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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*
*** See Appendix 2*

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.

# 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 1year 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 100µL 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 = $[10^{s}-1]\times100\%$ 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˚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 -20ºC, +4ºC, +20ºC, +37ºC, +45ºC and +56ºC for a period of approximately1 year. The potency of samples of the candidate IS 15/262 stored at the elevated temperatures were expressed relative to the material stored at –20ºC. 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 +37ºC and above being reliable, and the apparent drop in potency not being affected by problems of reconstitution. This was seen with +45ºC 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 +4ºC and +20ºC alone, as insufficient degradation has occurred which shows the candidate is stable at +20ºC 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 +37ºC 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 antibodiesof 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 1st IRR for Ebola antibodies<br>ARC convalescent plasma<br>1unit/mL | 100 µL frozen plasma |
| 38 | 15/284 | Candidate Panel Member 3: NOR<br>Anti-EBOV Convalescent Plasma | 0.25 mL plasma<br>Freeze-dried |
| 39 | 15/288 | Candidate panel Member<br>Negative Human Plasma (anti-EBOV) | 0.25 mL plasma<br>Freeze-dried |
| 66 | 15/282 | Candidate Panel Member 2: NHSBT<br>Anti-EBOV Convalescent Plasma | 0.25 mL plasma<br>Freeze-dried |
| 85 | 15/286 | Candidate Panel Member 4: INMI<br>Anti-EBOV Convalescent Plasma | 0.25 mL plasma<br>Freeze-dried |
| 92 | 15/262 | Candidate WHO 1st International Standard<br>Anti-EBOV Convalescent Plasma Pool<br>Sierra Leone | 0.5 mL plasma<br>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<br>Anti-EBOV Convalescent Plasma | 0.25 mL plasma<br>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<br>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 1st 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<br>n=84 | 0.2536<br>n=10 | 0.2548<br>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<br>n=6 | ND | ND |
| CV of dry weight (%) | 0.2800 | NA | NA |
| Mean residual moisture (%) | 0.1616<br>n=12 | ND | ND |
| CV of residual moisture (%) | 8.87 | NA | NA |
| Mean oxygen content (%) | 0.31<br>n=12 | 0.29<br>n=12 | 0.41<br>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<br>n=10 | 0.2552<br>n=10 | 0.2534<br>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<br>n=6 |
| CV of dry weight (%) | NA | NA | 3.36 |
| Mean residual moisture (%) | ND | ND | 0.363<br>n=12 |
| CV of residual moisture (%) | NA | NA | 22.43 |
| Mean oxygen content (%) | 0.12<br>n=12 | 0.31<br>n=12 | 0.26<br>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 | | 38 | 39 | 66 | 85 | 92 | 93 | 94 | 95 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NIBSC | 15/220 | 15/284 | 15/288 | 15/282 | 15/286 | 15/262 | NA | NA | 15/280 |
| Lab | Method | n | Analyte anti- | Virus | 1st 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,NI,P |
| 12 | rcVSV PsN | 3 | GP | EBOV | PPP | I I I# | NNN | I I I# | I I 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 1st 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 |
| 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).<br>The samples were not heat inactivated prior to use.<br>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.<br>During sample dilution, it was noted that the reconstituted samples were viscous after incubation. | | | | | | | | | | |
| 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 |
| *100% neutralisation titres reported.<br>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). | | | | | | | | | | |

**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 1st 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 1st 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 1st 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 1st 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<br>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 |

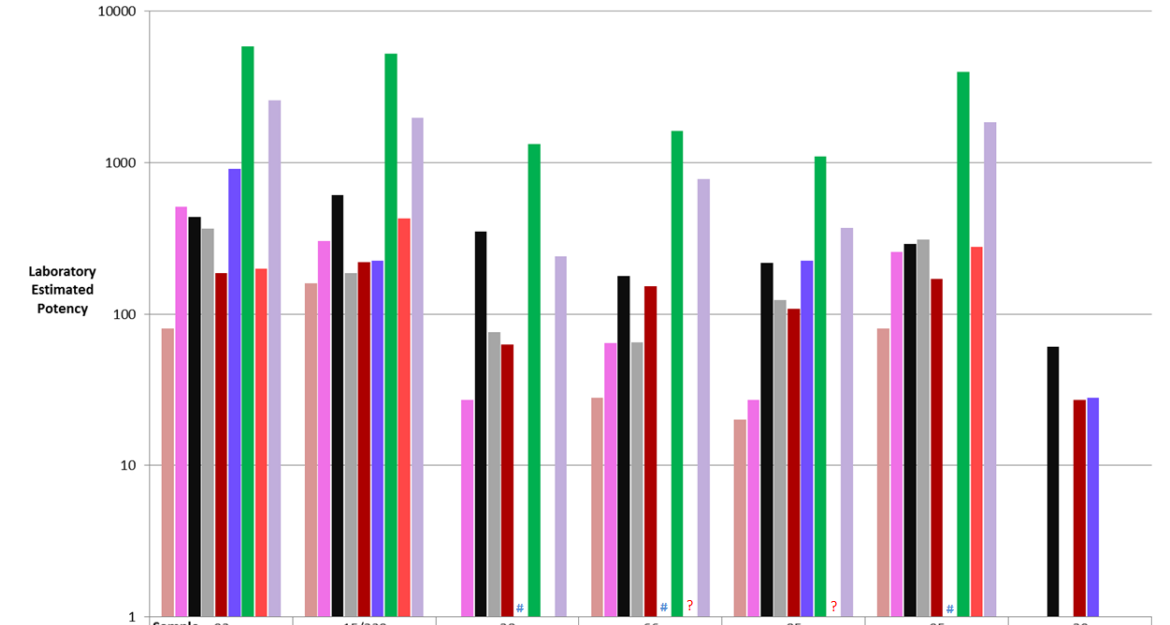
Abbreviations: GM = Geometric mean; GCV = geometric coefficient of variation

