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Collaborative Study for the Establishment of the First WHO International Standard for antibodies to Crimean-Congo haemorrhagic fever virus

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NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposal(s) contained therein. Written comments on the proposal(s) MUST be received in English by **23 March 2026** and should be addressed to:

Product Standards, Specifications and Nomenclature
Department of Medicines and Health Products Policies and Standards
World Health Organization
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Comments may also be submitted electronically to **Dr Ivana Knezevic** at email: knezevici@who.int.

The distribution of this document is intended to provide information to a broad audience of potential stakeholders and to improve the transparency of the consultation process. Following consideration of all comments received, the proposal(s) will then be considered by the WHO Expert Committee on Biological Standardization (ECBS) prior to a final decision being made and published in the WHO Technical Report Series.

Summary

Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne nairovirus with a wide geographical range and a potentially expanding distribution, with an estimated 3 billion people living in areas at risk of infection. Vaccines and treatments are in development, with a few candidates in early clinical stages, and reliable assays are needed for their evaluation. The availability of an International Standard (IS) for antibodies would facilitate the comparability of results from CCHFV serological assays and support the evaluation of vaccines and therapeutics. In this report a freeze-dried pool of plasma from CCHF-recovered individuals from Turkey was evaluated to serve as First WHO IS for antibodies to CCHFV. The production summary of candidate IS, NIBSC code 24/228, indicated a homogenous (based on mass CV%) and stable product (based on water activity and oxygen content). This was further supported by stability assessment with real-time data demonstrating that the product is suitable for shipping at ambient temperature, and a predicted loss of activity of 0.07% per year when stored at -20°C. The candidate WHO IS 24/228 was assessed in a multi-centre collaborative study involving 24 participants from 14 countries, representing three WHO Regions. Participants returned 46 datasets testing the candidate WHO IS, C-01, in methods detecting and quantifying neutralising and binding antibodies. The neutralisation tests included five methods using authentic CCHFV and seven methods using alternative systems based on a pseudotyped viruses or a transcription-competent virus-like particles. Most binding methods were qualitative or semi-quantitative and targeted the nucleoprotein. Seven commercial ELISA kits from 5 manufacturers were used, with only one manufacturer not disclosing the viral target. Four laboratories reported binding antibody titres against Gc using in-house methods. Twenty-seven of the 34 methods assessing binding activity were ELISA-based, with the remaining seven being immunofluorescence assays. Eight participants also returned results for other targets (*e.g*. GP38, whole virion, glycoprotein precursor) but there were insufficient datasets for statistical analysis. The candidate WHO IS C-01 was tested in parallel with a panel of samples that included pools of convalescent sera from Georgia or Uganda, another pool of plasma from Turkey, and individual plasma or serum donations from these three countries to act as clinical samples. The candidate WHO IS was found to be fit for purpose in all assays and ranked among the highest-titre samples. Expressing the neutralising and binding activities of the collaborative study samples relative to the candidate WHO IS, C-01, resulted in a lower inter-laboratory variation compared with the participants' reported values. Calibration effectiveness also supported the commutability of the candidate WHO IS. We proposed that sample C-01, code 24/228, is established as the First WHO International Standard for antibodies to CCHFV, with an arbitrary unitage of 25 IU/ampoule for neutralisation activity (24/228_NT) and 250 IU/ampoule for binding activity anti-NP IgG and 250 IU/ampoule for binding activity anti-Gc IgG (24/228_BA).

Introduction

Crimean-Congo haemorrhagic fever (CCHF) was first described in 1944-45 as a disease affecting Soviet Union soldiers in the Crimean Peninsula during the Second World War [1]. The causative agent was isolated almost 20 years later and found to be antigenically identical to Congo virus, identified in 1956 during a haemorrhagic fever outbreak in Zaire (now the Democratic Republic of Congo). The virus was subsequently renamed Crimean-Congo haemorrhagic fever virus (CCHFV) [1]. The geographical distribution of CCHFV mirrors that of its vector and main reservoir, ticks of *Hyalomma* species. The virus is endemic in Africa, the Balkans, the Middle East and Asian countries south of the 50th parallel north. CCHFV is transmitted to humans through bites from infected ticks or through handling infected animal carcasses. Human-to-human transmission has also been reported [2]. Most infections are asymptomatic or present with mild flu-like symptoms; however, severe cases progress to haemorrhagic fever 3-6 days from onset, with fatality rates that vary but may exceed 30% [3]. CCHFV has been identified as a priority and prototype pathogen within the Nairoviridae family in 2024 WHO pathogens prioritization framework for the high risk of causing a public health emergency combined with lack of medical counter measurements [4]. Indeed, although the antiviral drug ribavirin has been used as a treatment, the approach to managing CCHF is general supportive care. There are no vaccines licensed for CCHF, but many are in development with a couple of vectored candidate vaccines in clinical trial [5]. There are several commercial assays for the detection of CCHFV infection but are mainly used for diagnostics and epidemiological studies. Most laboratories have developed in house assays to evaluate immunological responses to CCHFV infection or vaccination.

International Standards are recognised as the highest order of reference material for biological substances and are usually assigned a potency in International Units (IU). They are intended to be used to calibrate assays to report the relative biological activity of samples in terms of IU, thereby enhancing comparability between laboratories and assays. Such standardisation supports the definition of analytical parameters, such as sensitivity, and clinical correlates, such as protective antibody levels. The availability of an IS for CCHFV antibodies will facilitate the standardisation of serological assays and support the interpretation of evaluation of epidemiological and clinical trial data during vaccine development, as outlined in the R&D Roadmap for CCHF [6]. The Expert Committee on Biological Standardization (ECBS) endorsed the development of a WHO International Standard (IS) for antibodies to CCHFV during the 69th WHO ECBS meeting in 2018.

This multi-centre international collaborative study evaluated a candidate preparation intended to serve as the First WHO IS for antibodies to CCHFV. The study is organized by the Medicines and Healthcare products Regulatory Agency (MHRA) on behalf of the World Health Organization (WHO) and facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI).

The aims of this collaborative study are to:

- Assess the suitability of the candidate to serve as a WHO International Standard and harmonise data in a range of typical assays performed in different laboratories.
- Characterise the candidate's reactivity/specificity in different assay systems.
- Assess commutability, i.e. establish the extent to which the candidate is suitable to serve as a standard for a variety of different samples.
- Recommend to the WHO ECBS the suitability of the candidate to serve as a WHO International Standard and propose an assigned International Unit.

Materials and Methods

Ethics Statement

The plasma donations from Turkey were collected as part of the CCHFVaccine project at the Sivas Cumhuriyet University Hospital from recalled individuals who had tested positive for CCHFV infection during the outbreaks in 2015 or 2017. The study was conducted in accordance with the declaration of Helsinki and approved by the local Research Ethics Committee of the Ankara Numune Education and Research Hospital, Turkey (Protocol # 17–1338). Ethical approvals from the samples collected from Georgia was provided by the National Centre for Disease Control and Public Health Institutional Review Board (IRB #2024-010) and from Uganda by the Uganda Virus Research Institute (UVRI) Research Ethics Committee (Ref. GC/127/351).
All the donors provided written informed consent, and the donations were shared with MHRA anonymised.

Candidate First WHO International Standard

The candidate WHO IS for antibodies to CCHFV (NIBSC code 24/228) is a freeze-dried preparation of a pool of plasma from five individuals from Turkey, collected as part of the CCHFVaccine project in 2019 from recovered patients from the 2015 or 2017 outbreaks [7]. Seven plasma donations were initially received at MHRA, tested and found negative for blood-borne pathogens (HIV Ab, HBsAg and HCV RNA). The samples were then tested for neutralising antibody titre using an in-house pseudotyped based method [8] and for binding antibodies by ELISA (see below). The five plasma samples with the highest combined antibody responses were selected and pooled to prepare the candidate WHO IS (24/228).

Filling and Lyophilization of the Candidate WHO International Standard

The definitive fill and lyophilization of 24/228 was performed by the Manufacturing team at MHRA under ISO9001 between 21st – 25th March 2025. Material was dispensed in 0.25 mL volumes into 2.5 mL glass DIN ampoules at 4°C on an AVF5090 filling line (Bausch & Stroebel, Ilshofen, Germany). The homogeneity of the fill was maintained by on-line check-weighing of a proportion of the filled ampoules. Filled ampoules were partially stoppered with halobutyl 13mm diameter igloo closures and lyophilised in a CS15 freezer drier. Ampoules were loaded onto the shelves at 4°C and primary freezing was performed to -50˚C over 4 hours. Primary drying was performed at -35˚C for 30 hours at 100 µbar vacuum followed by a ramp to 25˚C over 10 hours, then 30 hours secondary drying at 25°C and 30

μbar vacuum. Vials were back filled with dry nitrogen to atmospheric pressure and flame sealed. The sealed vials were stored at -20°C under continuous temperature monitoring. Assessments of residual moisture and oxygen content, as indicators of freeze-drying completion and vial integrity after sealing, were determined for twelve vials of freeze-dried product. Water activity and Oxygen content were measured non-invasively by frequency modulated spectroscopy in the vial/ampoule headspace using a tunable infrared laser (Lighthouse Instruments, Amsterdam, the Netherlands). Water activity was calculated from the equilibrium water vapour pressure relative to pure water at the measurement temperature (reported as the relative humidity, RH).

