	Candidate Panel Member 3:	Candidate Panel Member 4:	Candidate Panel Member
Source material	NOR Anti-EBOV Convalescent Plasma	INMI Anti-EBOV Convalescent Plasma	Negative Human Plasma (anti-EBOV)
Presentation	Freeze-dried in 3 ml DIN ampoule		
Appearance	Robust cake	Robust cake	Robust cake
Filling machine	Hamilton	Hamilton	Hamilton
Date of filling	18 March 2016	4 March 2016	18 March 2016
No. of ampoules filled	218	194	364
Mean fill weight (g)	0.2533 n=10	0.2552 n=10	0.2534 n=10
CV of fill weight (%)	0.3	0.222	1.1
Freeze dryer	Serail CS100 [#]	Serail CS15 [*]	Serail CS100 [#]
Date of completion of lyophilization	22 March 2016	4 March 2016	22 March 2016
Mean dry weight (g)	ND	ND	0.01762 n=6
CV of dry weight (%)	NA	NA	3.36
Mean residual moisture (%)	ND	ND	0.363 n=12
CV of residual moisture (%)	NA	NA	22.43
Mean oxygen content (%)	0.12 n=12	0.31 n=12	0.26 n=12
CV of oxygen content (%)	75.84	25.73	49.06
No. of ampoules available to WHO after quality control and collaborative study	~100	~100	~250

Abbreviations: ARC = American Red Cross; NHSBT= National Health Service Blood and Transplant; NOR = Norway; INMI = National Institute for Infectious Diseases Lazzaro Spallanzani; ND= Not Done; NA= Not Applicable.

* Products 15/280, 15/282 and 15/286 were loaded into the same freeze-dryer for lyophilisation.

Products 15/284 and 15/288 were loaded into the same freeze-dryer for lyophilisation.

Table 4. Samples scored as positive or negative for EBOV Ab.

Sample				CS code	15/220	38	39	66	85	92	93	94	95
				NIBSC		15/284	15/288	15/282	15/286	15/262	NA	NA	15/280
Lab	Method	n	Analyte anti-	Virus	1 st WHO IRR (ARC CP)	NOR CP	Negative plasma	UK NHSBT CP	INMI CP	Candidate WHO 1st IS Anti-EBOV SL CP	Mab 1-P6	Mab 1-2-66-4-C12	ARC CP
1a	EIA (screening at ¼ dilution)	3	GP IgG	EBOV	PPP	PPP	NNN	PPP	PPP	PPP	PPP	PPP	PPP
1b	EIA (quantitation at 1/50 dilution)	3	GP IgG	EBOV	PPP	ND*	ND	PPP	ND*	PPP	PPP	ND*	PPP
1c	EIA (competitive, tested neat)	3	GP IgG	EBOV	PPP	PPP	NNN	PPP	PPP	PPP	NNN	NNN	PPP
1d	EIA (IgG capture)	3	GP IgG	EBOV	PPP	PPP	NNN	PPP	PPP	PPP	PPP	PPP	PPP
2	LVV-PsN	3**	GP	EBOV	P	P	p	P	P	P	N	N	P
3	EIA	3	inactivated WV	EBOV	PPP	PPP	NNN	PPP	PPP	PPP	NNN	NNN	PPP
4	LVV-PsN	3	GP	Makona	PPP	PPP	NNN	PPP	PPP	PPP	PPP	PPP	PPP
5	EIA	3	GP	Makona	PPP	PPP	NNN	PPP	PPP	PPP	PPP	PPP	PPP
6a	EIA	3	GP	EBOV	PPP	PPP	PpI***	PPP	PPP	PPP	PPP	PPP	PPP
6b	EIA	3	NP	EBOV	PPP	PPP	ppN***	PPP	PPP	PPP	NNN	NNN	PPP
6c	EIA	3	NP	SUDV	PPP	PPP	NNp***	PPP	PPP	PPP	NNN	NNN	PPP
6d	Western	See Appendix 4											
7	EIA	3	VP40 & NP	EBOV	PPP	PPP	NNN	PPP	PPP	PPP	NNN	NNN	PPP
8	EIA	3	GP IgG	EBOV	PPP	PPP	NNN	PPP	PPP	PPP	PPP	PPP	PPP
9	LVV PsN	3	GP	Makona	PPP	PPP	ppp	PPP	PPP	PPP	PPP	NNN	PPP
10a	EIA	4	GP IgG	EBOV	PPPP	PPPP	NpNN	PPPP	PPPP	PPPP	PPPP	pPPP	PPPP
10b	VSV PsN	4	GP	EBOV	PPPP	PPPP	NNNN	PPPP	PPPP	PPPP	P,NR,NR,P	NR,P,N, NR	PPPP
11	LVV PsN	3	EBOV GP	Makona C15	PPP	pPp	ppN	PPP	PPP	PPP	PPP	PPP	P,Nl,P
12	rcVSV PsN	3	GP	EBOV	PPP	II I#	NNN	II I#	II I#	PPP	PPP	PPP	PPP
13	nrVSV PsN	3	GP	Kikwit	PPP	PPP	NNN	PPP	PPP	PPP	PPP	PPP	PPP
14	EIA	3	GP	Kikwit	PPP	PPP	NNN	PPP	PPP	PPP	PPP	PPP	PPP
15a	EIA	3	GP IgG	EBOV	PPP	PPP	NNN	PPP	PPP	PPP	PPP	NNN	PPP
15b	Neut	3	WV	Makona	PPP	NNN##	NNN	PpN##	ppN##	PPP	PPP	pNN	PPP
16a	Neut	3	WV	Mayinga	PPP	PPP	NNN	PPP	PPP	PPP	PPP	PPP	PPP
16b	EIA	3	WV	Makona	PPP	PPP	NNN	PPP	PPP	PPP	PPP	NNN	PPP
17	SPR	2	GP	EBOV	PP	PP	NN	PP	PP	PP	PP	NN	PP

Table 4. continued

Abbreviations: EIA = enzyme immunoassay; Neut = neutralisation assay; PsN = pseudotype neutralisation assay; LVV = lentiviral vector; VSV = vesicular stomatitis virus vector; rc = replication competent; nr = non-replicative; SPR = surface plasmon resonance; GP = glycoprotein; WV = whole virus; NP = nucleoprotein; VP40 = matrix protein; EBOV = variant not indicated; SUDV = Sudan virus; ARC = American Red Cross; CP = convalescent plasma; NOR = Norway; NHSBT= National Health Service Blood and Transplant; INMI = National Institute for Infectious Diseases Lazzaro Spallanzani; SL = Sierra Leone ; NA= not applicable; Mab = monoclonal antibody; N= negative, P= positive; p = weakly positive; I= indeterminate; ND = not detected; NI = non-interpretable; NR = not reportable;.

*Laboratory 1 stated that samples (38, 85 and 94), which scored reactive in the screening assay (1a), have a titre too low to be detected by the quantitation assay (1b).

** Laboratory 2 reported overall reactivity for the 3 assays.

***Laboratory 6 returned an overall result of negative for sample 39.

Laboratory 12 reported samples 38, 66, and 85 as negative/marginally positive.

Laboratory 15 reported that the ELISA (15a) was more sensitive than the fluorescence reduction neutralization assay (FRNA) (15b) in identifying anti-Ebola antibodies in the provided samples. ELISAs identify total anti-Ebola IgG antibodies whereas the FRNA only identifies neutralizing antibodies in a sample, a smaller fraction of the total antibodies produced, which most likely explains the difference in antibody detection between these assays.

Results highlighted in grey are inconsistent with the anticipated reactivity.

The MAb sample results in **BOLD** are expected to be negative in anti-NP and anti-VP40 EIA.

Results highlighted in pink indicate the methods by which one MAb sample is reported positive and the other negative.

Table 5. Individual and median titre estimates for neutralisation assays (Neut), lentiviral vector (LVV), pseudoneutralisation (PsN) assays and vesicular stomatitis virus vector (VSV) PsN, with potencies expressed relative to samples 15/220 and 92. Unless stated otherwise, the titres are 50% reduction neutralisation titres (RNT50).

Method	Lab	Analyte anti-	Virus	Sample	Log Median Titre			Median Estimate Titre	Relative to sample 15/220 (WHO IRR-ARC CP)	Relative to sample 92 (Candidate 1 st IS- SL CP)
					Assay 1	Assay 2	Assay 3			
Neut	15b	Whole virus	Makona isolate C05	15/220	2.20	2.20	1.90	160	1.00	2.00
				38	no neutralization (no neut.)			NA	NA	NA
				39	no neut.			NA	NA	NA
				66	1.60	1.30	no neut.	28	0.18	0.35
				85	1.30	1.30	no neut.	20	0.13	0.25
				92	1.90	1.90	1.60	80	0.50	1.00
				93	2.20	2.51	2.20	160	1.00	2.00
				94	1.30	no neut.		NA	NA	NA
95	1.90	2.20	1.60	80	0.50	1.00				
<p>Lab 15 comments: the starting sample dilution was 1/20. C05 isolate of Ebola Makona (full designation: Ebola virus/H.sapiens-wt/GIN/2014/Makona-C05, abbreviated name: EBOV/Mak-C05, GenBank accession no. KP096420.1) was kindly provided by Dr. Gary P. Kobinger (Public Health Agency of Canada). The samples were not heat inactivated prior to use. All nine samples were tested by ELISA and FRNA (see Appendix 7). The FRNA assay, which detects neutralizing antibodies rather than all Ebola GP specific antibodies, measured differing results based on criteria used (50 or 80% reduction). A FRNA50 measured four positive samples, three indeterminate samples, and two negative samples. When the neutralization criteria was more stringent (80%), four samples were FNRA positive and five were negative. A total of seven samples were positive for antibodies by ELISA and two samples were negative. During sample dilution, it was noted that the reconstituted samples were viscous after incubation.</p>										
Neut*	16a	Whole virus	Mayinga	15/220	2.48	2.26	2.56	304	1.00	0.60
				38	1.43	1.43	1.28	27	0.09	0.05
				39	0.60	0.83	0.60	4	0.01	0.01
				66	1.81	1.96	1.51	64	0.21	0.13
				85	1.43	1.35	1.51	27	0.09	0.05
				92	2.71	2.71	2.18	512	1.68	1.00
				93	3.01	3.01	3.16	1024	3.36	2.00
				94	2.03	3.01	1.81	108	0.35	0.21
95	2.41	2.78	2.18	256	0.84	0.50				
<p>*100% neutralisation titres reported.</p> <p>Lab 16 comments: All samples were evaluated by ELISA using inactivated Ebola Zaire Makona virus and virus neutralization assay using live Ebola Zaire Mayinga virus. All samples were tested in three independent experiments in both assays. With the exception for sample 94, ELISA and NT data correlated well with each other. For sample 94 we conclude that these antibodies most likely recognize conformation-dependent epitopes that are sensitive to SDS and heat treatment (see Appendix 8).</p>										