**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.

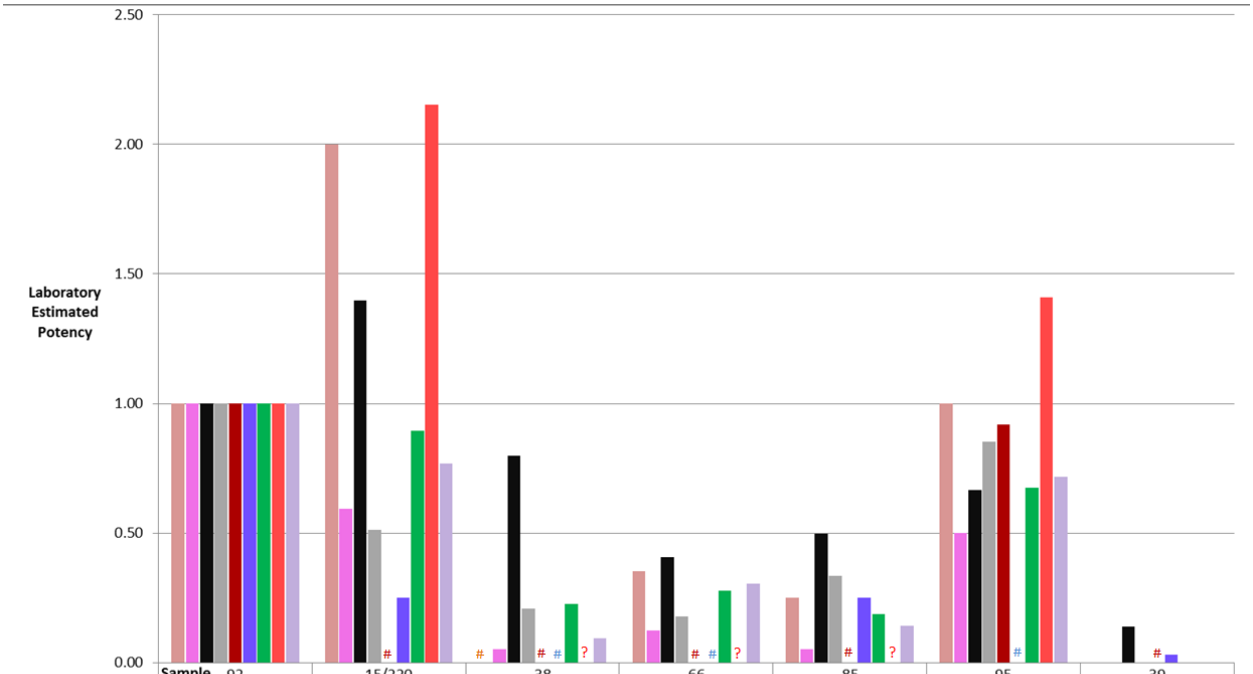
**Figure 1**. Median titre estimates for neutralisation assays (Neut) and pseudo-neutralisation (PsN) assays.




| Sample | 92 | 15/220 | 38 | 66 | 85 | 95 | 39 |
|---|---|---|---|---|---|---|---|
| Lab 15b Neut | 80 | 160 | Negative | 28 | 20 | 80 | Negative |
| Lab 16a Neut | 512 | 304 | 27 | 64 | 27 | 256 | Negative |
| Lab 2 LVV-PsN | 436 | 610 | 349 | 178 | 217 | 290 | 61 |
| Lab 4 LVV-PsN | 365 | 187 | 76 | 65 | 123 | 311 | Negative |
| Lab 9 LVV-PsN | 186 | 220 | 63 | 153 | 108 | 171 | 27 |
| Lab 11 LVV-PsN | 905 | 226 | ~~20~~* | ~~7241~~* | 226 | # | 28 |
| Lab 10b VSV-PsN | 5845 | 5230 | 1331 | 1619 | 1101 | 3953 | Negative |
| Lab 12 rcVSV-PsN | 198 | 426 | ? | ? | ? | 279 | Negative |
| Lab 13 nrVSV-PsN | 2572 | 1978 | 240 | 782 | 371 | 1843 | Negative |

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.