Study Sample Panel

The collaborative study included 14 additional samples for evaluation alongside the candidate WHO IS (Table 1). This included the bulk material for the candidate WHO IS, pre-lyophilisation (C-15), as well as individual samples from Turkey (C-05, C-06, C-13 and C-14); one of these was not used to prepare the candidate WHO IS pool (CS-14).
The additional samples were collected through Integrum Scientific by CEPI partners Uganda Virus Research Institute, Uganda from healthy individuals with previously exposure to CCHFV during outbreak in 2000 and 2016 and the National Centre for Disease Control and Public Health, Georgia, where CCHF outbreaks were recorded in 2022 and 2023 [9].
The remaining 9 study samples were prepared in the following way:

- Pooled plasma from 2 individuals from Turkey (Reference Panel #1, C-02)
- Pooled sera from 13 individuals from Georgia (Reference Panel #2, C-03)
- Pooled sera from 10 individuals from Uganda (Reference Panel #3, C-04)
- Three single donations from Georgia, not included in the Reference panel #2 (C-06, -07 and -08)
- One individual serum sample from Uganda, not included in the Reference panel#3 (C-11)
- Two pools of serum samples from individuals with no history of CCHFV exposure as negative samples from Georgia (C-10) and Uganda (C-12).

All the convalescent samples have been tested at the UKHSA using an in-house validated RT-PCR method [10] and no CCHFV RNA was detected. Samples were also solvent/detergent treated to minimise the risk of presence of enveloped viruses not tested for [11]. All samples were screened and tested negative for blood-borne pathogens (HIV antibodies, HBsAg and HCV RNA) with exception of sample C-12, negative pool from Uganda, which tested positive for HBsAg. As the preparation was solvent/detergent treated it was still considered not infectious.

Participants

Twenty-seven laboratories accepted to participate in the study however, three participants withdrew due to technical/procurement issues with the assays due to be performed. Overall, the 24 laboratories returning results were from 14 countries, representing 3 WHO Regions: China (2), France (3) Georgia (1), Germany (3), Italy (1), Japan (1), the Netherlands (1)

Slovenia (1), South Korea (2), Sweden (1), Switzerland (1), Turkey (2), Uganda (1) and United Kingdom (4) and are listed in Appendix 1. All laboratories' data are referred to by a code number randomly allocated and not reflected in the order presented in Appendix 1. Participating organizations included kit manufacturers, national control/reference laboratories and academic/research laboratories.

Study Design

The study protocol is provided in Appendix 2. Participants were requested to test the study samples using their established method(s) for the detection of antibodies against CCHFV. It was requested that three independent assays were performed, using a fresh set of samples for each assay and preparing serial dilutions of the samples to be tested, at least in duplicate. A results reporting sheet was provided for participants to record all essential information including the raw data from each assay. Participants were asked to return results by 10th October 2025.

Assay Methods

The participants analysed the collaborative study samples using methods to detect neutralising (Table 2) or binding antibodies (Table 3) against CCHFV. Where laboratories performed multiple assay methods, laboratory codes are followed by a letter indicating the different methods (e.g. Lab 1a, 1b). The detection of neutralising antibodies included five methods using authentic virus, five using pseudotyped virus (PV) and two laboratories used a transcription- and entry-competent virus-like particles (tecVLPs). Binding antibody methods included ELISA against mainly nucleoprotein (NP, n=20) or glycoprotein C (Gc, n=4), but also whole virion (n=1) and GP38 (n=1). Most of the results for anti-NP antibodies quantification (11/16) were obtained using a commercial kit. Assay kits from four different manufacturers were used in the study. A fifth commercial kit was used by two laboratories, but the CCHFV target was not disclosed. Five participants returned results from immunofluorescence assay (IFA) detecting NP or the precursor glycoprotein GCP or the whole virion (Table 3).

Statistical Methods

All test details and raw data were returned to MHRA using the provided summary form to permit independent analysis.

Potency estimates for each of the study samples was calculated as the geometric mean (GM) from the participant reported data for the three independent experiments, and data reported and included in the statistical analysis if the sample was determined as positive in at least two out of three measurements by the participants.

Relative potency estimates were calculated by parallel-line analysis [12] comparing transformed assay responses to log dose for results with sufficient dose-response data. Calculations were performed using the R software package [13]. For all assays, data for each test preparation was analysed separately against the reference preparation chosen for that analysis, and the resulting potency estimates are therefore based on direct pair-wise

comparisons. Model fit was assessed visually and by calculated R^2 value, and non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference sample.
For neutralisation assays reporting an end-point titres, potency estimates were calculated as the ratio of endpoint titres of the test and reference samples within an assay run.
Valid relative potency estimates were combined to generate an unweighted geometric mean potency per laboratory. The laboratory GM values were then also used to calculate overall unweighted geometric mean potencies and median potencies. Variability between assays and laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^s-1\} \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed potencies) and the anti-log of the median absolute deviation (10^{MAD}).

Stability Testing of the Lyophilized Candidate WHO IS
Accelerated thermal degradation studies commenced in April 2025 for the lyophilized candidate Standard 24/228 to predict the stability of the material. Fifteen ampoules of the candidate were stored at -20, +4, +20, +37 and +45°C. Three ampoules for each temperature have been retrieved at 2 weeks, 1 month, 3 months and 6 months. An additional timepoint is scheduled for retrieval at 12 months if required. Retrieved samples were held at -20°C before testing. Sample potencies relative to the baseline, -20°C sample, were assessed using an ELISA method targeting CCHFV Gc protein at least in duplicate in 3 independent assays. Relative potencies were calculated by sigmoid curve analysis using the R software package [13]. The long-term stability of the candidate material was predicted using the Arrhenius model for accelerated degradation studies with potencies expressed relative to the samples stored at -20˚C [14].

In house CCHFV Gc ELISA
Nunc Maxisorp 96-well plates were coated overnight at 4ºC with 42.3 µL of CCHFV Gc protein (SHFc-tag, 0.26mg/ml, Native Antigen) in PBS. Plates were washed 3 times with PBS containing 0.05% Tween-20 (PBST), then blocked with 5% milk (Marvel) in PBST for 1 hour at room temperature shaking (650 rpm). Plates were washed 3 times with PBST. Fifty microliters of serum/plasma samples diluted in blocking buffer were added to the plates in triplicate and incubated shaking (650 rpm) at room temperature for 1 hour. Wells were washed 3 times with PBST and 50 µL of horse radish peroxidase-conjugated anti-human IgG antibody (Sigma.) diluted 1:9000 in blocking buffer were added to each well. After an hour incubation at room temperature, plates were washed with PBST and developed by adding 3,3′,5,5′-Tetramethylbenzidine Substrate Systems (TMB, Neogen Europe, Ltd.). Reactions were stopped after 10 minutes by the addition of 2N H_2SO_4. For the analysis, absorbance from each well was subtracted from the average of absorbance of the diluent-only wells.

Results and Data Analysis

Production of the Candidate WHO International Standard 24/228

A product summary is provided in Table 4. The coefficient of variation of the mean fill mass was 0.62% across 99 ampoules, which is acceptable for a plasma fill at 0.25g per ampoule [15]. The product was successfully lyophilised with a water activity of 1.43% RH, indicating a stable product. The mean oxygen headspace measured by Frequency Modulated Spectrometry was 0.35% across 12 ampoules, indicating nitrogen gas back-fill and container closure integrity. Microbiological testing returned a negative result for bacteria and mould/yeast contamination.

Stability Assessment

Ampoules of the candidate WHO IS, NIBSC code 24/228, stored at different temperatures -20 (baseline), +4, +20, +37 and +45°C, were retrieved at 2 weeks,1 month, 3 months, 6 months. Real time data on the degradation samples are reported as titration curves and showed no evidence of potency loss up to a month even at 37°C (Figure 1). This suggests that the preparation 24/228 can be shipped at ambient temperature. This aligns with previous stability assessment for other WHO IS for antibodies to priority pathogens. The long-term stability of the candidate WHO IS was estimated by fitting an Arrhenius model to calculate a predicted loss of activity per year at different storage temperatures. This prediction was 0.07% when stored at -20°C, which indicates that the product is stable for long term storage.

Collaborative Study Data Received

Overall, 24 laboratories returned results which cover 46 datasets (Table 2 and 3). This includes twelve datasets detecting neutralising antibodies, of which five were conducted with authentic CCHFV, two used transcription-competent VLPs and five pseudotyped vesicular stomatitis virus-based methods. Both the tcVLP and the PV incorporated a firefly luciferase reporter gene, with the methods providing a readout in relative light units (RLU) and reporting 50% neutralisation titres (NT_{50}), except for laboratory 5 using a NT_{75}. Three of the neutralisation methods using authentic virus detected infection by foci formation and reported the antibody titre based on a foci reduction neutralisation test (FRNT). The remaining two laboratories determined neutralising titre by reduction of cytopathic effect (CPE) or immunostaining of the cells and absorbance detection (optical density, OD). In all the cases the titre was reported as $NT_{50,}$ The CCHFV strain used by the laboratory is reported in Table 2. The methods for the detection of binding antibodies were mainly based on ELISA. Four laboratory returned results from in-house ELISA quantifying IgG against CCHFV Gc. Twenty datasets were a mix of in house and commercial assays detecting anti-nucleoprotein (NP) antibodies. Two of these methods were immunofluorescence assay (IFA, lab 1b and 25b) while the remaining methods were ELISA. Six commercial kits were used by nine participants. Most of the anti-NP methods were detecting IgG, with only two targeting IgM and four laboratories using the ID Screen® CCHF Double Antigen Multi-species ELISA detecting both IgG and IgM. Two laboratories (6 and 23b) used another commercial kit VectoCrimean-CHF with the target antigen not disclosed by the manufacturer. Two laboratories detected antibodies against the whole virion by IFA (lab 24) or indirect ELISA (Lab 17). Laboratories 25 and 13 returned data derived by IFA for antibodies against the

glycoprotein precursor GPC, either as single (lab 25a) or coupled with NP (lab 13) using the EUROIMMUN Mosaic 2 slides. Finally, lab 27d tested the samples for antibodies against GP38 protein.