Table 5 continued

Method	Lab	Analyte anti-	Virus	Sample	Log Median Titre			Median Estimate	Relative to sample 15/220 (WHO IRR-ARC CP)	Relative to sample 92 (Candidate 1 st IS- SL CP)		
					Assay 1	Assay 2	Assay 3					
LVV-PsN	2	GP	EBOV	15/220*	2.47	2.95	2.79	610	1.00	1.40		
				38*	2.34	2.65	2.54	349	0.57	0.80		
				39*	1.73	1.78	1.79	61	0.10	0.14		
				66*	2.22	2.51	2.25	178	0.29	0.41		
				85	2.12	2.53	2.34	217	0.36	0.50		
				92*	2.16	2.64	2.72	436	0.72	1.00		
				93	1.77	1.35	1.87	58	0.10	0.13		
				94	0.71	no neut.	1.17	9	0.01	0.02		
95*	2.37	2.46	2.50	290	0.48	0.67						
Lab 2 comments: Some pipetting problems due to viscosity of some samples. *Concentration-dependent inhibition was noted for these samples.												
LVV-PsN	4	Gp	Makona	15/220	2.25	2.27	2.57	187	1.00	0.51		
				38	NA*	1.77	2.00	76	0.41	0.21		
				39	No neutralization shown			NA	NA	NA		
				66	1.81	1.90	1.77	65	0.35	0.18		
				85	2.09	1.89	2.19	123	0.66	0.34		
				92	2.68	2.56	2.52	365	1.95	1.00		
				93	2.95	2.77	2.80	627	3.35	1.72		
				94	3.45	3.49	3.74	3117	16.67	8.54		
95	2.32	2.52	2.49	311	1.66	0.85						
NA = Not assessed. Lab 4 comments: * Although we couldn't produce a titration curve, on the single point analysis 2 out of 3 replicates neutralise 50% at dilution 1/20.												
LVV-PsN	9	GP	Makona	15/220	2.34	2.69	2.27	220	1.00	NA		
				38	1.81	1.80	1.65	63	0.28	NA		
				39	1.69	1.40	1.43	27	0.12	NA		
				66	2.19	2.28	2.08	153	0.70	NA		
				85	2.03	2.15	1.97	108	0.49	NA		
				Assays were split across 2 plates								
				92	2.43	2.24	2.27	186	NA	1.00		
				93	2.83	2.84	2.69	683	NA	3.67		
				94	<1.60	1.36	<1.60	NA	NA	NA		
				95	2.32	2.16	2.23	171	NA	0.92		
NA = Not assessed. Lab 9 comments: We heat-inactivate antibodies at 56°C x 30 minutes prior to use and do not use complement in our assay. Based on use of negative sera in our prior experiments, we expect that titers < 40 (i.e. Log 1.60) are negative.												

Table 5 continued

Method	Lab	Analyte anti-	Virus	Sample	Log Median Titre			Median Estimate	Relative to sample 15/220 (WHO IRR-ARC CP)	Relative to sample 92 (Candidate 1 st IS- SL CP)	
					Assay 1	Assay 2	Assay 3				
LVV-PsN	11	GP	Makona isolate C15	15/220	2.35	2.35	2.20	226	1.00	0.25	
				38*	1.30	1.90	1.30	20	0.09	0.02	
				39	1.75	1.45	1.30	28	0.12	0.03	
				66*	3.71	4.01	3.86	7241	32.00	8.00	
				85	2.66	2.35	2.35	226	1.00	0.25	
				92	2.81	2.96	3.11	905	4.00	1.00	
				93	2.51	3.11	2.51	320	1.41	0.35	
				94	3.56	4.16	3.56	3620	16.00	4.00	
			95*	3.11	uninterpretable	2.35	NA	NA	NA		
NA = not assessed. Lab 11 comments: * Samples 38, 66 and 95 produced dilution curves that were difficult to interpret.											
Method	Lab	Analyte anti-	Virus	Sample	Log Median Titre				Median Estimate	Relative to sample 15/220 (WHO IRR-ARC CP)	Relative to sample 92 (Candidate 1 st IS- SL CP)
					Assay 1	Assay 2	Assay 3	Assay 4			
VSV-PsN	10b*	GP	EBOV	15/220	3.72	3.91	3.61	NR	5230	1.00	0.90
				38	3.18	3.20	3.07	3.03	1331	0.26	0.23
				39	<1.30	<1.30	<1.30	<1.30	NA	NA	NA
				66	3.23	3.19	3.23	3.17	1619	0.31	0.28
				85	3.12	3.06	3.03	2.89	1101	0.21	0.19
				92	3.72	3.84	3.81	3.72	5845	1.12	1.00
				93	3.82	NR	NR	3.26	NA	NA	NA
				94	NR	2.84	<1.30	NR	NA	NA	NA
			95	3.61	3.78	3.57	3.59	3953	0.76	0.68	
* PRNT60 titres reported. NA = not assessed; NR= not reportable. Lab 10 comments: Additional participant's comments about results obtained with their ELISA and PRNT are given in Appendix 5.											

Table 5 continued

Method	Lab	Analyte anti-	Virus	Sample	Log Median Titre			Median Estimate	Relative to sample 15/220 (WHO IRR-ARC CP)	Relative to sample 92 (Candidate 1 st IS- SL CP)
					Assay 1	Assay 2	Assay 3			
rcVSV-PsN	12	GP	EBOV	15/220	2.63	2.60	2.77	426	1.00	2.15
				38	*	*	*	NA	NA	NA
				39	negative	negative	negative	NA	NA	NA
				66	*	*	*	NA	NA	NA
				85	*	*	*	NA	NA	NA
				92	2.47	2.27	2.30	198	0.46	1.00
				93	2.29	2.02	2.12	131	0.31	0.66
				94	3.31	3.35	3.47	2225	5.22	11.24
95	2.45	2.41	2.58	279	0.65	1.41				
NA = not assessed										
Lab 12 comments: *Samples 38, 66, 85 are negative or marginally positive (equivocal). FRNT50 cannot be determined.										
nrVSV-PsN	13	GP	Kikwit	15/220	3.30	3.27	3.37	1978	1.00	0.77
				38	2.38	2.38	2.51	240	0.12	0.09
				39	<	<	<	NA	NA	NA
				66	2.71	2.89	3.05	782	0.40	0.30
				85	2.45	2.57	2.99	371	0.19	0.14
				92	3.22	3.41	3.76	2572	1.30	1.00
				93	3.88	3.98	4.36	9632	4.87	3.74
				94	4.67	4.48	4.87	46513	23.51	18.09
95	3.03	3.27	3.39	1843	0.93	0.72				
Lab 13 comments Additional participant's comments about the PRNT50 and PRNT80 results are given in Appendix 6.										

Table 6. Summary of median neutralizing antibody titres for convalescent plasmas tested in neutralisation (Neut), lentiviral vector (LVV) pseudoneutralisation (PsN) and vesicular stomatitis virus vector (VSV) PsN assays. Also shown are overall geometric mean titres and geometric coefficients of variation. Unless stated otherwise, the titres are 50% reduction neutralisation titres (RNT50).

Neutralisation Method	Lab code	Median Neut/PsN titres RNT50 unless stated otherwise						Comments
		15/220	38	66	85	92	95	
Neut, Whole virus Makona isolate C05	15b	160	n/a	28	20	80	80	
Neut, Whole virus, Mayinga	16a	304	27	64	27	512	256	100% neutralisation titre
LVV-PsN, EBOV	2	610	349	178	217	436	290	
LVV-PsN, Makona	4	187	76	65	123	365	311	
LVV-PsN, Makona	9	220	63	153	108	186	171	
LVV-PsN, Makona isolate C15	11	226	20#	7241#	226	905	n/a#	
VSV-PsN, EBOV	10b	5230	1331	1619	1101	5845	3953	RNT60
rcVSV-PsN, EBOV	12	426	n/a	n/a	n/a	198	279	
nrVSV-PsN, EBOV	13	1978	240	782	371	2572	1843	
	Overall GM	474	116	271	139	529	399	
	Overall GCV	228%	348%	558%	275%	282%	258%	
Except samples 38 and 66 for laboratory 11	Overall GM		156	170				
	Overall GCV		308%	324%				

Abbreviations: GM = Geometric mean; GCV = geometric coefficient of variation.

n/a = Median titre not assessed.

Titration curves were difficult to interpret for these samples.

Table 7. Summary of individual laboratory median potencies expressed relative to the candidate 1st IS for EBOV Ab (Sample 92) for convalescent plasma samples tested in neutralisation (Neut), lentiviral vector (LVV) pseudoneutralisation (PsN) and vesicular stomatitis virus vector (VSV) PsN assays. Also shown are overall geometric mean potencies and geometric coefficients of variation.

Neutralisation Method	Lab code	Potencies relative to sample 92					
		15/220	38	66	85	92	95
Neut, Whole virus Makona isolate C05	15b	2.00	n/a	0.35	0.25	1.00	1.00
Neut, Whole virus, Mayinga	16a	0.59	0.05	0.13	0.05	1.00	0.50
LVV-PsN, EBOV	2	1.40	0.80	0.41	0.50	1.00	0.67
LVV-PsN, Makona	4	0.51	0.21	0.18	0.34	1.00	0.85
LVV-PsN, Makona	9*	n/a	n/a	n/a	n/a	1.00	0.92
LVV-PsN, Makona isolate C15	11	0.25	0.02#	8.00#	0.25	1.00	n/a#
VSV-PsN, EBOV	10b	0.89	0.23	0.28	0.19	1.00	0.68
rcVSV-PsN, EBOV	12	2.15	n/a	n/a	n/a	1.00	1.41
nrVSV-PsN, EBOV	13	0.77	0.09	0.30	0.14	1.00	0.72
	Overall GM	0.87	0.13	0.42	0.20	1.00	0.81
	Overall GCV	108%	251%	292%	105%	0%	37%
Except samples 38 and 66 for laboratory 11	Overall GM		0.18	0.25			
	Overall GCV		180%	56%			

Abbreviations: GM = Geometric mean; GCV = geometric coefficient of variation.

* Samples not included on the same plate as sample 92 were not assessed.

n/a = Relative potency not assessed;.

Titration curves were difficult to interpret for this sample.

Table 8. Summary of individual laboratory median potencies expressed relative to the current WHO IRR for EBOV Ab (Sample 15/220) for convalescent plasma samples tested in neutralisation (Neut), lentiviral vector (LVV) pseudoneutralisation (PsN) and vesicular stomatitis virus vector (VSV) PsN assays. Also shown are overall geometric mean potencies and geometric coefficients of variation.