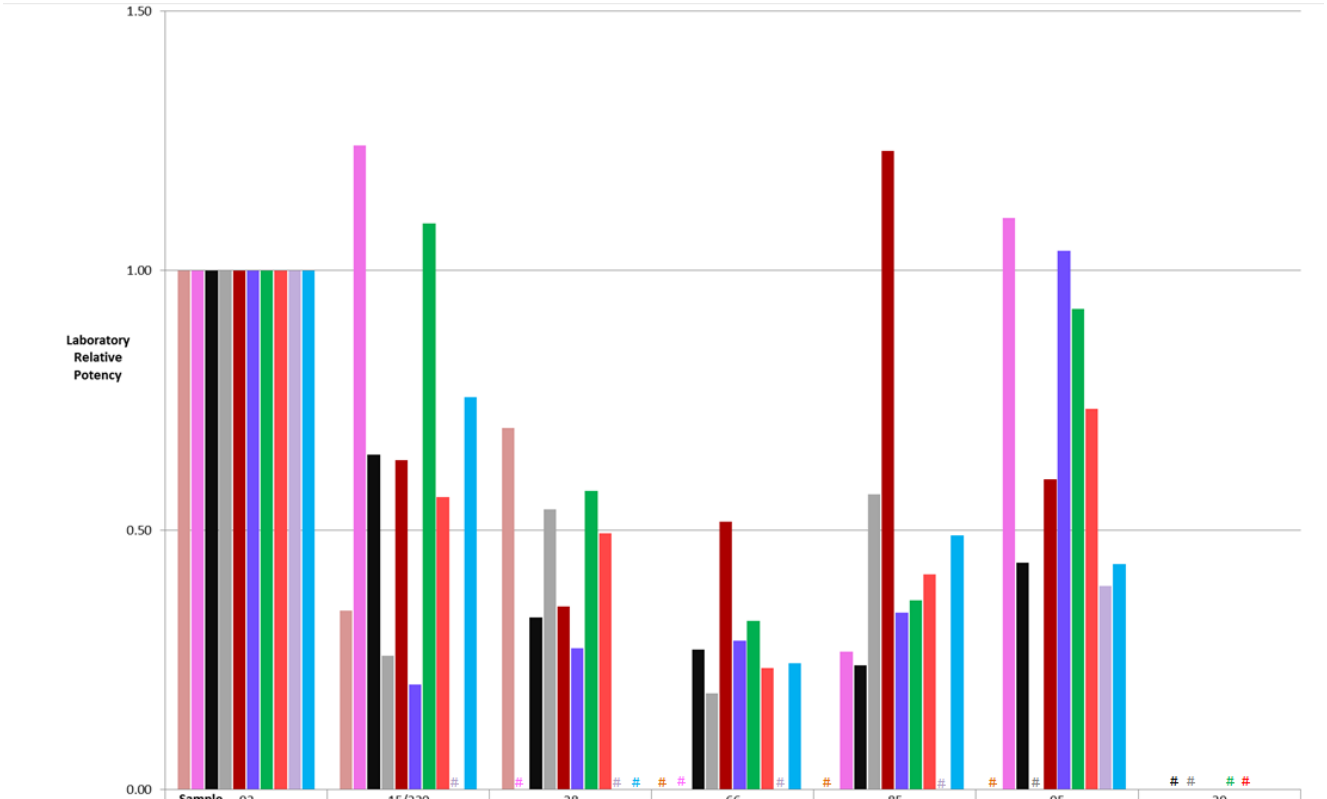
**Figure 2**. Laboratory potencies relative to sample 92 for neutralisation assays (Neut) and pseudoneutralisation (PsN) assays.




| Sample | 92 | 15/220 | 38 | 66 | 85 | 95 | 39 |
|---|---|---|---|---|---|---|---|
| Lab 15b Neut | 1.00 | 2.00 | # | 0.35 | 0.25 | 1.00 | Negative |
| Lab 16a Neut | 1.00 | 0.60 | 0.05 | 0.13 | 0.05 | 0.50 | Negative |
| Lab 2 LVV-PsN | 1.00 | 1.40 | 0.80 | 0.41 | 0.50 | 0.67 | 0.14 |
| Lab 4 LVV-PsN | 1.00 | 0.51 | 0.21 | 0.18 | 0.34 | 0.85 | Negative |
| Lab 9 LVV-PsN | 1.00 | **# | **# | **# | **# | 0.92 | **# |
| Lab 11 LVV-PsN | 1.00 | 0.25 | ~~0.02~~* | ~~8.0~~* | 0.25 | # | 0.03 |
| Lab 10b VSV-PsN | 1.00 | 0.90 | 0.23 | 0.28 | 0.19 | 0.68 | Negative |
| Lab 12 rcVSV-PsN | 1.00 | 2.15 | ? | ? | ? | 1.41 | Negative |
| Lab 13 nrVSV-PsN | 1.00 | 0.77 | 0.09 | 0.30 | 0.14 | 0.72 | Negative |

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.