Evaluation of the Neutralising antibodies Potencies

The geometric mean of the neutralisation assay results reported by the participants are summarised in Table 5. A potency value was calculated if the sample tested positive in at least 2 out of 3 independent experiments. Ranking of the samples varied between the laboratories; the most potent samples were always the Reference Panel member #2 (C-03), pool of sera from Georgia or the clinical sample C-11 from Uganda. The clinical sample C-07 from Georgia had the lowest neutralising antibody titre in all the laboratories, and lab 1a reported it as positive in only one of three experiments. The only exception was laboratory 10, that reported the candidate IS, C-01 as the sample with the lowest titre; however, the liquid bulk IS preparation C-15 scored 5th for neutralising antibody potency. The negative samples C-10 and C-12 were correctly identified as negative by all laboratory except lab 18a which reported the sample as low positive in 2 out of 3 experiments. Laboratory 15 tested the samples at only two dilution points (1:50 and 1:200) and therefore their results capped at NT_{50} of 200. The spread of results between the laboratory was up to 59-fold different for some samples (e.g. clinical sample C-06, table 5 and Figure 2A). In general, the titres reported by using authentic CCHFV were lower than those using VLPs or PV-based methods (Figure 2C).

Evaluation of Neutralisation Activity Relative to the Candidate WHO International Standard

To evaluate the suitability of the candidate WHO IS (C-01) and its ability to harmonise data reporting, potencies of the study samples were calculated relative to the candidate for each dataset using an arbitrarily assigned potency of 100 IU/mL (Table 6). For the neutralisation assays, the reduction in the spread of data is presented graphically in Figure 2B. The spread in the reported potency of the samples was reduced to within 10-fold when reported relative to the candidate WHO IS (C-01). The harmonisation of the data when reported relative to the candidate WHO IS is also highlighted by a lower percentage coefficient of variation (%GCV) and median absolute deviation (MAD; Table 6). Also, by expressing the neutralising activity of the samples relative to the candidate WHO IS the potency estimates were within 8-fold from the median (Table 8) while the geometric mean values from the titres as reported by the participants were over 8-fold-change from study median values (Table 7). Finally, the pattern observed in figure 2C, results obtained by the authentic neutralisation test trending lower than those obtained by tcVLP and PV-based neutralisation methods, disappears by expressing the neutralisation titres of the samples relative to the candidate IS, demonstrating harmonisation not only across laboratories but also across methods (Figure 2D).

Evaluation of Binding antibodies titres

Thirty-four datasets were returned for methods detecting binding antibodies as listed in Table 3. Five methods were specific for detection of IgM (lab 6a, 13a, 21c, 24b and 24d). Two of

these methods targeted NP (21c and 24d), one a combination of GPC and NP (13a), one the whole virion (24b) and one unknown (6a). All the samples were negative for IgM except the high titre sample C-03, which was equivocal in the µ-Capture ELISA Panadea CCHFV (NP) IgM Kit (lab 24d) and positive the VectoCrimean-CHF-IgM (lab 6a). This result is expected as the source plasma/serum used to generate the study samples was derived from convalescent individuals. The candidate WHO IS C-01, but not the liquid bulk preparation C-15, and samples C-06, C-09 and C-13 were also positive for IgM in the in-house IFA targeting the whole virion (lab 24b).
The results provided by the four participants that used an ELISA detecting IgG against CCHFV Gc are reported in table 9. The data were reported using different arbitrary units and numerically were over 77-fold different. Five datasets for the anti-NP IgG (Table 9) have been obtained using qualitative kits and reported as POS/NEG; however, using the raw data from the dilution series performed for each sample, the relative values of each sample against candidate IS C-01 was calculated and reported in Table 10. Overall, most of the samples were correctly identified as positive or negative with few exceptions (Table 9). Two laboratories scored positive both negative samples C-10 and C-12 using a commercial kit not used by any other laboratory (lab 12) or an in-house ELISA (lab 18b). Sample C-09 was reported as false negative in 5/16 methods including both in-house and commercial kits (lab 7c, 20, 26a,26b,27c). Indeed, it was ranked as the lowest titre sample by the remaining 11 assays with the only two exceptions being the same labs which reported the false positive results (lab 12 and 18b). Similar to the CCHFV Gc ELISAs, the results were reported using different units resulting in a 1000-fold difference in the numeric values which rendered the calculation of a median or average value meaningless. The spread of the results is visualised in Figure 3.

In Table 13 the results from assays targeting different antigens or using different methods have been recorded as reported by the participants. As there were fewer than 2 assays per antigen/method no statistical analysis was possible, and the data have been reported for information.

Evaluation of Binding activities Relative to the Candidate WHO International Standard
Some ELISA activity estimates were excluded due to non-overlapping response ranges, non-linearity or poor model fit (R^2 value lower than 0.95). With no coded duplicate samples, a slope ratio acceptance range could not be derived using the returned data, and a range of 0.80 - 1.25 was chosen as the parallelism acceptance range after assessing the outcome of this and some wider ranges. Slope ratios for analyses with suitable overlapping response ranges, linearity and model fit ranged from 0.20 to 2.55, with 94% within the range of 0.80 - 1.25. For the Gc ELISA methods (Lab 4a, 7b, 15c and 27b), the spread in the titre estimates across the study samples is reduced from up to 77-fold as reported (Table 9) to be within 3-fold when reported relative to the candidate WHO IS (Table 10) and thus improving comparability between the datasets. This can be observed in Figure 3 where the anti-Gc antibody titres between the laboratories are harmonised and clustered together (Figure 3a vs B). Lab 15c was an exception due to the use of only 2 dilution points (1:100 and 1:400) which limited the upper limit of detection to 400. This dataset was therefore excluded by the

statistical analysis. Despite the limited number of datasets (n=3) reporting the Gc binding potencies to the candidate WHO IS, resulted in a low GCV and MAD (Table 10) and the relative binding activity estimates are all within 2-fold from the median values, except for lab 15c, as already discussed (Table 11).
The results returned for the detection of IgG against CCHFV NP showed the wide spread of results due to different units being used (Figure 3C). Several assays for anti-NP antibodies, were qualitative or semi-qualitative; in these cases, the relative titres have been calculated based on the raw data provided for the dilution series from each sample compared to the candidate WHO IS dilutions (Table 10). These greatly improves harmonisation of the results between laboratories as shown in Figure 3D, and by a low GCV and MAD. Most of the relative activity estimates were within 2-fold from the study median values with few exceptions for samples with a low antibody titre (C-09) and lab 4b whose relative potencies were calculated as ratio rather than parallel line analysis (Table 12).

Assessment of commutability

Commutability of the candidate WHO IS, sample C-01, was assessed using a "calibration effectiveness" approach [16]. The analysis uses bias values calculated from reported estimates and from estimates calculated relative to C-01. Bias was defined as the laboratory geometric mean estimate for the sample as a ratio to the study consensus value for the sample (the study median value for the samples was used as sample target value for the purposes of this analysis), expressed as a percentage. A value of 100% indicates complete agreement with the sample target value, and the relative activity of test sample and standard is consistent with other labs and therefore the standard is commutable in such cases.
For the neutralising activity there is a clear harmonisation of the data from reported (Figure 4A) to the relative values (Figure 4B) with all the values within the acceptable range. To calculate the acceptable range for this study, the standard deviation of the bias values was calculated within each laboratory, and a median value, *msd*, was calculated across laboratories. The acceptable range was then set as ±3msd and reference lines with these values as shown in Figure 4. For neutralisation assays, the range was 37-270%. For the NP binding activity, as the reported data are expressed in different units it was not sensible to fit in a graph, but most of the relative values fell within the acceptable range set as 54-184% (Figure 4C).

Discussion

Within this study, the suitability of a candidate preparation to serve as the First WHO IS for antibodies to Crimean-Congo haemorrhagic fever virus (NIBSC code 24/228) has been evaluated. The source material for the candidate was a pool of plasma from recovered individuals from Turkey. The aim of this study was to assess whether the candidate could harmonise results from serological assays detecting anti-CCHFV antibodies.
A total of 24 laboratories from 14 countries, representing three WHO regions, participated in the study returning 46 datasets from methods detecting functional antibodies (neutralising) and binding antibodies targeting several CCHFV proteins. The neutralisation methods included in-house assays using authentic virus or alternative systems based on transcription-competent virus like particles or replication incompetent pseudotyped virus. Most

laboratories used the prototype IbAr10200 strain from Nigeria; others used isolates from Afghanistan, Russia and Turkey. The candidate WHO IS was tested in parallel with pools of sera from Uganda and Georgia and individual donations from the each of these three regions. The collaborative study results demonstrated that neutralisation titres could be effectively harmonised when expressed relative to the candidate IS (Figure 2). At least six CCHFV genotypes have been identified, distributed according to geographical region [17,18]. Therefore, it was important to show that the Turkey-derived candidate WHO IS could reduce interlaboratory variation for samples from other geographical regions (Uganda and Georgia), as indicated by the low %GCV and MAD values (Table 6).