Neutralisation Method	Lab code	Potencies relative to sample 15/220					
		15/220	38	66	85	92	95
Neut, Whole virus, Makona isolate C05	15b	1.00	n/a	0.18	0.13	0.50	0.50
Neut, Whole virus, Mayinga	16a	1.00	0.09	0.21	0.09	1.68	0.84
LVV-PsN, EBOV	2	1.00	0.57	0.29	0.36	0.72	0.48
LVV-PsN, Makona	4	1.00	0.41	0.35	0.66	1.95	1.66
LVV-PsN, Makona	9*	1.00	0.29	0.70	0.49	n/a	n/a
LVV-PsN, Makona isolate C15	11	1.00	0.09	32.00	1.00	4.00	n/a
VSV-PsN, EBOV	10b	1.00	0.25	0.31	0.21	1.12	0.76
rcVSV-PsN, EBOV	12	1.00	n/a	n/a	n/a	0.46	0.65
nrVSV-PsN, EBOV	13	1.00	0.12	0.40	0.19	1.30	0.93
	Overall GM	1.00	0.21	0.56	0.29	1.16	0.77
	Overall GCV	0%	111%	438%	131%	108%	53%
Except samples 38 and 66 for laboratory 11	Overall GM		0.24	0.32			
	Overall GCV		104%	56%			

Abbreviations: GM = Geometric mean; GCV = geometric coefficient of variation.

*Samples not included on the same plate as sample 15/220 were not assessed.

n/a = Relative potency not assessed.

Titration curves were difficult to interpret for these samples.

Table 9. Individual ELISA potency estimates relative to sample 92.

Lab	Assay	15/220	38	66	85	93	94	95
1a, 1b, 1c, 1d	Not amenable to parallel line analysis							
3	1	0.136	0.243	Non-parallel	Invalid*	ND	ND	Non-parallel
	2	0.795	1.505	5.403	Non-linear	ND	ND	Non-parallel
	3	0.379	0.927	Non-parallel	Non-parallel	ND	ND	Non-parallel
5	1	1.119	Not assessed**	Not assessed**	0.256	Not assessed**	Not assessed**	1.075
	2	1.402			0.265			1.110
	3	1.219			0.273			1.118
6a	1	0.581	0.392	0.255	0.210	4.746	OOR	0.484
	2	0.508	0.241	0.218	0.219	Non-parallel	OOR	0.421
	3	0.910	0.383	0.352	0.296	6.685	OOR	0.409
6b	1	Non-parallel	0.464	0.185	0.493	ND	ND	OOR
	2	Non-parallel	Non-parallel	Non-parallel	Non-parallel	ND	ND	OOR
	3	0.257	0.630	Non-parallel	0.658	ND	ND	OOR
7	1	0.689	0.499	0.626	1.447	OOR	OOR	0.542
	2	0.651	0.233	0.524	1.222	OOR	OOR	Non-parallel
	3	0.571	0.377	0.418	1.053	OOR	OOR	0.659
8	1	0.267	0.291	0.201	0.315	7.053	0.040	0.888
	2	Non-linear	0.310	0.408	Non-parallel	Non-parallel	0.053	0.810
	3	0.153	0.224	Non-parallel	0.367	Non-linear	0.032	1.555
10	1	0.962	0.469	0.320	0.329	Non-parallel	OOR	0.856
	2	1.381	0.709	0.309	0.448	Non-parallel	0.052	1.090
	3	0.979	0.570	0.347	0.330	5.581	0.042	0.850
	4	Non-linear	Invalid#	Invalid#	Invalid#	Invalid#	Invalid#	Invalid#
14	1	0.417	0.460	0.199	0.412	Non-parallel	0.041	0.668
	2	0.761	0.662	0.296	0.489	Non-parallel	0.053	0.924
	3	Non-parallel	0.396	0.217	0.352	Non-parallel	0.060	0.640
15	1	Not assessed**	Not assessed**	Not assessed**	Not assessed**	1.206	OOR	0.402
	2					1.236	OOR	0.387
	3					1.236	OOR	0.387
16b	Reported at 1/200 dilution. Not amenable to parallel line analysis.							

OOR = out of range-high. Sample not assessed

* Invalid due to variability between replicates; ** Samples not included on the same plate as sample 92 were not assessed. # Non-linear reference sample.

Table 10. ELISA results summary. Geometric mean potencies expressed relative to sample 92

Sample			15/220			38			66			85			93			94			95		
Lab Code	Analyte anti-	Virus	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV
3	inactivated WV	EBOV	3	0.345	143%	3	0.697	157%	1	5.403*	NA	0	IV	NA		ND	NA		ND	NA	0	IV	NA
5**	GP IgG	Makona	3	1.241	12%	3	NA	NA	3	NA	NA	3	0.265	3%	0	NA	NA	0	NA	NA	3	1.101	2%
6a	GP	EBOV	3	0.645	36%	3	0.331	31%	3	0.270	28%	3	0.239	21%	2	5.633	NA	0	OOR	NA	3	0.437	9%
6b	NP	EBOV	1	0.257	NA	2	0.540	NA	1	0.185	NA	2	0.569	NA	0	NA	NA	0	NA	NA	0	OOR	NA
7	VP40 & NP	EBOV	3	0.635	10%	3	0.353	47%	3	0.516	23%	3	1.230	17%		ND	NA		ND	NA	2	0.598	NA
8	GP IgG	EBOV	2	0.202	NA	3	0.272	19%	2	0.287	NA	2	0.340	NA	1	7.053	NA	3	0.041	28%	3	1.038	42%
10	GP IgG	EBOV	3	1.091	23%	3	0.575	23%	3	0.325	6%	3	0.365	19%	1	5.581	NA	2	0.047	NA	3	0.926	15%
14	GP	Kikwit	2	0.563	NA	3	0.494	30%	3	0.234	23%	3	0.414	18%	0	IV	NA	3	0.051	21%	3	0.734	22%
15a**	GP IgG	EBOV	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	3	1.117	1%		ND	NA	3	0.392	2%
Overall GM			0.52			0.44			0.29			0.42			4.06			0.05			0.70		
Overall GCV			92%			41%			41%			74%			124%			12%			51%		
Overall GM excluding labs 6b and 7			0.57			0.48			0.28			0.32			4.06			0.05			0.72		
Overall GCV excluding labs 6b and 7			99%			48%			15%			26%			124%			12%			53%		

Abbreviations: GM = Geometric mean; GCV = geometric coefficient of variation; WV = whole virus; GP = glycoprotein; NP = nucleoprotein; VP40 = matrix protein ; IV = all assays invalid for this sample; NA= not assessed; ND= not detected; OOR = out of range-high;

* The discrepant value from a single assay was not included in calculation of overall GM and overall %GCV.

** Samples not included on the same plate as sample 92 were not assessed.

Table 11. ELISA results summary. Geometric mean potencies expressed relative to sample 15/220.

Sample			GM						
Lab Code	Analyte anti-	Virus	92	38	66	85	93	94	95
3	inactivated WV	EBOV	2.901	2.022	15.674*		ND	ND	
5**	GP IgG	Makona	0.806			0.213			0.887
6a	GP	EBOV	1.550	0.513	0.418	0.370	8.731		0.678
6b	NP	EBOV	3.893	2.103	0.720	2.217			
7	VP40 & NP	EBOV	1.574	0.555	0.812	1.937			0.941
8	GP IgG	EBOV	4.949	1.348	1.419	1.683	34.904*	0.201	5.136
10	GP IgG	EBOV	0.916	0.527	0.298	0.334	5.114	0.043	0.848
14	GP	Kikwit	1.776	0.877	0.416	0.735		0.090	1.303
15a**	GP IgG	EBOV							
Overall GM			1.91	0.96	0.59	0.76	6.68	0.09	1.22
Overall GCV			92%	87%	77%	160%	46%	117%	109%
Overall GM excluding labs 6b and 7			1.76	0.92	0.52	0.50			1.28
Overall GCV excluding labs 6b and 7			99%	81%	99%	124%			125%

Abbreviations: GM = Geometric mean; GCV = geometric coefficient of variation; WV = whole virus; GP = glycoprotein; NP = nucleoprotein; VP40 = matrix protein ; IV = all assays invalid for this sample; NA= not assessed; ND= not detected; OOR = out of range-high;

* The discrepant value from a single assay was not included in the calculation of overall GM and overall %GCV.

** Samples not included on the same plate as sample 92 were not assessed.

Table 12. Surface Plasmon Resonance results summary. Potencies expressed relative to samples 92 and 15/220. See appendix 9 for the SPR raw data.

Relative to Sample 92								
Sample	92	15/220	38	66	85	93	94	95
Assay 1	1.000	0.756	Non-linear	0.244	0.490	0.221	ND	0.425
Assay 2	1.000	0.755	Non-linear	0.243	0.490	0.222	ND	0.443
Mean	1.00	0.76	NA	0.24	0.49	0.22	NA	0.43
Relative to Sample 15/220								
Sample	92	15/220	38	66	85	93	94	95
Assay 1	1.322	1.000	Non-linear	0.322	0.648	0.292	ND	0.562
Assay 2	1.324	1.000	Non-linear	0.322	0.649	0.294	ND	0.587
Mean	1.32	1.00	NA	0.32	0.65	0.29	NA	0.57

NA = relative potency not assessed

ND = Not detected

Table 13. Overall geometric mean potencies across all assays expressed relative to the candidate IS (sample 92) and IRR (15/220).

lower 95% confidence limit	0.57	0.24	0.24	0.25		0.66
Overall GM relative to 92	0.68	0.30	0.27	0.30	1.00	0.73
upper 95% confidence limit	0.80	0.38	0.30	0.36		0.80
Overall GCV	100%	130%	44%	105%		45%
lower 95% confidence limit		0.35	0.36	0.37	1.25	0.98
Overall GM relative to 15/220	1.00	0.45	0.40	0.45	1.47	1.07
upper 95% confidence limit		0.57	0.44	0.54	1.75	1.18