| Sample | 92 | 15/220 | 38 | 66 | 85 | 95 | 39 |
|---|---|---|---|---|---|---|---|
| Lab 3 (inactivated wv) | 1.00 | 0.35 | 0.70 | ~~5.40~~* | # | # | Negative |
| Lab 5 (αGP) | 1.00 | 1.24 | **# | **# | 0.27 | 1.10 | Negative |
| Lab 6a (αGP) | 1.00 | 0.65 | 0.33 | 0.27 | 0.24 | 0.44 | # |
| Lab 6b (αNP) | 1.00 | 0.26 | 0.54 | 0.19 | 0.57 | # | # |
| Lab 7 (αVP40 & NP) | 1.00 | 0.64 | 0.35 | 0.52 | 1.23 | 0.60 | Negative |
| Lab 8 (αGP) | 1.00 | 0.20 | 0.27 | 0.29 | 0.34 | 1.04 | Negative |
| Lab 10a (αGP) | 1.00 | 1.09 | 0.58 | 0.33 | 0.37 | 0.93 | Negative/Positive |
| Lab 14 (αGP) | 1.00 | 0.56 | 0.49 | 0.23 | 0.41 | 0.73 | # |
| Lab 15a (αGP) | 1.00 | **# | **# | **# | **# | 0.39 | Negative |
| Lab 17 SPR | 1.00 | 0.76 | # | 0.24 | 0.49 | 0.43 | Negative |

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**

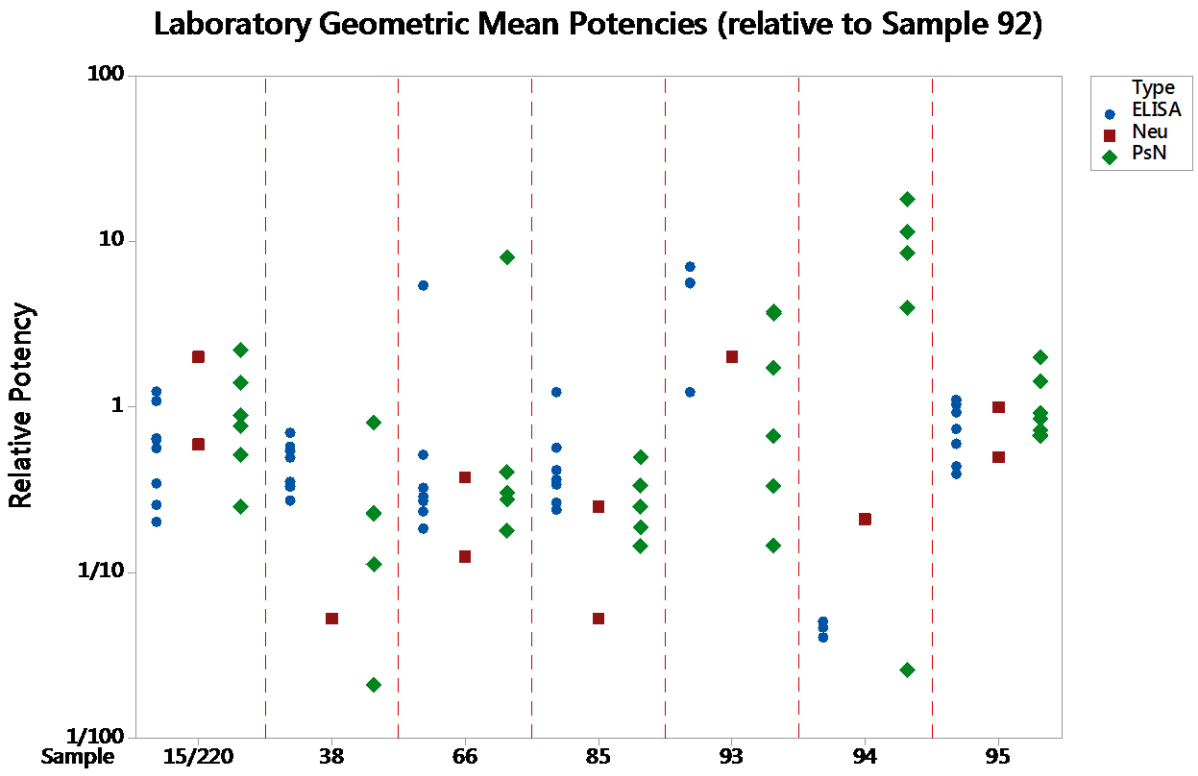
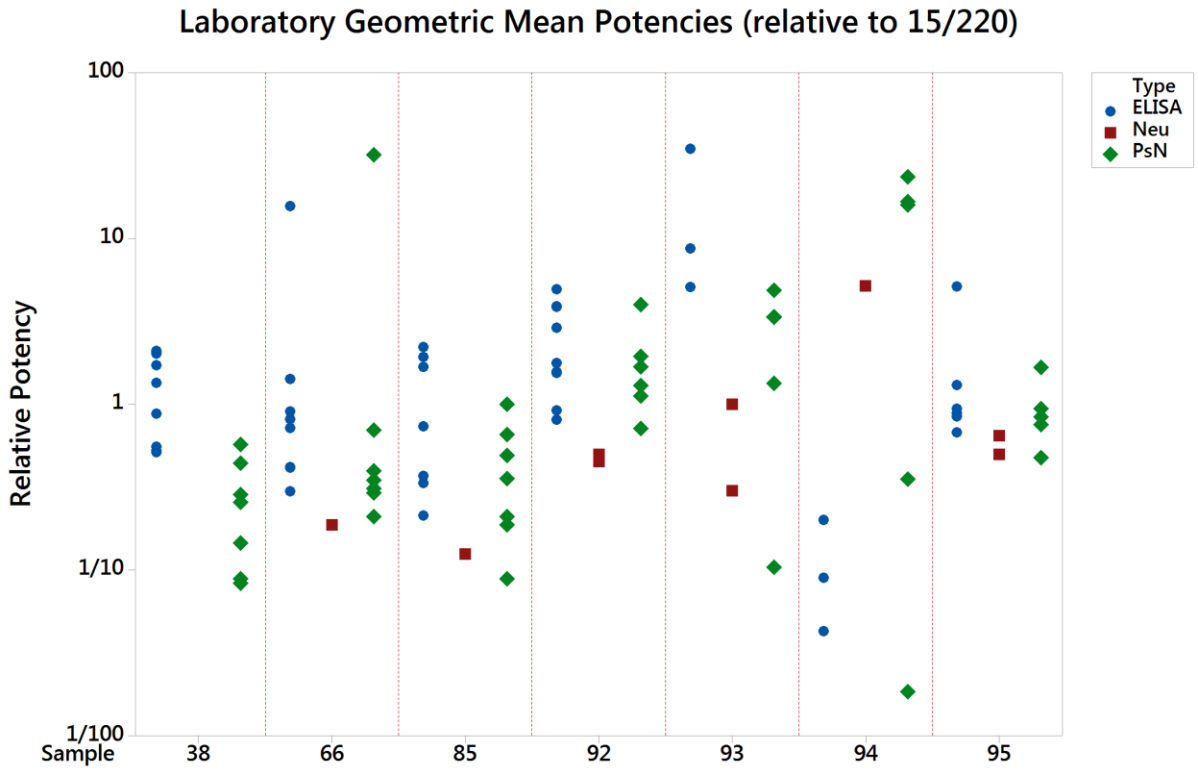