The binding activity of the WHO IS to CCHFV antigens was evaluated in 23 different methods, including in-house ELISAs and IFAs, as well as 7 commercial ELISA kits and commercially available IFA slides. All but one of the commercial ELISA kits targeted NP; the remaining kit manufacturer did not disclose the CCHFV antigen. The intended use of these assays was qualitative or semi-qualitative. However, other methods used in this study for the detection of anti-NP IgG were quantitative. Quantification of anti-NP antibodies is important because antibody levels change over time, aiding epidemiological interpretation. It also improves the diagnostic accuracy (e.g. defining limit of detection) and increases comparability of results across laboratories and assays. The four in-house ELISA used to detect anti-Gc IgG were all quantitative. Interestingly, the ranking of the samples differed between assays targeting Gc *versus* NP, with Gc ELISA showing patterns more consistent with neutralising titres; for example, sample C-09 had the 3rd or 4th highest mean titre (excluding the C-01) in neutralisation and Gc ELISA, but lowest mean titre in the NP assays (see the ranking of samples in Tables 8, 11 and 12).

In both assays (Gc and NP) using a common unitage by expressing potencies relative to the candidate WHO IS improved comparability, allowing consensus geometric means to be calculated with low interlaboratory variation (%GCV and MAD, table 10). This comparison was not possible for other antigen targets due to insufficient datasets ($n < 3$). Those results are reported for information in Table 11. Finally, the WHO IS was tested in five laboratories using four methods to detect IgM against NP alone or in combination with GPC. All but one method returned negative results. Only one lab has detected IgM by IFA against the whole virion in the candidate WHO IS, C-01, however, the respective liquid bulk preparation C-15 tested negative. Given that the Turkish donations used to prepare the WHO IS were collected 2–4 years post outbreak, the absence of detectable IgM is expected.

Proposal

It is proposed that the sample C-01, NIBSC product code 24/228, is established as the First WHO International Standard for antibodies to Crimean-Congo haemorrhagic fever virus for neutralisation assays with an assigned potency of 25 IU/ampoule (24/228_NT) and 250 IU/ampoule for binding antibodies (IgG) to Gc and 250 IU/ampoule for binding antibodies (IgG) to NP (24/228_BA). The proposed Instructions for Use (IFU) are presented in Appendices 3 and 4, respectively. There are approximately 2500 ampoules (0.25 mL/ampoule) available for distribution. Based on the real time stability study results, we

propose that the International Standard can be shipped at ambient temperature. MHRA is the custodian laboratory, and the standard will be kept at -20˚C.

Comments from Participants

Lab 1, 4, 9, 11 and 21 revised the manuscript and had no comments
Lab 8 provided useful corrections and suggestions

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Tables

Table 1. Collaborative Study Panel
Samples were shipped under study reference CS751

Sample Code	Sample Description	Abbreviation	Appearance	Volume (mL)
C-01	24/228 - Candidate First WHO IS for antibodies to CCHFV	WHO IS	f/d	0.25
C-02	Pool of convalescent plasma from Turkey-Reference Panel #1	RP#1(T)	Liquid	0.25
C-03	Pool of convalescent sera from Georgia-Reference Panel #2	RP#2(G)	Liquid	0.25
C-04	Pool of convalescent sera from Uganda-Reference Panel #3	RP#3(U)	Liquid	0.25
C-05	Clinical sample – plasma Turkey	C-05(T)	Liquid	0.1
C-06	Clinical sample – plasma Turkey	C-06(T)	Liquid	0.1
C-07	Clinical sample – serum Georgia	C-07(G)	Liquid	0.1
C-08	Clinical sample – serum Georgia	C-08(G)	Liquid	0.1
C-09	Clinical sample – serum Georgia	C-09(G)	Liquid	0.1
C-10	Negative serum pool from Georgia	Neg (G)	Liquid	0.1
C-11	Clinical sample – serum Uganda	C-11 (U)	Liquid	0.1
C-12	Negative serum pool from Uganda	Neg (U)	Liquid	0.1
C-13	Clinical sample – plasma Turkey	C-13(T)	Liquid	0.1
C-14	Clinical sample – plasma Turkey	C-14(T)	Liquid	0.1
C-15	Candidate WHO IS liquid bulk preparation	IS bulk	Liquid	0.1

f/d: freeze-dried; IS: International Standard.

Table 2. Neutralisation Methods

Lab	Assay	Strain	Readout	Output
1a	FRNT	UK	Foci	NT50
2	Neut	nd	CPE	NT50
4c	tec-VLP	IbAr10200	RLU	NT50
5	VSV-PV	IbAr10200	RLU	**NT75**
7a	tc-VLP	IbAr10200	RLU	NT50
9	VSV-PV	IbAr10200	RLU	NT50
10	VSV-PV	Afg09	RLU	NT50
11	VSV-PV	1-CR/HU-2015	RLU	NT50
15a	FRNT	nd	Foci	NT50
18a	FRNT	Turkey-Kelkit06	Foci	NT50
21b	Microneut	Hoti	OD	NT50
27a	VSV-PV	IbAr10200	RLU	NT50

VSV-PV: vesicular stomatitis virus pseudotyped virus; FRNT: foci reduction neutralisation test; tec (or tc)-VLP: transcription- (and entry-) competent virus-like particles; CPE: cytopathic effect; RLU: relative light units; OD: optical density; NT_{50}: 50% neutralisation titre. Nd: not disclosed

Table 3. Methods for the detection of binding antibodies

Lab	Target	Assay	Ig class	Isolate	Readout	Output
4a	**Gc**	In-direct ELISA	IgG	IbAr10200	OD	EU
7b		In-direct ELISA	IgG	nd	OD	Endpoint
15c		Direct ELISA	IgG	nd	OD	pos/neg
27b		In-direct ELISA	IgG	IbAr10200	OD	Endpoint
1b	**NP**	IFA	IgG	IbAr10200	Fluorescence	endpoint
4b		In-direct ELISA	IgG	IbAr10200	OD	EU
7c		In-direct ELISA	IgG	nd	OD	Endpoint
8		In-direct ELISA EUROIMMUN (EI 279a-9601 G)	IgG	nd	OD	RU
12		In-direct ELISA RayBiotech (IEQ-CCHFNP-IgG-1)	IgG	nd	OD	EU
15b		Direct ELISA	IgG	nd	OD	pos/neg
16		In-direct ELISA EUROIMMUN (EI 279a-9601 G)	IgG	nd	OD	RU
18b		In-direct ELISA	IgG	nd	OD	Endpoint

19a		Double Antigen ELISA ID Screen® CCHF Double Antigen Multi-species ELISA	IgG+IgM	nd	OD	S/P
19b		In-direct ELISA EUROIMMUN (EI 279a-9601 G)	IgG	nd	OD	RU
20		Double Antigen ELISA ID Screen® CCHF Double Antigen Multi-species ELISA	IgG+IgM	nd	OD	S/P
21a		FcγR ELISA Panadea CCHFV (NP) IgG ELISA Kit (ELG.001)	IgG	nd	OD	Index value
21c		µ-Capture ELISA Panadea CCHFV (NP) IgM ELISA Kit (ELM.001)	IgM	nd	OD	Index value
23a		Double Antigen ELISA ID Screen® CCHF Double Antigen Multi-species ELISA	IgG+IgM	nd	OD	S/P
24c		FcγR ELISA Panadea CCHFV (NP) IgG ELISA Kit (ELG.001)	IgG	nd	OD	Index value
24d		µ-Capture ELISA Panadea CCHFV (NP) IgM ELISA Kit (ELM.001)	IgM	nd	OD	Index value
25b		IFA (EUROIMMUN Mosaic 2)	IgG	nd	Fluorescence	endpoint
26a		In-direct ELISA EUROIMMUN (EI 279a-9601 G)	IgG	nd	OD	ratio
26b		Double Antigen ELISA ID Screen® CCHF Double Antigen Multi-species ELISA	IgG+IgM	nd	OD	S/P
27c		In-direct ELISA	IgG	IbAr10200	OD	Endpoint
25a	**GPC**	IFA (EUROIMMUN Mosaic 2)	IgG	nd	Fluorescence	endpoint
13a	**GPC & NP**	IFA (EUROIMMUN Mosaic 2)	IgM	nd	Fluorescence	endpoint

13b	**GPC & NP**	IFA (EUROIMMUN Mosaic 2)	IgG	nd	Fluorescence	endpoint
24a	**whole virion**	IFA	IgG	nd	Fluorescence	endpoint
24b	**whole virion**	IFA	IgM	nd	Fluorescence	endpoint
6a	**nd**	ELISA kits VectoCrimean-CHF-IgM	IgM	nd	OD	pos/neg
6b	**nd**	ELISA kits VectoCrimean-CHF-IgG	IgG	nd	OD	pos/neg
17	**whole virion**	in-direct ELISA	IgG+IgM	IbAr10200	OD	pos/neg
23b	**nd**	ELISA kits VectoCrimean-CHF-IgG	IgG	nd	OD	pos/neg
27d	**GP38**	in-direct ELISA	IgG	IbAr10200	OD	Endpoint

GPC: glycoprotein precursor; Gc: Glycoprotein C; NP: nucleoprotein; IFA: immunofluorescence assay; nd: not disclosed; EU: ELISA units; RU: relative units; OD: optical density; S/P: standard to positive ratio.