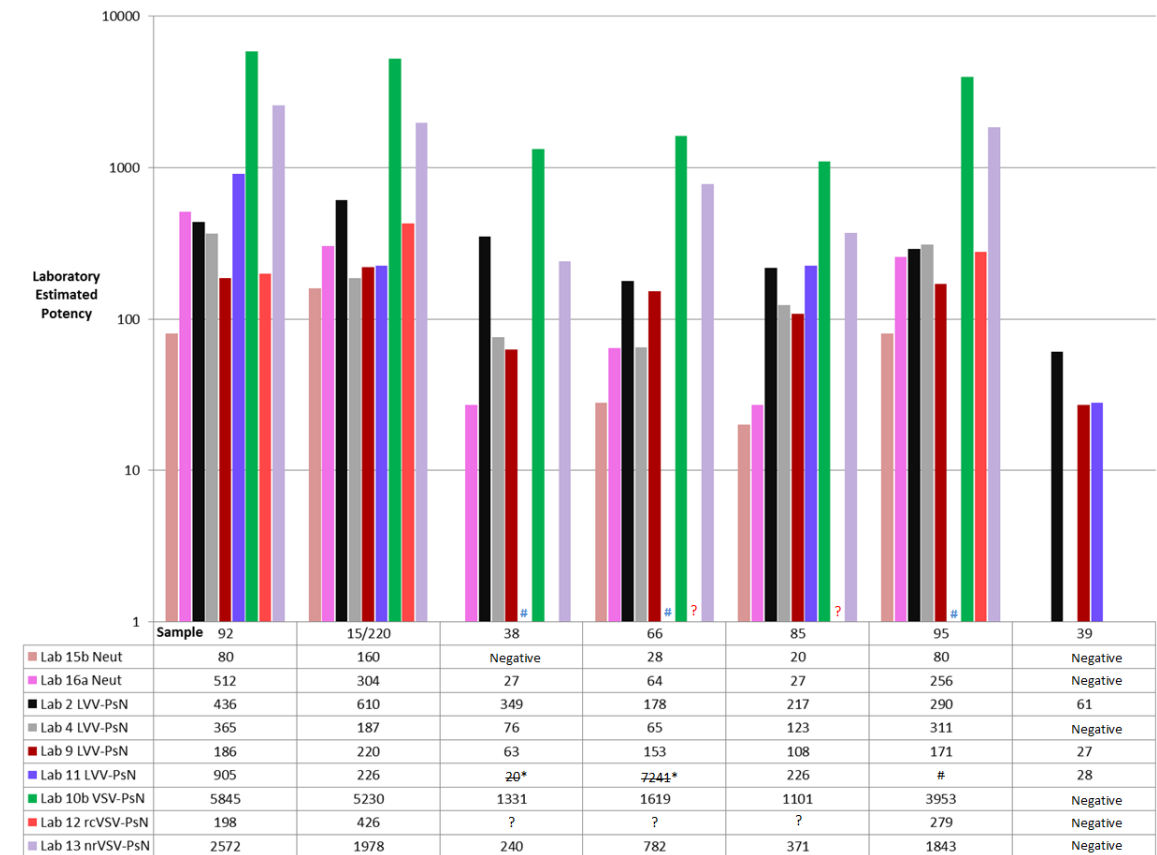
Abbreviations: GM = Geometric mean; GCV = geometric coefficient of variation

Table 14. Predicted degradation rates for candidate WHO 1st IS for EBOV Ab 15/262.

Temp	K	S.E. (K)	% Loss per Week	95% UCL % Loss
-150	0	0	0	0
-70	0	0	0	0
-20	0	0	0	0
4	0.00001	0.00001	0.001	0.006
20	0.00023	0.00019	0.023	0.118
37	0.00492	0.00176	0.491	1.363

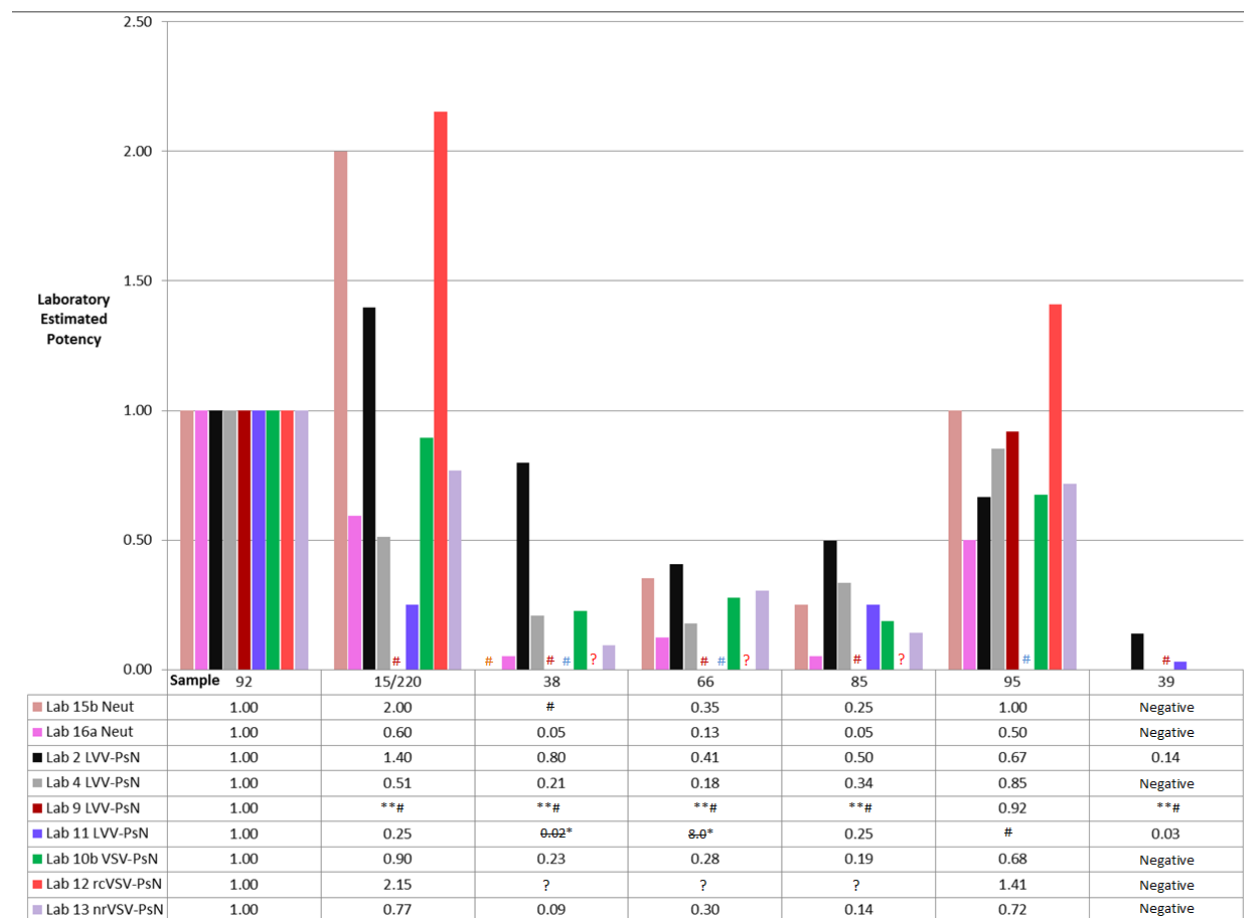
The Upper Confidence Limit for Potency Loss is based on 5 times the standard error of the Degradation Rate.

Figure 1. Median titre estimates for neutralisation assays (Neut) and pseudo-neutralisation (PsN) assays.



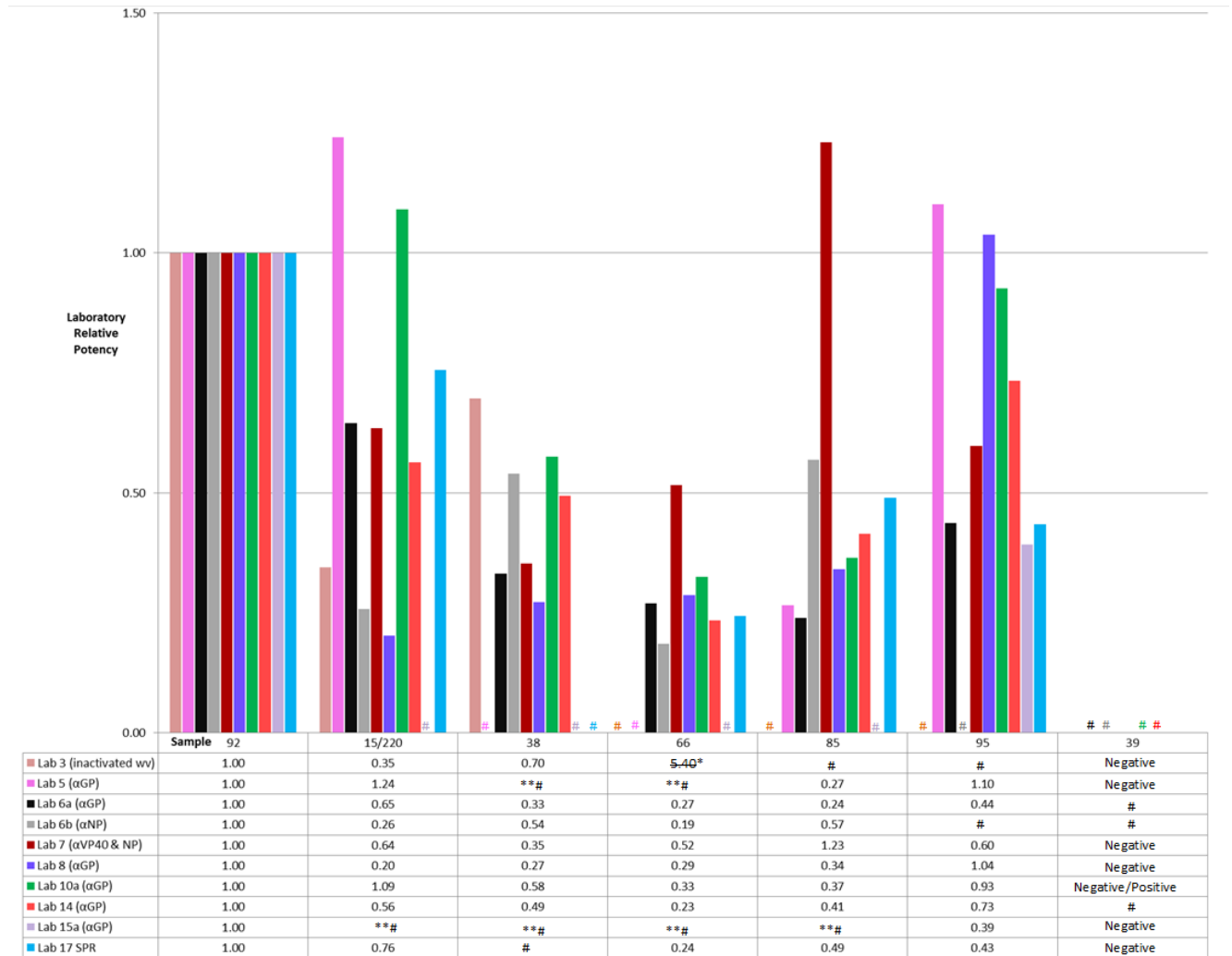
Notes: * Discrepant value from a single assay was not included in graph; # Positive- relative potency not determined; ** Relative potency was not determined for samples not included on the same plate as sample 92.

Figure 2. Laboratory potencies relative to sample 92 for neutralisation assays (Neut) and pseudoneutralisation (PsN) assays.



Notes: * Discrepant value from a single assay was not included in graph; # Positive- relative potency not determined; ** Relative potency was not determined for samples not included on the same plate as sample 92.

Figure 3. Laboratory potencies relative to sample 92 for enzyme immunoassays and SPR.



Notes: * Discrepant value from a single assay was not included in graph; # Positive- relative potency not determined; ** Relative potency was not determined for samples not included on the same plate as sample 92.