**Figure 5**



**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 (OVRR), CBER, FDA | USA |
| Carol D. Weiss | OVRR, 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.

**Appendix 2**

**Ebola CP Consortium investigators**

| Name | Affiliation | Country |
|---|---|---|
| MG Semple, (Consortium Lead Investigator)<br>JT Scott | Institute of Translational Medicine and NIHR Health Protection Research Unit in Emerging and Zoonotic Infections University of Liverpool, Liverpool | UK |
| SM Gevao (Country Lead Investigator)<br>F Sahr (Country Deputy Lead Investigator)<br>CP Cole, J Russell | College of Medicine and Allied Health Sciences, Freetown | Sierra Leone |
| S Baker, O Kargbo, P Kamara | National Safe Blood Service, Connaught Hospital, Ministry of Health & Sanitation, Freetown | Sierra Leone |
| M Lado, CS Brown | King's Sierra Leone Health Partnership, King's Health Partners & King's College London, London | UK |
| J van Griensven, R Ravinetto, Y Claeys | Institute of Tropical Medicine, Antwerp | Belgium |
| RS Tedder, R Gopal, TJG Brooks | National Infection Service, Public Health England, London | UK |
| CC Smith | Health Protection Scotland | UK |
| HA Doughty | NHS Blood and Transplant, & College of Medical and Dental Sciences, University of Birmingham, Birmingham | UK |
| A Mari Saez, M Borchert | Institute for Tropical Medicine and International Health, Charité, Berlin | Germany |
| AH Kelly | Department of Sociology, Philosophy and Anthropology, Exeter University, Exeter | UK |
| JK Baillie | The Roslin Institute, University of Edinburgh, Edinburgh | UK |
| N Shindo, D Pfeifer | Department of Pandemic and Epidemic Diseases, World Health Organization Geneva | Switzerland |
| DL Hoover | ClinicalRM Inc., Ohio | USA |
| WA Fischer II, DA Wohl | Department of Medicine, University of North Carolina, Chapel Hill | USA |
| NM Thielman | Duke University School of Medicine, Durham | USA |
| PW Horby, L Merson | Nuffield Department of Medicine, University of Oxford, Oxford | UK |
| PG Smith, T Edwards | MRC Tropical Epidemiology Group, London School of Hygiene & Tropical Medicine, London | UK |

**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. Olaussen, 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 NIBCS, 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 where 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.