Table 4. Production Summary of the Candidate WHO IS, 24/228

NIBSC Product Code	**24/228**
Product Description	First WHO IS for antibodies to CCHFV
Dates of processing	Filling; 21 March 2025 Lyophilisation; 21-25 March 2025
Presentation	Freeze-dried preparation in 2.5mL DIN ampoules
No. vials filled	2640
Mean fill mass (g)	0.27 (n = 99)
CV of fill mass (%)	0.62
Mean relative moisture (%)	1.43 (n = 12)
CV of mean relative moisture (%)	6.3
Mean of oxygen content (%)	0.35 (n = 12)
CV of oxygen content (%)	57.36

n = number of samples tested

Table 5. Laboratory Reported Neutralising Potency Estimates of Study Samples

Results are the geometric mean of three independent experiments. Units are the individual assay output provided in Table 2. False positive and false negative results are highlighted in red. In blue results which are not used in the statistical calculations.

Lab	C-01 WHO IS	C-02 RP#1(T)	C-03 RP#2(G)	C-04 RP#3 (U)	C-05 (T)	C-06 (T)	C-07 (G)	C-08 (G)	C-09 (G)	C-10 Neg (G)	C-11 (U)	C-12 Neg (U)	C-13 (T)	C-14 (T)	C-15 IS bulk
1a	25	16	40	16	10	10	10	10	10	-	40	-	10	40	16
2	73	62	302	43	92	33	31	46	86	-	115	-	69	132	139
4c	764	328	876	379	281	594	124	261	541	-	1375	-	228	395	342
5	77	52	205	67	62	96	50	156	112	-	108	-	65	119	183
7a	160	119	499	136	107	128	50	143	184	-	408	-	245	146	197
9	492	250	1279	541	298	371	157	268	506	-	1174	-	441	447	458
10	283	413	842	356	321	352	486	360	390	-	1627	-	483	460	425
11	106	51	246	81	72	74	23	77	106	-	86	-	63	72	93
15a	*200**	*159**	*200**	*159**	*200**	*100**	*50**	*100**	*159**	-	*200**	-	*159**	*200**	*159**
18a	64	50	143	22	40	40	20	63	45	-	63	32	45	57	63
21b	22	12	36	17	16	11	11	14	16	-	44	-	14	41	24
27a	302	88	589	214	133	183	80	126	262	-	879	-	174	225	214
GM	124	77	281	92	81	86	56	87	113		233		91	135	129
Median	106	62	302	105	92	96	50	126	112		115		69	132	183
GCV	216%	214%	231%	286%	215%	298%	210%	224%	280%		330%		266%	151%	204%
10^{MAD}	2.66	1.93	2.12	3.49	2.29	2.9	2.31	2.08	2.48		2.88		3.29	2.33	1.96
n	11	11	11	10	11	11	10	11	11		11		11	11	11

GM: geometric mean; GCV: geometric coefficient of variation; MAD: ; n:number

Table 6. Potency Estimates of Study Samples Expressed Relative to the candidate WHO IS (C-01)

Potency estimated were determined using an arbitrary potency of 100 IU/mL.

Lab	C-02 RP#1(T)	C-03 RP#2(G)	C-04 RP#3 (U)	C-05 (T)	C-06 (T)	C-07 (G)	C-08 (G)	C-09 (G)	C-11 (U)	C-13 (T)	C-14 (T)	C-15 IS bulk
1a	63	159	63	40	40		40	40	159	40	159	63
2	85	420	59	126	45	42	63	118	159	95	182	191
4c	43	115	50	37	78	16	34	71	180	30	52	45
5	67	266		80	124	65	202	144	140	84	154	236
7a	75	310	85	67	80	31	89	108	256	154	92	123
9	51	260	110	61	75	32	54	103	239	90	91	93
10	146	297	126	113	124	172	127	138	575	171	163	150
11	48	231	77	67	70	22	73	100	81	59	68	88
18a	79	224	35	63	63	31	100	71	100	71	89	100
21b	53	161	76	70	48	49	61	72	197	61	184	109
27a	36	243	88	55	76	23	36	75	251	54	69	66
GM	63	231	72	66	70	38	69	89	185	73	108	103
Median	63	243	76	67	75	32	63	100	180	71	92	100
GCV	47%	43%	46%	45%	45%	95%	74%	45%	69%	69%	57%	64%
10^{MAD}	1.26	1.22	1.25	1.19	1.2	1.42	1.59	1.38	1.33	1.32	1.68	1.5
n	11	11	10	11	11	10	11	11	11	11	11	11

GM: geometric mean; GCV: geometric coefficient of variation; 10^{MAD}: anti-log of the median absolute deviation; n:number

Table 7. Laboratory geometric mean reported neutralising activity estimates as fold-change from study median values

	Laboratory										
Sample	**1a**	**2**	**4c**	**5**	**7a**	**9**	**10**	**11**	**18a**	**21b**	**27a**
C-01	4.22	1.46	7.18	1.38	1.50	4.62	2.66	1.00	1.68	4.79	2.84
C-03	7.63	1.00	2.87	1.49	1.64	4.19	2.76	1.24	2.14	8.53	1.93
C-11	2.88	1.00	11.91	1.07	3.53	10.17	14.10	1.34	1.82	2.64	7.62
C-15	11.50	1.32	1.87	1.00	1.08	2.51	2.33	1.96	2.88	7.52	1.17
C-09	11.15	1.30	4.85	1.00	1.65	4.54	3.50	1.05	2.48	6.95	2.35
C-14	3.30	1.00	2.99	1.11	1.11	3.39	3.48	1.84	2.33	3.23	1.71
C-04	6.64	2.46	3.59		1.29	5.13	3.38	1.29	4.69	6.25	2.03
C-06	9.58	2.90	6.20	1.00	1.34	3.87	3.68	1.29	2.40	8.99	1.91
C-13	6.93	1.00	3.29	1.06	3.54	6.36	6.98	1.11	1.54	5.13	2.52
C-05	9.16	1.00	3.07	1.48	1.17	3.26	3.50	1.28	2.29	5.88	1.45
C-08	12.56	2.74	2.08	1.24	1.14	2.13	2.87	1.62	1.98	9.21	1.00
C-02	3.89	1.00	5.31	1.19	1.93	4.05	6.70	1.20	1.22	5.22	1.43
C-07		1.61	2.49	1.00	1.00	3.15	9.76	2.14	2.49	4.63	1.61
% < 2	0%	77%	8%	100%	85%	0%	0%	92%	38%	0%	62%
% < 4	25%	100%	62%	100%	100%	46%	69%	100%	92%	15%	92%
% < 8	58%	100%	92%	100%	100%	92%	85%	100%	100%	77%	100%

1.00 : X < 2 3.00 : 2 < X < 4 6.00 : 4 < X < 8 10.00 : X > 8

Table 8. Laboratory geometric mean relative to C-01 neutralising activity estimates as fold-change from study median values

Collaborative study samples are reported in order of potency (median) from Table 6

	Laboratory										
Sample	**1a**	**2**	**4c**	**5**	**7a**	**9**	**10**	**11**	**18a**	**21b**	**27a**
C-03	1.53	1.73	2.12	1.09	1.28	1.07	1.22	1.05	1.08	1.51	1.00
C-11	1.13	1.13	1.00	1.29	1.42	1.33	3.19	2.23	1.80	1.09	1.39
C-15	1.59	1.91	2.23	2.36	1.23	1.07	1.50	1.14	1.00	1.09	1.52
C-09	2.51	1.18	1.41	1.45	1.08	1.03	1.38	1.00	1.41	1.38	1.34
C-14	1.73	1.98	1.77	1.68	1.00	1.01	1.77	1.36	1.03	2.01	1.32
C-04	1.21	1.29	1.54		1.12	1.44	1.65	1.00	2.16	1.00	1.16
C-06	1.90	1.66	1.03	1.65	1.06	1.00	1.65	1.08	1.20	1.57	1.00
C-13	1.78	1.35	2.37	1.19	2.17	1.27	2.41	1.20	1.00	1.16	1.32
C-05	1.69	1.88	1.82	1.19	1.00	1.11	1.69	1.00	1.07	1.04	1.23
C-08	1.59	1.00	1.84	3.21	1.42	1.16	2.02	1.16	1.59	1.02	1.76
C-02	1.00	1.35	1.47	1.07	1.19	1.24	2.32	1.30	1.26	1.18	1.73
C-07		1.34	1.95	2.04	1.02	1.01	5.41	1.45	1.01	1.53	1.39
% < 2	91%	100%	75%	73%	92%	100%	58%	92%	92%	92%	100%
% < 4	100%	100%	100%	100%	100%	100%	92%	100%	100%	100%	100%
% < 8	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

1.00 : X < 2 3.00 : 2 < X < 4 6.00 : 4 < X < 8 10.00 : X > 8

Table 9. Laboratory Reported Binding Potency Estimates of Study Samples

Results are the geometric mean of three independent experiments. Units are the individual assay output provided in Table 3. False positive and false negative results are highlighted in red. In blue results which are not used in the statistical calculations.