Figure 4

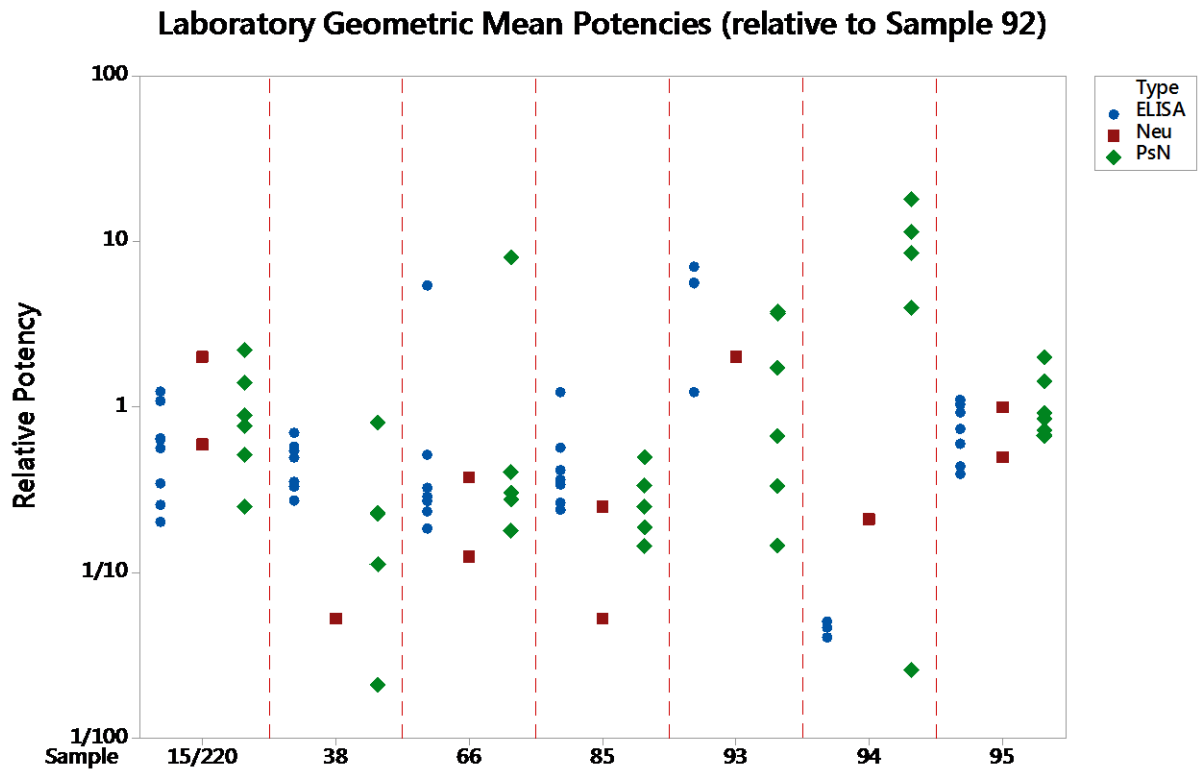
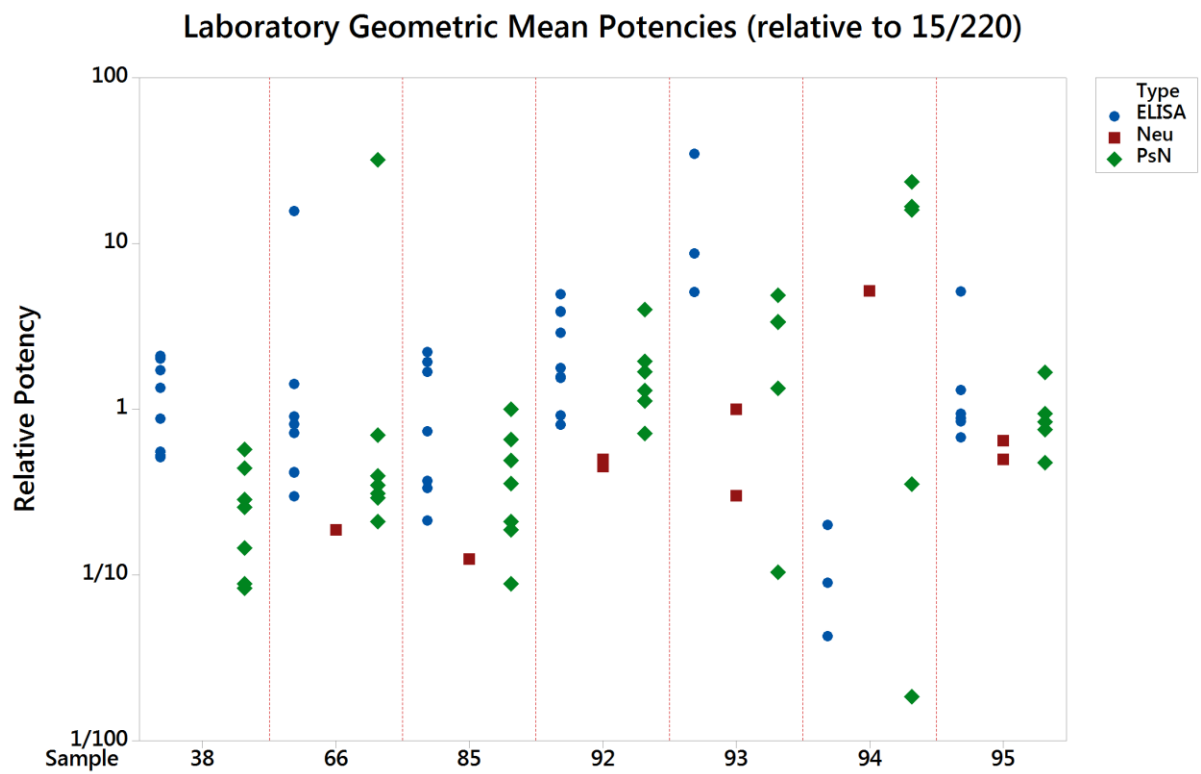


Figure 5



Appendix 1**Collaborative study participants**

(In alphabetical order by country)

Name	Laboratory	Country
Béatrice Labrosse, Christophe Léculier, Delphine Pannetier	Laboratoire P4 Inserm Jean Mérieux	France
Thomas Strecker, Verena Krähling, Sarah Katharina Fehling	Institute of Virology of the Philipps-University, Marburg	Germany
Barbara Schnierle, Lisa Henss	Paul-Ehrlich-Institut	Germany
Derek Gatherer, Lisa Bishop, Katharina Hartman, Robert Lauder, Marcell Mullner, Hoang Son Tran	Lancaster University	UK
Sophie Myhill, Giada Mattiuzzo	NIBSC	UK
Edward Wright, Emma Bentley	University of Westminster	UK
Richard Tedder, Dhan Samuel, Steve Dicks	Virus Reference Department, Public Health England, Colindale	UK
Thomas Rudge	Battelle-BBRC	USA*
Matthew L. Boisen	Zalgen Labs LLC	USA
Anna N Honko, Richard S. Bennett, Jamie Pettitt, Krisztina Janosko, Jonathan Marchand, Elena Postnikova,	Integrated Research Facility, National Institutes of Allergy and Infectious Diseases	USA
Gerardo Kaplan, Krishnamurthy Konduru,	Office of Blood Research and Review, CBER-FDA	USA
Surender Khurana	Office of Vaccine Research and Review (OVR), CBER, FDA	USA
Carol D. Weiss	OVR, CBER, FDA	USA
Wayne R. Hogrefe, Nicole Rodriguez, Rohini G. Sandesara, Sarah L. Daijogo	Q2 Focus Diagnostics Clinical Trials.	USA
Hua Wu	SAB Biotherapeutics Inc.	USA
Jay W. Hooper, Steve A. Kwilas, Meagan Wisniewski	United States Army Medical Research Institute of Infectious Diseases (USAMRIID)	USA
Peter M. Silvera	USAMRIID	USA

*Steven A. Rubin, FDA/CBER facilitated sample permits and shipments to laboratories in the USA.

Ebola CP Consortium investigators

Name	Affiliation	Country
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Appendix 3 Collaborative Study Protocol

WHO International Collaborative Study to Establish the 1st International Standard for Ebola Virus Antibodies

In October 2015, the WHO Expert Committee on Biological Standardization (ECBS) established a preparation of plasma (NIBSC product code 15/220) obtained from a patient recovered from Ebola virus disease (EVD) to serve as the WHO 1st International Reference Reagent for Ebola antibodies with an assigned potency of 1 unit per mL. NIBSC 15/220 was assessed in a WHO international collaborative study and is known in that study as EBOV Ab Sample Code 79 [1, 2].

15/220 is serving as the interim WHO Reference Reagent for EBOV antibodies

(https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=15/220) while we undertake further work to develop a candidate preparation and evaluate its suitability to serve as the WHO 1st International Standard for EBOV antibodies. WHO International Standards 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 [3]. In addition to the 1st International Standard, we are developing a WHO Reference Panel of 5 to 7 EBOV antibody samples for testing and comparison across assays and laboratories.

Participation in the study will involve the testing of the candidate International Standard and other antibody preparations alongside the WHO International Reference Reagent (15/220) in assay(s) that are in routine use in the participant's laboratory. The aims [3] of this WHO international collaborative study are to

- assess the suitability of the candidate to serve as the International Standard with an assigned unitage in IU per vial for use in the harmonization of EBOV antibody assays. There is no international conventional reference measurement procedure for EBOV antibodies and the IU is not traceable to the International System of Units (SI) of quantity
- identify additional candidate EBOV antibody preparations for inclusion in a WHO International Reference Panel for EBOV antibodies.
- characterise each candidate preparation in terms of reactivity/specificity.
- assess each candidate'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 candidate is suitable to serve as an international standard for the variety of different samples and assay types.
- recommend to WHO ECBS for establishment the candidate found to be suitable to serve as the 1st International Standard.
- recommend to WHO ECBS the establishment of the EBOV antibody reference panel.

Study Samples

1. Candidate 1st WHO International Standard for antibodies to EBOV

The candidate material is a pool of plasmas obtained from six Sierra Leone patients recovered from EVD (Provided by Dr Calum Semple, University of Liverpool, UK). The treatment histories of the Sierra Leone patients are not known. Approximately 1000 ampoules containing the freeze-dried equivalent of 0.5mL plasma have been prepared at NIBSC.

2. Candidate WHO Reference Panel Samples for EBOV antibodies

The candidate panel members will be evaluated alongside the candidate International Standard. Members of the panel may include negative samples, reactive samples from convalescent patients and monoclonal antibody formulations. We currently do **not** have samples from human recipients of Ebola vaccines for inclusion in the study.

- **National Institute for Infectious Diseases Lazzaro Spallanzani (INMI) donor (provided by Maria Rosaria Capobianchi, INMI, Italy):** The INMI patient had received Favipiravir, 3 treatments of convalescent plasma from 2 donors, ZMAb and Melanocortin (TCS 10).