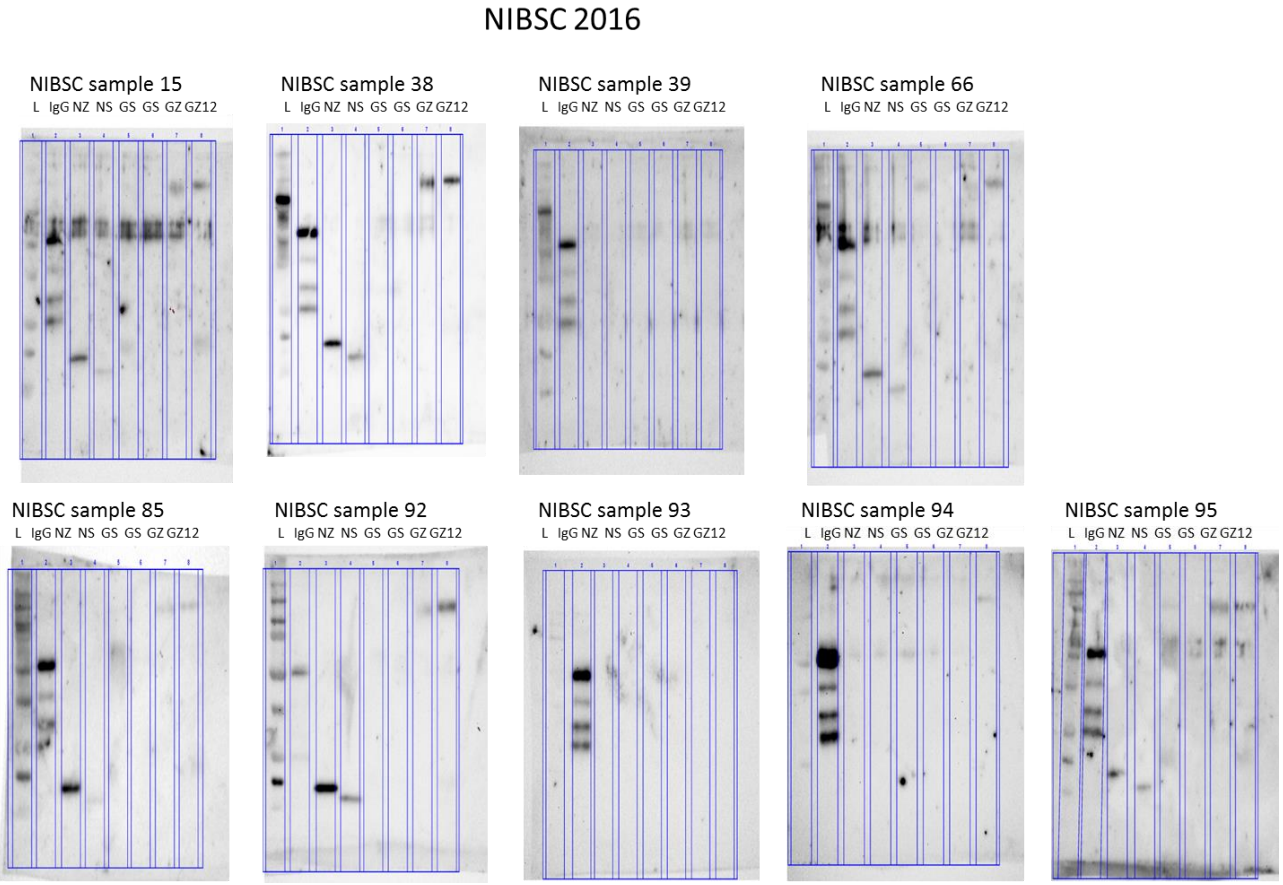
**Deadline for completed results spread sheets is 6 weeks from receipt of study materials. If it is not practicable to return results within 6 weeks, please inform Dianna Wilkinson.**

**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

**Appendix 4**

**Western blot analysis of study samples performed by Laboratory 6.**




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 $PRNT_{60}$ 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 $PRNT_{60}$ 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.