Antigen	Lab	C-01 WHO IS	C-02 RP#1(T)	C-03 RP#2(G)	C-04 RP#3 (U)	C-05 (T)	C-06 (T)	C-07 (G)	C-08 (G)	C-09 (G)	C-10 Neg (G)	C-11 (U)	C-12 Neg (U)	C-13 (T)	C-14 (T)	C-15 IS bulk
Gc	4a	7299	3944	16798	7242	3686	5131	2253	5454	8457	-	30859	-	6243	5645	7338
	7b	431	342	1368	862	342	609	171	609	862		1724		543	431	684
	15c	*400*	*400*	*400*	*400*	*400*	*252*	*400*	*400*	*400*		*400*	*100*	*400*	*400*	*400*
	27b	2691	1600	6400	2263	2691	2263	951	3200	5382		9051		3200	1903	3805
NP	4b	191	303	1128	842	317	331	873	131	57	-	521	-	144	202	187
	7c	271	100	1086	271	100	171	431	200	-	-	171	-	100	305	271
	8	>200	>200	>200	>200	>200	>200	>200	>200	68	-	>200	-	184	>200	>200
	12	62001	40708	94960	69374	38748	26069	35618	25316	41242	25849	28815	37742	26745	49879	61404
	15b	*400*	*252*	*400*	*400*	*400*	*159*	*400*	*159*	*100*		*252*		*400*	*400*	*252*
	16	561	500	4000	1000	1000	1000	2000	1000	200		630		500	1260	1000
	18b	1016	806	2281	1016	640	570	1280	640	453	90	640	160	718	508	403
	19a	POS	POS	POS	POS	POS	POS	POS	POS	POS	-	POS	-	POS	POS	POS
	19b	386	307	1433	413	402	386	845	>200	56	-	417	-	184	481	416
	20	46.2	29.5	104.3	58.5	30.4	37.2	74.4	38.7	-	-	57.1	-	31.0	55.7	45.0
	21a	POS	POS	POS	POS	POS	POS	POS	POS	POS	-	POS	-	POS	POS	POS
	23a	POS	POS	POS	POS	POS	POS	POS	POS	POS	-	POS	-	POS	POS	POS
	24c	14.0	13.7	14.8	13.8	12.0	14.6	14.7	14.3	7.8	-	14.2	-	13.8	14.5	13.3
	26a	*-*	*-*	*2.3*	*-*	*-*	*-*	*1*	*-*	*-*	*-*	*-*	*-*	*-*	*0.95*	*-*
	26b	60.3	43.4	125.7	69.1	42.6	43.7	89.1	40.3	-	-	46.9	-	32.9	54.9	37.1
	27c	305	224	1425	635	800	252	566	252	-	-	317	-	159	356	283

Table 10. Laboratory Reported Binding Potency Estimates of Study Samples

Potency estimated were determined using an arbitrary potency of 1000 IU/mL.

Antigen	Lab	C-02 RP#1(T)	C-03 RP#2(G)	C-04 RP#3 (U)	C-05 (T)	C-06 (T)	C-07 (G)	C-08 (G)	C-09 (G)	C-11 (U)	C-13 (T)	C-14 (T)	C-15 IS bulk
Gc	4a	540	2301	992	505	703	309	747	1159	4228	855	773	1005
	7b	613	3010	1287	534	950	395	882	1165	4452	945	752	1058
	27b	884	2941	1138	1363	1345		823	1528	3626	854	703	1137
	GM	664	2731	1133	717	965	349	816	1273	4087	884	742	1066
	Median	613	2941	1138	534	950	349	823	1165	4228	855	752	1058
	GCV	29%	16%	14%	75%	38%	19%	9%	17%	11%	6%	5%	6%
	10^{MAD}	1.14	1.02	1.13	1.06	1.35	1.13	1.07	1.01	1.05	1	1.03	1.05
	n	3	3	3	3	3	2	3	3	3	3	3	3
NP	4b*	*1586*	*5905*	*4406*	*1659*	*1734*	*4570*	*684*	*296*	*2726*	*753*	*1057*	*980*
	7c	590	3444	1376	727	888	1882	431		956	622	1453	1069
	8	448	2925	745	470	714	1313	514	96	658	459	1054	951
	12	614	1703	1177	615	369	518	1021	722	434	412	1135	1222
	16	789	5041	1451	1164	1570	3165	1330	304	980	751	2013	1602
	18b	734	1807	1060	566	549	1336	568	618	639	611	573	704
	19a	668	2457	1163	663	826	1647	918		1237	742	1405	1054
	19b	793	3690	1133	1085	1030	2178	644	162	1030	565	1412	1051
	20	560	3094	1049	778	824	1454	674		1255	605	1309	1140
	21a	512	3181	902	528	727	1324	623	60	816	476	1228	927
	23a	562	2465	904	664	663	1268	702	138	634	469	881	647
	24c	621	2891	914	530	765	1494	629	103	866	628	1338	870
	26a	521	2709	1531	1511	673	1642	754	151	845	554	1151	1072
	27c	540	2301	992	505	703	309	747	1159	4228	855	773	1005
	GM	655	2999	1217	774	806	1621	700	195	907	578	1186	998
	Median	614	2925	1133	664	765	1494	674	156	866	605	1228	1051
	GCV	37%	42%	55%	51%	50%	67%	34%	125%	56%	22%	34%	26%
	10^{MAD}	1.18	1.19	1.24	1.25	1.16	1.14	1.12	1.75	1.32	1.23	1.15	1.11
	n	13	13	13	13	13	13	13	10	13	13	13	13

GM: geometric mean; GCV: geometric coefficient of variation; 10^{MAD}: anti-log of the median absolute deviation; n:number* excluded from statistical calculations as estimates were derived as ratios from reported end-point dilutions

Table 11. Laboratory geometric mean relative activity estimates as fold-change from study median values (Gc ELISA)

Collaborative study samples are reported in order of potency (median) from Table 10.

	Laboratory			
Sample	**4a**	**7b**	**15c**	**27b**
C-11	1.08	1.14	3.92	1.08
C-03	1.13	1.16	2.60	1.13
C-09	1.00	1.00	1.16	1.32
C-04	1.08	1.21	1.07	1.07
C-15	1.03	1.03	1.03	1.10
C-06	1.16	1.16	1.30	1.65
C-13	1.05	1.05	1.11	1.05
C-08	1.14	1.04	1.17	1.04
C-14	1.01	1.01	1.31	1.08
C-02	1.36	1.20	1.36	1.20
C-05	1.45	1.37	1.37	1.86
C-07	1.28	1.00	2.53	
% < 2	100%	100%	75%	100%
% < 4	100%	100%	100%	100%
% < 8	100%	100%	100%	100%

: X < 2 : 2 < X < 4

: 4 < X < 8 : X > 8

Table 12. Laboratory geometric mean relative activity estimates as fold-change from study median values (NP ELISA)

	Laboratory													
Sample	**4b**	**7c**	**8**	**12**	**15b**	**16**	**18b**	**19a**	**19b**	**20**	**21a**	**23a**	**24c**	**27c**
C-03	2.03	1.18	1.01	1.71	2.91	1.73	1.61	1.18	1.27	1.06	1.09	1.18	1.01	1.07
C-07	3.10	1.28	1.12	2.84	1.47	2.15	1.10	1.12	1.48	1.01	1.11	1.16	1.01	1.11
C-14	1.13	1.22	1.13	1.05	1.19	1.69	2.07	1.18	1.19	1.10	1.03	1.35	1.13	1.03
C-04	4.02	1.26	1.47	1.07	1.10	1.32	1.03	1.06	1.03	1.04	1.21	1.21	1.20	1.40
C-15	1.04	1.05	1.07	1.20	1.61	1.58	1.44	1.04	1.04	1.12	1.09	1.57	1.17	1.06
C-11	3.19	1.12	1.30	1.97	1.36	1.15	1.34	1.45	1.20	1.47	1.05	1.35	1.01	1.01
C-06	2.33	1.19	1.04	2.02	1.88	2.10	1.36	1.11	1.38	1.10	1.03	1.13	1.03	1.11
C-05	2.39	1.05	1.48	1.13	1.44	1.67	1.23	1.05	1.56	1.12	1.32	1.05	1.31	2.17
C-08	1.04	1.53	1.28	1.55	1.66	2.02	1.16	1.39	1.02	1.02	1.06	1.07	1.05	1.14
C-02	2.57	1.05	1.38	1.01	1.02	1.28	1.19	1.08	1.28	1.10	1.21	1.10	1.01	1.19
C-13	1.24	1.02	1.32	1.47	1.64	1.24	1.01	1.22	1.08	1.01	1.28	1.30	1.03	1.10
C-09	1.83		1.68	4.47	1.55	1.88	3.82		1.00		2.68	1.17	1.57	1.07
% < 2	42%	100%	100%	75%	92%	75%	83%	100%	100%	100%	92%	100%	100%	92%
% < 4	92%	100%	100%	92%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
% < 8	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

: X < 2 : 2 < X < 4 : 4 < X < 8 : X > 8

Table 13. Laboratory reported binding antibodies estimates of study samples

Antigen	Method	Lab	C-01 WHO IS	C-02 RP#1 (T)	C-03 RP#2 (G)	C-04 RP#3 (U)	C-05 (T)	C-06 (T)	C-07 (G)	C-08 (G)	C-09 (G)	C-10 Neg (G)	C-11 (U)	C-12 Neg(U)	C-13 (T)	C-14 (T)	C-15 IS bulk
NP	IFA	1b	80	80	202	113	160	403	160	-	28		40		202	127	160
NP	IFA	25b	141	141	1600	-	141	200	566	400	-		141		200	566	566
GPC	IFA	25a	141	141	566	-	141	141	-	141	-		566		141	141	141
GPC & NP	IFA	13b	113	113	453	20	113	80	113	141	80		226		113	113	160
whole virion	ELISA	17	POS	POS	POS	POS	POS	POS	POS	POS	POS		POS		POS	POS	POS
whole virion	IFA	24a	1280	1280	10240	2560	1280	2560	2560	1280	2560		10240		2560	2560	2560
nd	ELISA VectoCrimean-CHF-IgG	6b	4.25	4.16	4.09	4.20	4.21	4.17	4.05	4.25	3.34		4.15		4.19	4.14	4.15
nd	ELISA VectoCrimean-CHF-IgG	23b	POS	POS	POS	POS	POS	POS	POS	POS	POS		POS		POS	POS	POS
GP38	ELISA	27d	294	159	1008	159	317	152	100	713	141		566		100	141	283