- **Norway (NOR) donor (provided by Dr Richard W. Olausson, Oslo University):** The Ebola-specific therapeutic treatments given to the NOR patient include ZMAb (anti-GP), Favipiravir (RNA pol inhibitor) and TKM-100802 (iRNA).
- **American Red Cross (ARC) donor (provided by Dr Susan L. Stramer, ARC):** The ARC patient received 2 aliquots of convalescent plasma 24 hours apart, as well as 7 nightly infusions of TKM-Ebola (iRNA). The ARC donor did not receive monoclonal antibody treatments.
- **UK National Health Service Blood and Transplant (NHSBT) donor (provided by Dr Sheila MacLennan, Leeds):** The NHSBT patient received Brincidifovar sp.? (anti-viral) and convalescent plasma treatments.

Prior to shipment to the UK, all convalescent plasmas were tested and found negative for EBOV RNA. Prior to receipt at NIBSC, the convalescent plasmas were held at Public Health England (PHE), Colindale, UK until confirmed by PCR that no EBOV RNA could be detected in the materials. After receipt at NIBSC, plasma donations were solvent-detergent extracted as described in the ECBS collaborative study report [1]. For each panel member, approximately 200 to 600 ampoules containing the freeze-dried plasma have been prepared at NIBSC.

Please note that, while the NOR, ARC and NHSBT source materials listed above are the same as those described in the ECBS report [1], they have been prepared independently for assessment in this collaborative. For example, the NOR, ARC and NHSBT samples for this collaborative study have undergone separate solvent-detergent treatments with respect to Sample 43,79 and 79 from the previous study. Furthermore, the NOR, ARC and NHSBT have been freeze-dried for this collaborative study, whereas the corresponding samples in the earlier study were liquid presentations.

- **Purified monoclonal antibodies donated by Prof A. Townsend, University of Oxford, UK:** These were cloned from B cells of volunteers participating in the Oxford, UK vaccine trial who have been primed with the monovalent formulation of the chimpanzee adenovirus 3 (ChAd3)-vectored vaccine encoding the surface glycoprotein of Ebola virus (GSK/NIH vaccine candidate) [4].

Two MAbs have been prepared in sterile PBS-Ca²⁺-Mg²⁺ supplemented with 5% human serum albumin. One-hundred vials of each MAb in 100µL liquid aliquots have been prepared.

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 Ebola. Laboratories may use multiple methods to test the study materials provided that the study design is followed for each method.

Design of study

Participants are requested to:

- perform 3 independent assays on different days for antibodies against Ebola. Laboratories will receive at least 3 sets of study samples which should allow for 3 independent assays by one method. Laboratories with more than one assay method will receive additional sample sets to allow 3 independent assays per method (subject to availability). Based on availability, spare vials/ampoules of certain samples will be provided to allow participants to run a preliminary assay in order to ascertain working dilutions.
- 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 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 4 steps including one step 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 assay readout (e.g. from the spectrophotometer/luminometer/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 whether each sample dilution tested is considered positive or negative according to assay criteria.

- Include all study samples in each assay so that the potency of antibodies relative to one another may be calculated. Please note in the reporting sheet if it is not practicable to test all samples concurrently indicating which samples were tested concurrently.
- 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 6 weeks of receipt of the study materials. If it is not practicable to turn around results within 6 weeks, please inform dianna.wilkinson@nibsc.org. An excel spread sheet will be 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 spread sheet facilitates the analysis and interpretation of results. If multiple assay methods are undertaken, a separate worksheet for each method should be completed. The reporting spread sheet will be e-mailed to each participant closer to the launch of the collaborative study. 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 to the WHO ECBS on the selection, use and unitage of the 1st International Reference Reagent and Reference Panel for use in assays for Ebola virus (EBOV) antibodies. The finalised report will then be submitted to ECBS, 2016 who will decide on the recommendations.

Participation in the WHO collaborative study is conducted under the following conditions:

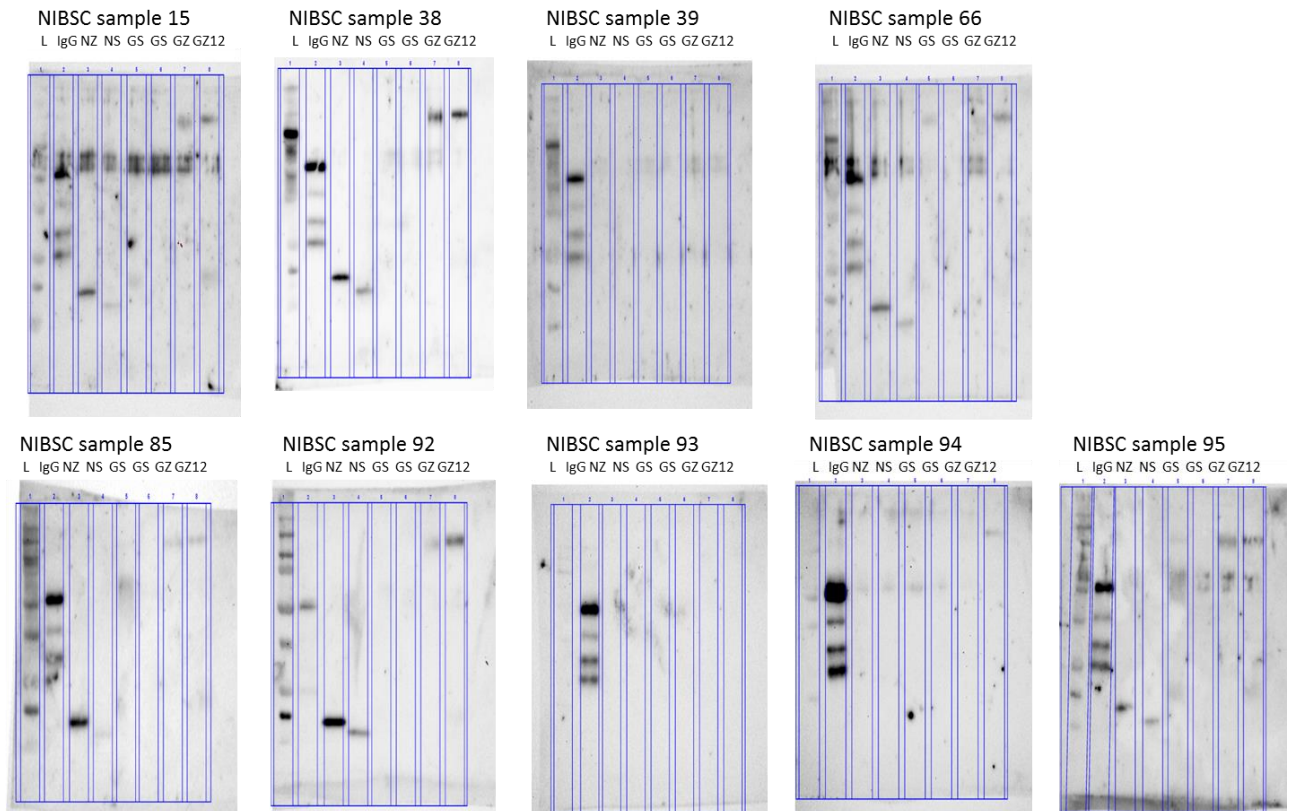
- The data obtained in the collaborative study should not be published or cited before the formal establishment of the standard by WHO, without the expressed permission of the NIBSC Study organizer.
- In order to address the immediate need for Ebola serology standards, participants are permitted to use the study samples for purposes that fall outside of the collaborative study. To better inform the subsequent implementation of any standard, participants are encouraged to provide to the study organizer any information gained through their use of the study materials. THE CANDIDATE MATERIALS ARE PROVIDED TO PARTICIPANTS “AS IS”, WITHOUT ANY REPRESENTATION OR WARRANTY OF SATISFACTORY QUALITY OR FITNESS FOR A PARTICULAR PURPOSE.
- 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 also in subsequent publications.
- Participants will receive a copy of the report of the study and 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 spread sheets is 6 weeks from receipt of study materials. If it is not practicable to return results within 6 weeks, please inform Dianna Wilkinson.

All completed results spread sheets should be returned electronically to:

Dr Dianna Wilkinson
Principal Scientist
Viral Vaccines Section
Division of Virology
National Institute for Biological Standards and Control
Blanche Lane, South Mimms
Hertfordshire, EN6 3QG
UK
Tel. +44(0)1707 641314
dianna.wilkinson@nibsc.org

NIBSC 2016



NZ – Zaire NP
NS – Sudan NP
GS – Sudan GP (two lanes)
GZ – Zaire GP isoform 1
GZ 1,2- Zaire GP isoform 1,2

Appendix 5

Laboratory 10: Results of WHO EBOV Ab study CS570

FANG ELISA Results

Sample 93 showed high variability over the 4 days of ELISA testing (Appendix 5, Table 1). As the final concentration and endpoint titer result for Sample 93 was well above the ULOQ of our assay, a pre-dilution of 1:1024 was required to bring the sample within range of the assay. Testing of high titered samples with this assay has been shown to be precise and accurate with predilutions up to 1:128. Evaluation of larger dilution factors has not been performed. Because dilution of small volumes of sample into large volumes of assay diluent are required to pre-dilute to 1:1024, dilution bias may be a contributing factor in the high variability of sample 93.

rVSV-ZEBOV GP PRNT Results

Samples 93 and 94 both had poor titration curves in our PRNT assay on all 4 days they were tested. Below is a graph depicting plaque counts from one day of running for samples 85, 92, 93 and 94. For samples 85 and 92, the titration was as expected with an increase in plaques as the sample became more dilute. However, for samples 93 and 94, the plaque counts hovered around our 40% virus cutoff for many of the starting dilutions and did not increase until much later in the dilution series (see Appendix 5, Figure 1). As such, the slope was relatively flat. This resulted in significantly different PRNT₆₀ titers being calculated for both of these samples (6590 vs. 1806 for sample 93 and 690 vs. <20 for sample 94) when they passed the correlation coefficient criteria. The other two days these samples failed these criteria because the plaque counts bounced above and below the virus control 40% cutoff. Per our TSOP, correlation coefficient failures would be repeated, however, due to sample constraints, this was not possible. As such, there are only 2 data points for samples 93 and 94 that are available and these should be considered within the context of the flat titration seen on all days of testing. It should be noted that although our assay does begin at a 1:10 dilution of sample, the PRNT₆₀ results start at the 1:20 dilution and exclude the initial 1:10 dilution due to a “plaque explosion phenomenon” that has been seen in our assay where there is an excessive number of plaques in the first dilution only.

Figure 1. Graphs depicting the plaque counts of samples 85, 92, 93 and 94. All samples had been pre-diluted 1:4 before being tested and as such, the values pertaining to the dilution of the sample on the x-axis should be multiplied by 4 for the correct dilution.