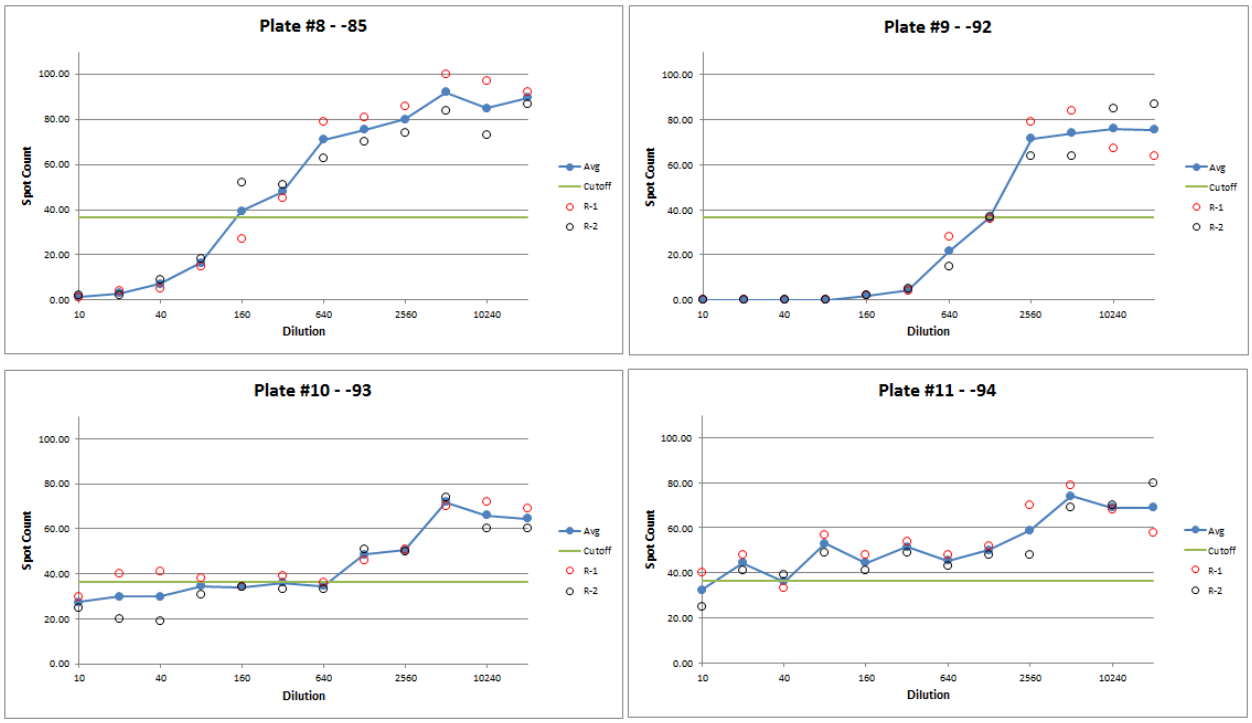
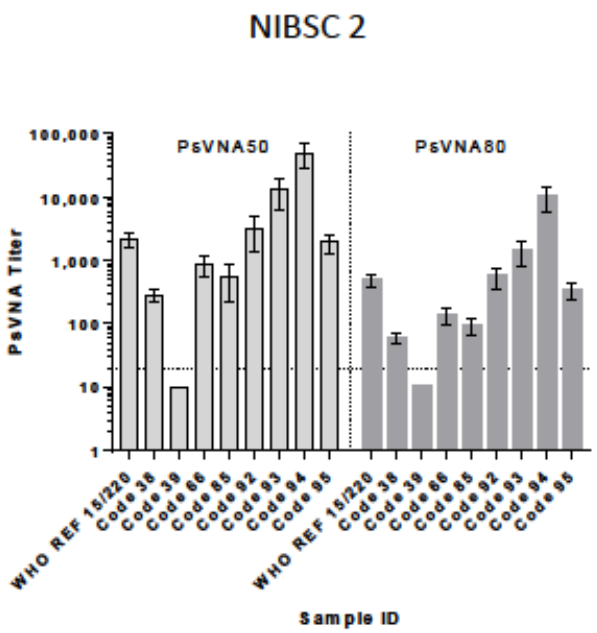


**Table 1. ELISA and PRNT Results for NIBSC Study CS570 Sample Panel.** Each sample was tested on 4 separate run days in both the FANG ELISA and rVSV-ZEBOV GP PRNT assays. Samples with higher than expected variability are indicated in yellow. The mean concentration, mean end point titer and mean $PRNT_{60}$ 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.

## Appendix 7

## Summary of EIA and neutralisation assays provided by Laboratory 15.

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

| | 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 | |
| Sample code # | 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).

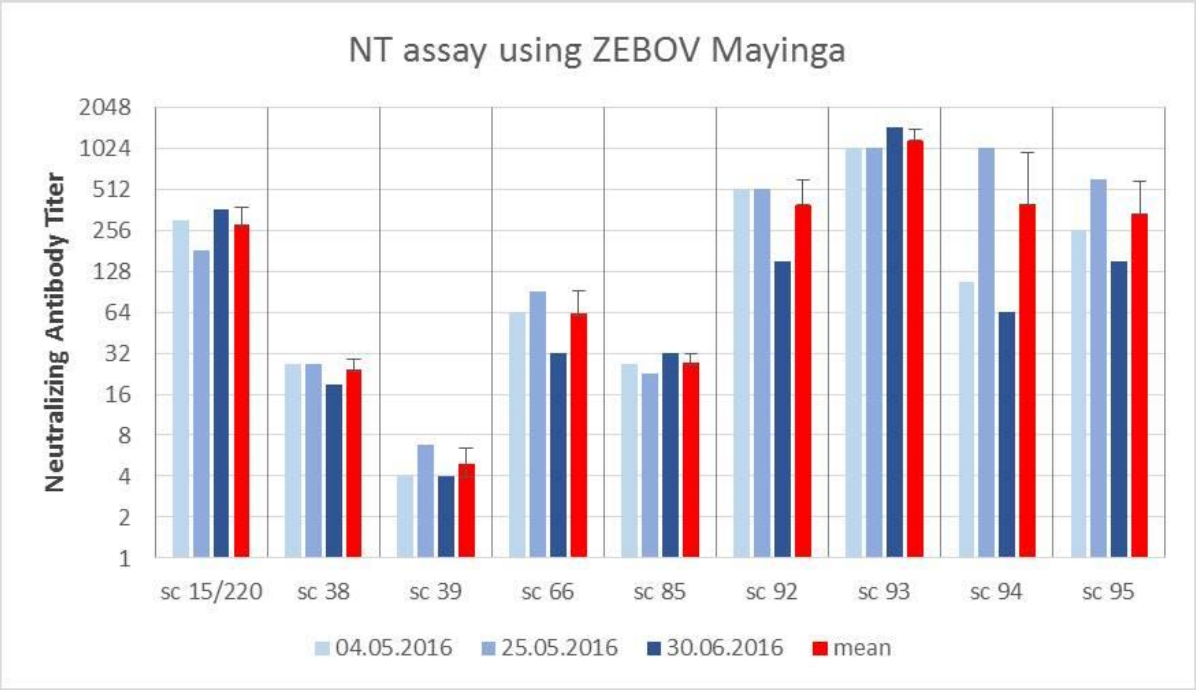
The FRNA assay, which detects neutralizing antibodies rather than GP specific antibodies, measured differing results based on criteria used (50 or 80% reduction). A FRNA50 measured four positive samples, three indeterminate samples, and two negative samples. When the neutralization criteria was more 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**