Figures

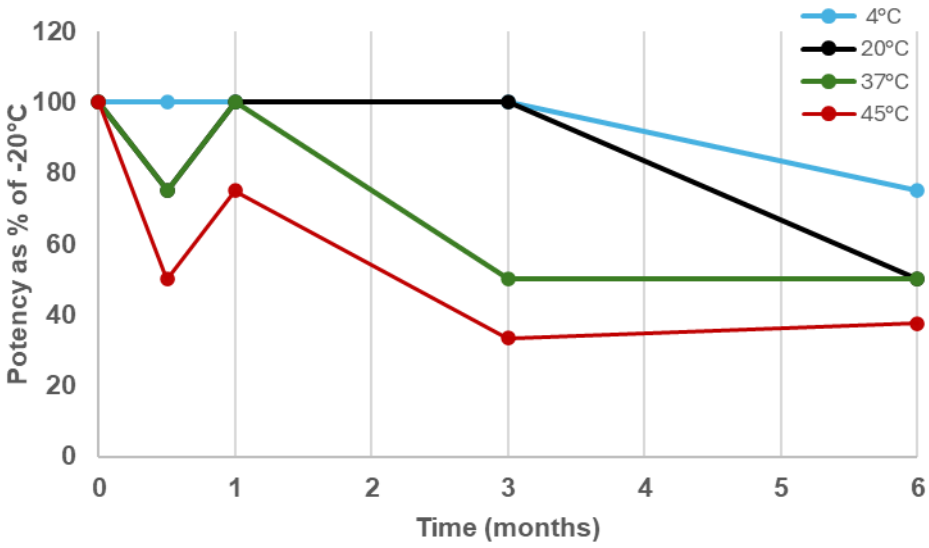

Figure 1. Stability assessment of the candidate International Standard for antibodies to CCHFV. Freeze-dried ampoules of sample C-01, NIBSC code 24/228 were stored at five different temperatures (-20, 4, 20, 37 and 45˚C). At each time point, two vials were retrieved and reconstituted with 0.25 mL of molecular grade water. Each vial was assessed in duplicate in an ELISA method for the detection of anti-CCHFV Gc antibodies. Data are reported as percentage activity of each sample relative to the ones stored at -20˚C (baseline) for each time point.

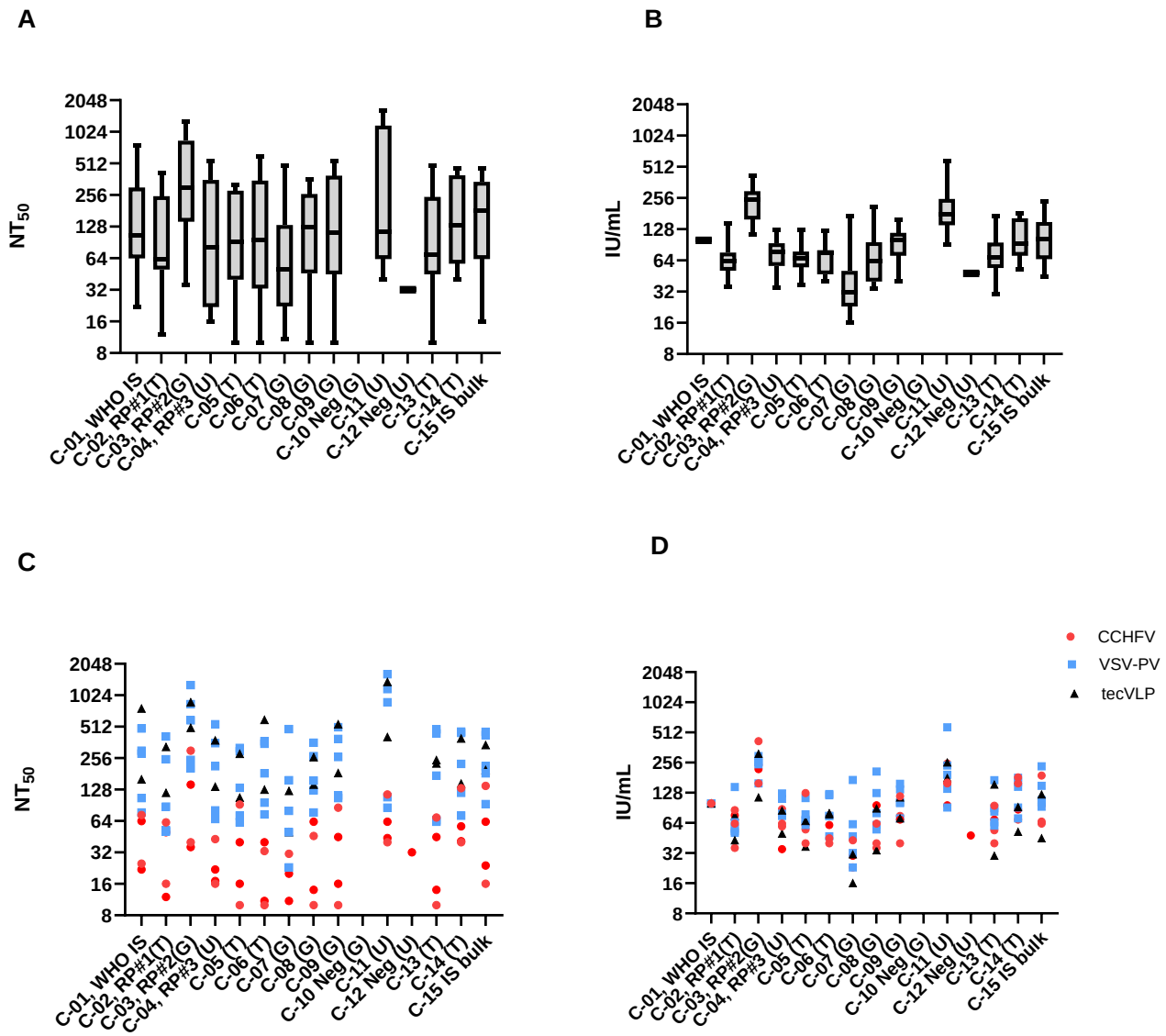

Figure 2. Harmonisation of Neutralising Antibody Titres when Reported Relative to the Candidate WHO IS

A and C) The laboratory reported geometric mean neutralising antibody titres for each sample (Table 5) in NT_{50}; **B and D)** potencies reported relative to the candidate WHO IS (C-01) in IU/mL with an assigned value of 100 IU/mL(Table 6). Square: PV neutralisation; Triangle: transcription competent VLP neutralisation; Circle: Authentic virus neutralisation

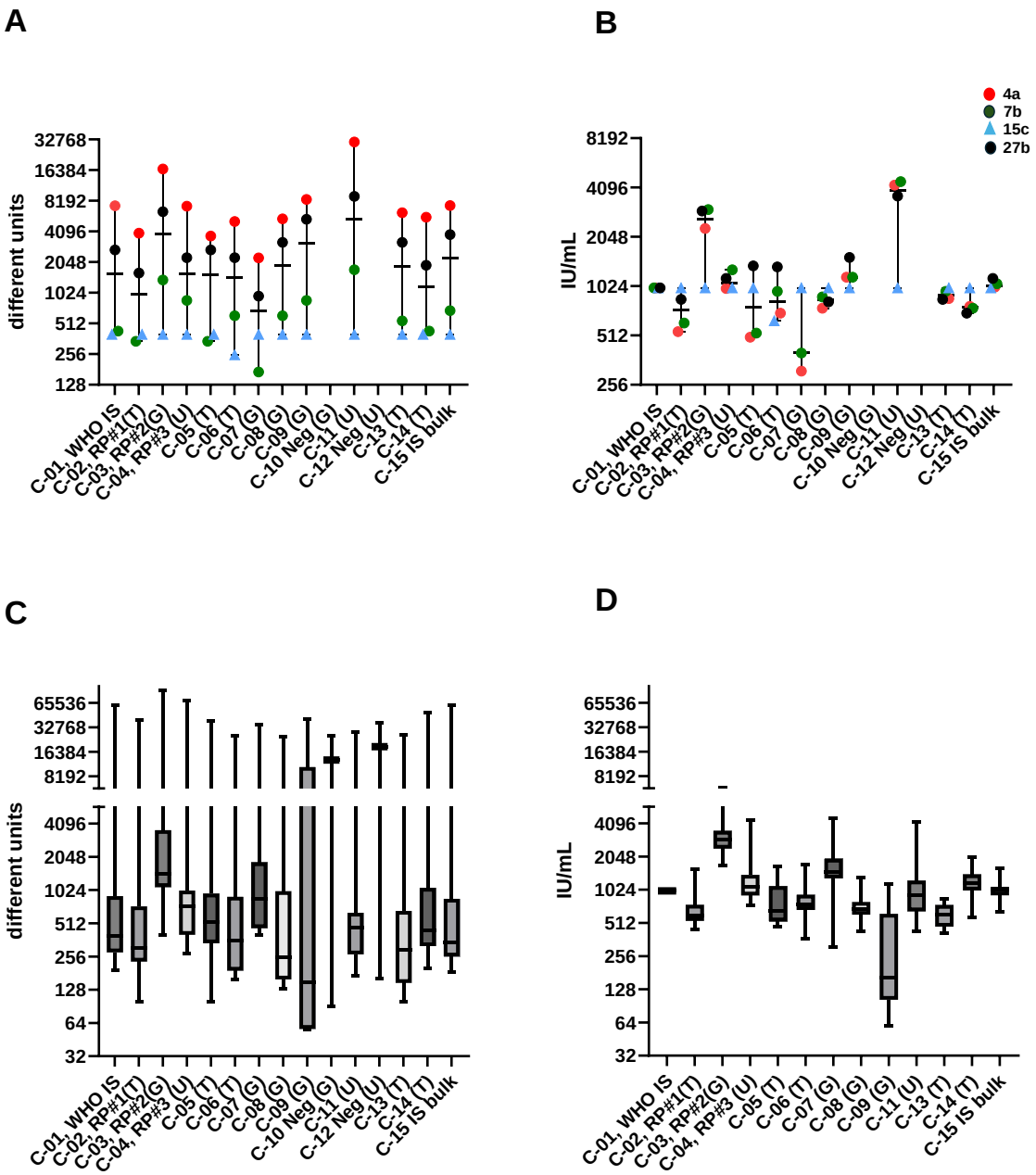

Figure 3. Harmonisation of Binding Antibody Titres when Reported Relative to the Candidate WHO IS