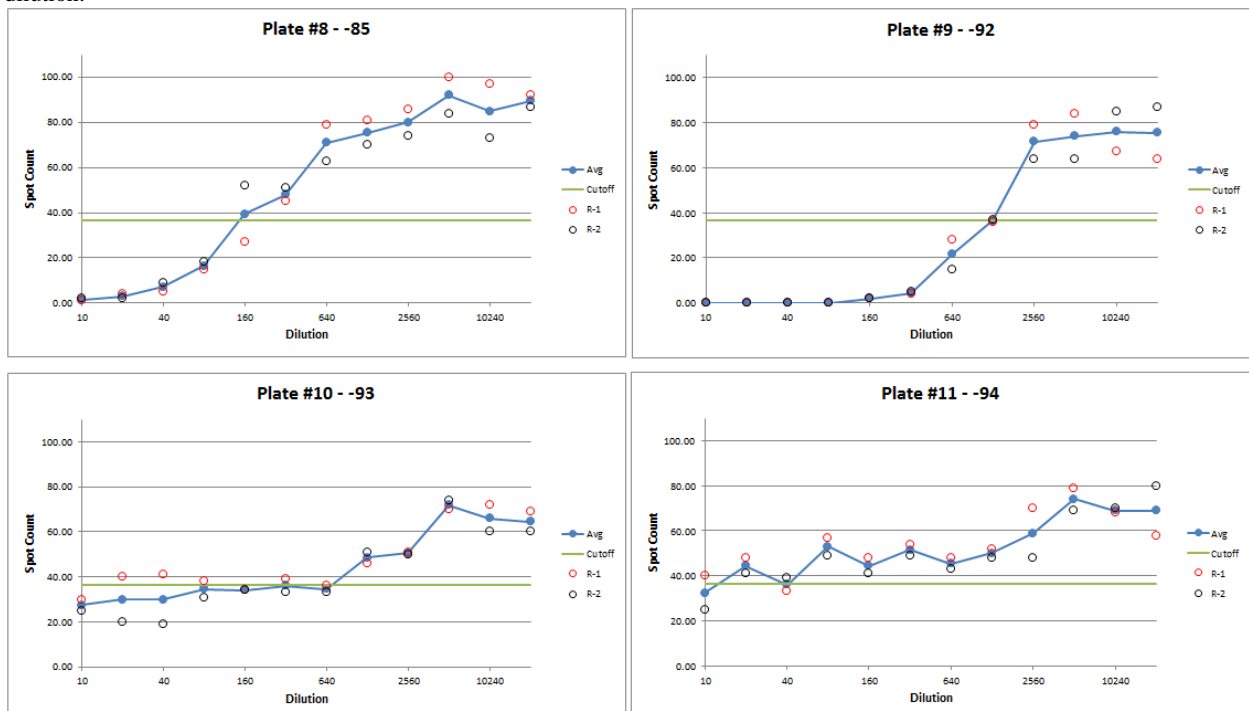
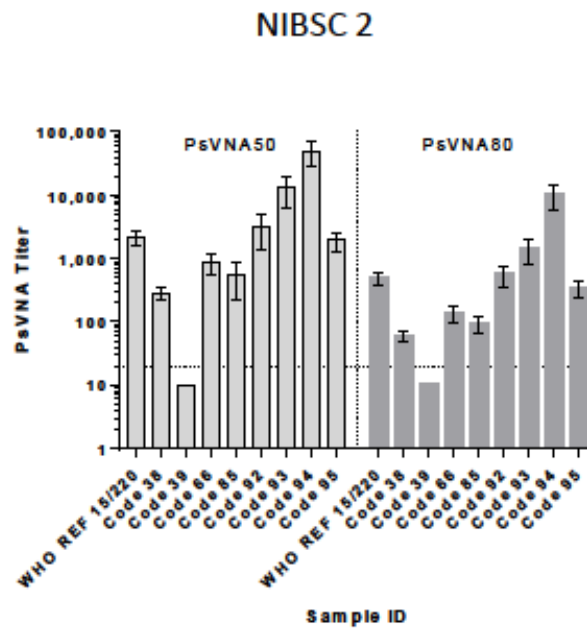


Table 1. ELISA and PRNT Results for NIBSC Study CS570 Sample Panel. Each sample was tested on 4 separate run days in both the FANG ELISA and rVSV-ZEBOV GP PRNT assays. Samples with higher than expected variability are indicated in yellow. The mean concentration, mean end point titer and mean PRNT₆₀ are shown....

ELISA-PRNT Combined Overall Results						
Sample ID	ELISA				PRNT	
	Mean Conc. (EU/ml)	Conc. %CV	Mean Endpoint Titer	Endpoint Titer %CV	Mean	%CV
15/220	29458	13.1%	29257	33%	5802	30%
38	16343	19.3%	19200	37%	1333	19%
39	38	NA	50	NA	< 20	NA
65	9758	23.8%	10971	28%	1609	7%
85	10507	11.6%	12800	0%	1078	21%
92	28561	22.4%	23040	25%	5989	14%
93	250323	66.8%	332800	99%	4198	81%
94	1189	14.3%	1200	47%	690	NA
95	24873	19.0%	25600	0%	4402	24%

Appendix 6

PsVNA50 and PsVNA80 data provided by Laboratory 13.



	PsVNA50			PsVNA80		
	Mean	%CV	N	Mean	%CV	N
WHO REF 15/220	2160.111	27.327	9	486.000	22.144	9
Code 38	281.333	18.995	9	58.889	18.100	9
Code 39	10.000	0.000	9	10.000	0.000	9
Code 66	855.778	36.387	9	139.111	29.553	9
Code 85	555.444	60.183	9	94.778	29.428	9
Code 92	3225.556	58.098	9	561.444	36.531	9
Code 93	13130.556	52.222	9	1372.556	43.834	9
Code 94	49441.000	44.530	9	10312.778	41.681	9
Code 95	1943.222	32.570	9	334.444	27.803	9

Avg % CV

41.289

31.134

Fig. 200. NIBSC 2 data analysis. All 9 values are used to determine mean and %CV. The average %CV was approximately 41 for PsVNA50 and 31 for PsVNA80. The limit of quantitation was 20 (dashed line). A value of 10 was given to samples with titers of <20. Samples with 0% CV were not included in determination of average %CV.

Summary of EIA and neutralisation assays provided by Laboratory 15.

Table 3. Summary of FRNA50 and FRNA80 results compared to ELISA titers (EU/mL).

Sample code #	FRNA ₅₀ sample results for each independent run / dilution series			FRNA ₈₀ sample results for each independent run / dilution series			ELISA sample results for each independent run / dilution series (U/mL) ^a					
	Assay 1	Assay 2	Assay 3	Assay 1	Assay 2	Assay 3	Assay 1		Assay 2		Assay 3	
	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 1	Dil 2	Dil 1	Dil 2	Dil 1	Dil 2
15/220	160	160	80	40	40	20	5182.50	5348.01	3873.12	4078.39	5506.45	5910.47
38	<20	<20	<20	<20	<20	<20	5290.48	5500.64	4194.60	5049.54	4864.97	5666.08
39	<20	<20	<20	<20	<20	<20	<100	<100	<100	<100	<100	<100
66	40	20	<20	<20	<20	<20	2618.02	2707.24	2143.08	1758.55	2408.30	2555.98
85	20	20	<20	<20	<20	<20	4261.05	3881.85	3260.15	3164.95	3736.47	3353.92
92	80	80	40	40	40	20	9751.01	8953.29	6489.32	6724.31	8782.90	8547.88
93	160	320	160	160	160	160	12464.14	12921.47	6493.49	7217.63	12967.6	13434.64
94	20	<20	<20	<20	<20	<20	<100	<100	<100	<100	<100	<100
95	80	160	40	40	20	20	3634.94	3901.14	3219.13	3236.37	3892.92	3909.23
Internal Pos. Control ^c	640	640	320	320	320	160	254888	205068	208040	193815	226101	222783

^a Titer is expressed as the first dilution (reciprocal) at which 50% or 80% reduction in virus infected cells are measured.

^b Titers below the lowest dilution tested, 1/20, are indicated with <20.

^c Commercially available anti-Ebola virus antibody from IBT Bioservices (Lot # 1302001) produced against VLPs was used as a positive control.

^d A standard curve was run on each plate (0.5-10 U/mL) allowing calculation of U/mL for each sample. Limits of quantification were calculated as follows: LLOQ = 0.5 U x 200 = 100 U/mL, ULOQ = 10 U x highest dilution (6400 or 12800).

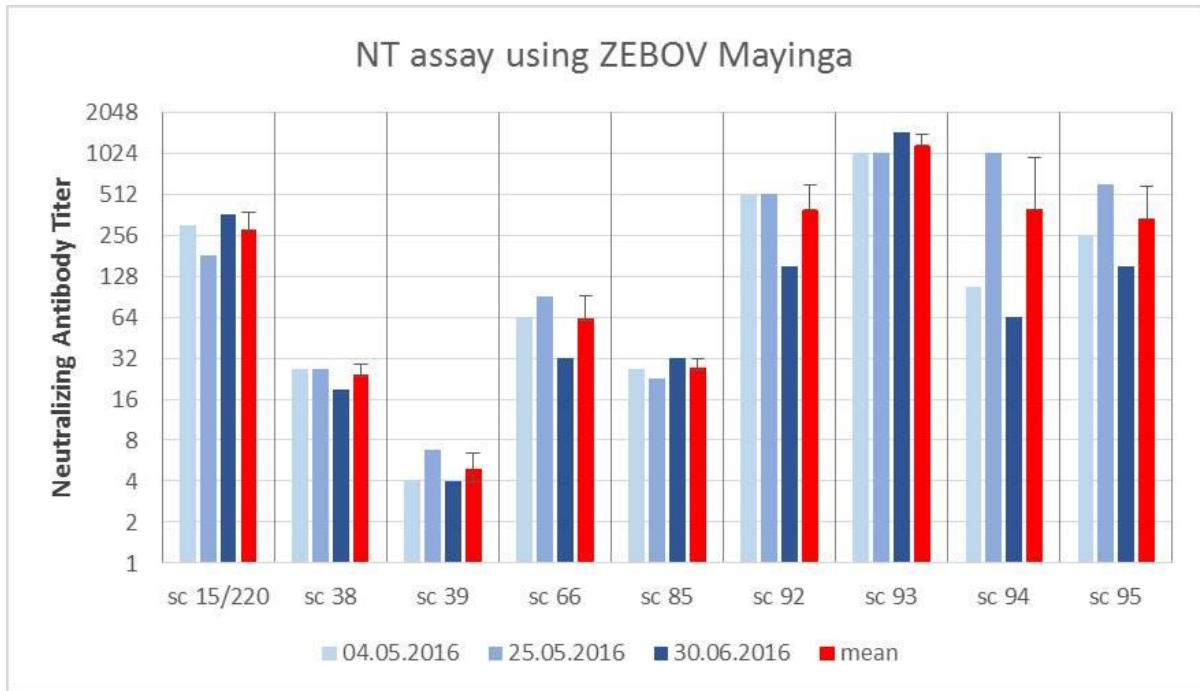
The FRNA assay, which detects neutralizing antibodies rather than GP specific antibodies, measured differing results based on criteria used (50 or 80% reduction). A FRNA₅₀ measured four positive samples, three indeterminate samples, and two negative samples. When the neutralization criteria was more stringent (80%), four samples were FNRA positive and five were negative. A total of seven samples were positive for antibodies by ELISA and two samples were negative.