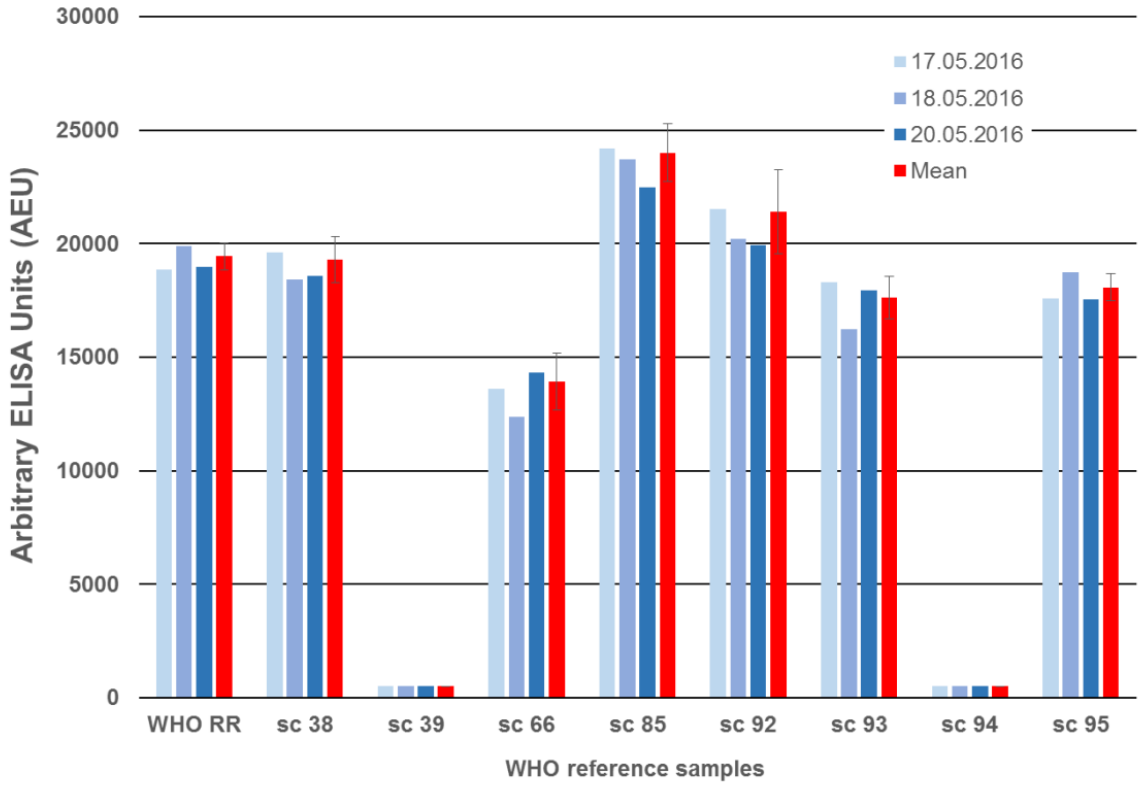
**Laboratory 16**

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.



## 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 –20ºC 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**

We gratefully acknowledge the important contributions of the collaborative study participants. We would also like to thank NIBSC Standards Production and Development for distribution of the candidate materials. We also thank David Wood and Micha Nuebling of the WHO and participants of teleconferences for their support, guidance and advice. We also thank colleagues Maria Zambon, Angie Lackenby, Simon Carne, Pamela Saunders, Meera Chand and Kevin Brown at Public Health England, Colindale, UK for PCR testing of plasma samples. We also thank Steven A. Rubin, FDA/CBER, USA for facilitating the sample permits and shipments to laboratories in the USA.

**11. FURTHER INFORMATION**

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/products/biological_reference_materials/frequently_asked_questions/how_are_international_units.aspx
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http://www.nibsc.org/products/ordering_information/frequently_asked_qu estions.aspx
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| **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.<br>Absorbent materials used to treat spillage should be treated as biological waste. | | | |

**15. LIABILITY AND LOSS**

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|---|
| **16. INFORMATION FOR CUSTOMS USE ONLY**<br>**Country of origin for customs purposes*:** United Kingdom<br>* 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**.**<br>**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 –20ºC 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.**

**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**

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http://www.nibsc.org/products/biological_reference_materials/frequently_asked_questions/how_are_international_units.aspx
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| **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.<br>Absorbent materials used to treat spillage should be treated as biological waste. | | | |

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| **Net weight:** 0.25g per ampoule |
| **Toxicity Statement:** Non-toxic |
| **Veterinary certificate or other statement** if applicable**.**<br>**Attached:** No |

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