Appendix 8

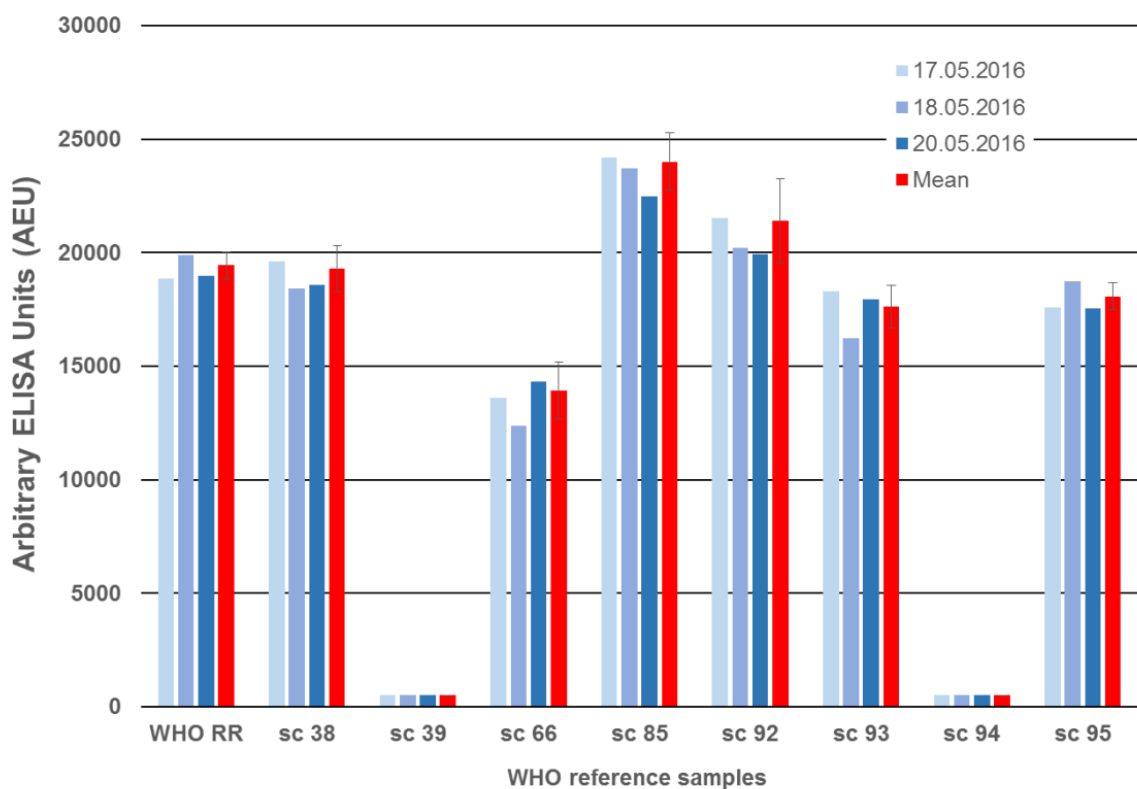
Laboratory 16

Comparison of results obtained with the neutralisation assay against live Ebola virus Mayinga (lab code 16a) and EIA against inactivated Ebola virus Makona (16b).

Method 16a. Endpoint titres for neutralisation.



Method 16b. EIA of collaborative study samples diluted 1/200.



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Appendix 9

Laboratory 17

Data returned for samples assessed by Surface Plasmon Resonance. With <20 Resonance Units (RU) at the 1:40 dilution, sample 39 scored overall negative.

	Assay 1			Assay 2	
	Dilution		RU		RU
WHO Reference Reagent- NIBSC code 15/220	10x	+	832.89	+	843.82
	20x	+	442.95	+	447.07
	40x	+	258.45	+	258
	80x	+	163.63	+	167.05
	160x	+	107.84	+	106.14
38	10x	+	468.92	+	478.36
	20x	+	314.75	+	316.65
	40x	+	286.70	+	287.30
	80x	+	187.98	+	185.03
	160x	+	72.09	+	71.08
39	10x	+	23.19	+	26.70
	20x	+	15.63	+	16.73
	40x	-	7.15	-	7.71
	80x	-	5.03	-	5.13
	160x	-	3.11	-	3.20
66	10x	+	353.13	+	353.63
	20x	+	192.93	+	192.57
	40x	+	105.09	+	107.19
	80x	+	72.18	+	73.17
	160x	+	53.32	+	52.75
85	10x	+	551.05	+	552.13
	20x	+	331.42	+	335.94
	40x	+	205.74	+	209.59
	80x	+	125.23	+	127.28
	160x	+	68.16	+	66.54
92	10x	+	1038.24	+	1040.31
	20x	+	599.62	+	605.21
	40x	+	322.75	+	324.53
	80x	+	195.13	+	196.59
	160x	+	123.2	+	124.45
93	10x	+	265.29	+	274.37
	20x	+	193.73	+	194.07
	40x	+	124.34	+	123.49
	80x	+	80.56	+	79.45
	160x	+	45.8	+	46.03
94	10x	-	3.82	-	4.94
	20x	-	3.01	-	3.62
	40x	-	-0.05	-	1.93
	80x	-	-0.08	-	1.59
	160x	-	0.22	-	1.37
95	10x	+	496.3	+	523.58
	20x	+	286.25	+	291.48
	40x	+	169.51	+	177.68
	80x	+	118.02	+	120.39
	160x	+	79	+	82.35

Appendix 10

Accelerated degradation data for the candidate WHO 1st International Standard Anti-EBOV Convalescent Plasma Pool-Sierra Leone (NIBSC 15/262).

Degtest-R Report

Assay: 15/262 DEGTEST CS570

Data Input

Temp	Potency	Time	LCL	UCL	df
4	1.36637	2	0.52986	3.52352	2
4	1.04729	4	0.75432	1.45405	2
4	1.07313	13	0.60421	1.90598	2
4	1.65497	26	1.18924	2.3031	2
4	0.92286	52	0.70783	1.20321	2
20	1.22696	2	0.76848	1.95896	2
20	1.02098	4	0.46861	2.22447	2
20	0.92739	13	0.7804	1.10207	2
20	1.49435	26	1.24897	1.78794	2
20	0.97127	52	0.54691	1.7249	2
37	0.92276	2	0.18035	4.72131	2
37	0.88099	4	0.3115	2.49163	2
37	0.96784	13	0.62894	1.48935	2
37	0.85836	26	0.1078	6.83469	2
37	0.58985	52	0.27837	1.24987	2
45	1.18504	2	0.58002	2.42116	2
45	1.22017	4	0.37776	3.94117	2
45	0.67161	13	0.00063	711.72681	1
45	0.5994	26	0.05287	6.79513	2
45	0.35931	52	0.13675	0.94409	2
56	0.2812	2	0.00766	10.32708	2
56	0.68484	4	0.11696	4.01013	1
56	0.33919	13	0.0362	3.17842	2

Reference Temperature (Celsius)

-20

Individual contribution chi-squared table

Temp	Time	Observed	Predicted	Chi-Squared
4	2	136.64	100	2.01
4	4	104.73	100	0.368
4	13	107.31	99.99	0.28
4	26	165.5	99.98	43.064
4	52	92.29	99.95	1.675
20	2	122.7	99.95	3.554
20	4	102.1	99.91	0.014
20	13	92.74	99.7	3.255
20	26	149.43	99.4	95.649
20	52	97.13	98.8	0.016
37	2	92.28	99.02	0.035
37	4	88.1	98.05	0.196
37	13	96.78	93.8	0.097
37	26	85.84	87.99	0.003
37	52	58.98	77.43	2.43
45	2	118.5	96.37	1.551
45	4	122.02	92.87	1.003
45	13	67.16	78.63	0.083
45	26	59.94	61.82	0.003
45	52	35.93	38.22	0.076
56	2	28.12	81.41	1.611
56	4	68.48	66.27	0.056
56	13	33.92	26.26	0.242

Chi-Squared table (Goodness of fit)

	Value	DF	P-Value
Total Chi-Squared	157.272	21	0

Appendix 11

Proposed instructions for use

WHO International Standard

1st WHO International Standard for EBOV antibodies

NIBSC code: 15/262

Instructions for use

(Version 1.00, Dated)

1. INTENDED USE

The 1st WHO International Standard for EBOV antibodies is intended to be used in the standardization of assays used in the detection and quantitation of EBOV antibodies

2. CAUTION

This preparation is not for administration to humans.

The preparation contains material of human origin. The convalescent plasmas are confirmed PCR-negative for EBOV and were solvent detergent-extracted prior to their development into the 1st WHO IS. 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

1st WHO International Standard for EBOV antibodies an assigned unitage of 1.5 IU/mL.

4. CONTENTS

Country of origin of biological material: Sierra Leone

Each ampoule contains the freeze-dried equivalent of 0.5 mL pooled convalescent plasma obtained from six Sierra Leone patients recovered from ebola virus disease (EVD).

5. STORAGE

15/262 should be stored at –20°C on receipt. The contents of the ampoule should be reconstituted with 0.1mL distilled water using safety precautions as described above.

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

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

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

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.

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

9. REFERENCES

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:

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NIBSC Terms & Conditions:

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12. CUSTOMER FEEDBACK

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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 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):	Contains material of human origin.		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
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: 0.5g per ampoule
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

Proposed instructions for use

WHO International Standard

1st WHO International Reference Panel for EBOV antibodies NIBSC code: 16/344

Instructions for use

(Version 1.00, Dated)

1. INTENDED USE

The 1st WHO International Reference Panel **for EBOV antibodies** is intended to be used in the assessment of assays used in the detection and quantitation of EBOV antibodies

2. CAUTION

This preparation is not for administration to humans.

The preparation contains material of human origin. The panel consists of freeze-dried preparations of EVD convalescent plasma samples obtained from four patients and a negative control plasma. The convalescent plasmas are confirmed PCR-negative for EBOV and, along with the negative plasma, were solvent detergent-extracted prior to their development into the 1st WHO International Reference Panel. 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

It is proposed that ARC sample is assigned a unitage of 1.1 IU/mL as calibrated against the IS. The NHSBT, NOR and INMI panel members have not been assigned a unitage.

4. CONTENTS

Country of origin of biological material: Italy, USA, UK, Norway

Each ampoule contains the freeze-dried equivalent of 0.25 mL pooled convalescent plasma obtained from four patients recovered from ebola virus disease (EVD).

5. STORAGE

16/344 should be stored at –20°C on receipt. The contents of the ampoule should be reconstituted with 250 uL distilled water using safety precautions as described above.

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

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