A and C) The laboratory reported geometric mean binding antibody titres for each sample (Table 7) with different units for CCHFV Gc (A) and NP protein (C); **B and D)** potencies reported relative to the candidate WHO IS (C-01) in IU/mL with an assigned value of 1000 IU/mL (Table 8) for Gc (B) and NP proteins (D).

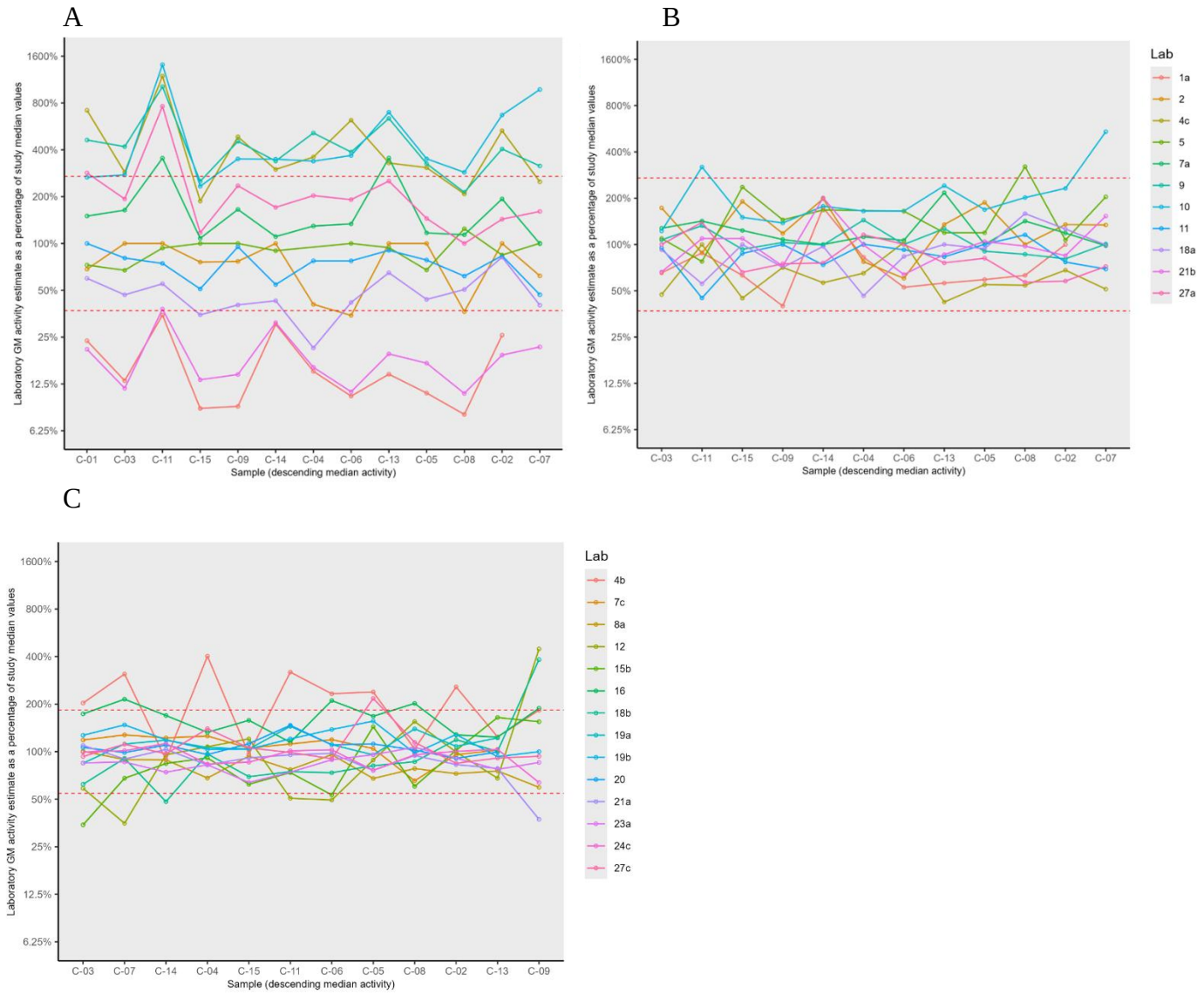

Figure 4. Assessment of commutability by calibration effectiveness. Laboratory geometric mean relative activity estimated as percentage of study median values for neutralising antibodies as reported by the participants (A) or relative to candidate WHO IS C-01 (B) and for binding antibodies to CCHFV NP (C). The reference lines (dotted red) showed the acceptable range calculated as ±3 median value of the standard deviation across laboratories.

Appendix 1

Collaborative Study Participants

(in alphabetic order by organisation)

Participant	Organisation	Country
Aykut Ozkul	Ankara University, Faculty of Veterinary Medicine, Department of Virology, AVZAL	Turkey
Martin Gabriel , Christina Deschermeier, Petra Emmerich, Stephan Günther, Nadine Petersen, Toni Rieger	Bernhard Nocht Institute for Tropical Medicine (BNITM)	Germany
Nazli Ayhan, Remi Charel	Centre National de Référence (CNR) des Arbovirus, Marseille University	France
Aykut Ozdarendeli	Vaccine Research and Development Center (ERAGEM) Erciyes University	Turkey
Corine Geurts van Kessel, Sandra Scherbeijn, Boaz Eimers	Erasmus MC	The Netherlands
Julia M. Klemens	Euroimmun Medizinische Labordiagnostika AG	Germany
BAIZE Sylvain and PANNETIER Delphine	French National Reference Center for VHF / Unit of Biology of Emerging Viral Infections, Institut Pasteur and Laboratoire P4 Jean Mérieux INSERM	France
Markus Keller, Kerstin Fischer, Patrick Slowikowski, Katrin Schwabe	Friedrich-Loeffler-Institut	Germany
Francisco J. Pérez Rodríguez, Camille Escadafal	Geneva University Hospitals (HUG)	Switzerland
Damien Gely, Valentin Ollivier, Fabien Donnet	Innovative Diagnostics	France
Qianqian Li, Youchun Wang	Institute of Medical Biology, Chinese Academy of Medical Science and Peking Union Medical College (IMBCAMS)	China
Seong Eun Bae, Jonghyun Bae, Young-Shin Park, Min Ji Kang, and Jae-Ouk Kim	International Vaccine Institute	South Korea
M Shimojima, T Kurosu, T Yoshikawa, K Oishi, M Kawahara, H Nakano	Japan Institute of Health Security (JIHS)	Japan
Brian Willett	MRC–University of Glasgow Centre for Virus Research (CVR)	United Kingdom
Shirin Ashraf, Emma Thomson	MRC–University of Glasgow Centre for Virus Research (CVR)	United Kingdom

Tom Malone, Chitra Tejpal	Medicines and Healthcare products Regulatory Agency (MHRA)	United Kingdom
Hongyoon Kim, Chulhyun Lee	Ministry of Food and Drug Safety	South Korea
Huang Weijin, Nie Jianhui, Qin Haiyang	National Institutes for Food and Drug Control (NIFDC)	China
Francesca Colavita, Silvia Meschi, Fabrizio Maggi	National Institute for Infectious Diseases (INMI) IRCCS "Lazzaro Spallanzani"	Italy
Jack Saunders, Teresa Lambe	Oxford Vaccine Group, University of Oxford	United Kingdom
Sofia Appelberg, Stéphanie Devignot, Ali Mirazimi	Public Health Agency of Sweden	Sweden
Nazibrola Chitadze, Giorgi Chakhunashvili	R. G. Lugar Center for Public Health Research, National Center for Disease Control and Public Health (NCDC) of Georgia	Georgia
Stephen Balinandi, Jocelyn Kigozi, Julius Lutwama	Uganda Virus Research Institute	Uganda
Misa Korva, Katarina Resman Rus	University of Ljubljana	Slovenia

Appendix 2. CS751 Study Protocol

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STUDY PROTOCOL

Collaborative Study CS751
Development of a WHO International Standard for Antibodies to Crimean-Congo haemorrhagic fever virus

Study Background and Aims

This multi-center International collaborative study will evaluate a candidate preparation to serve as the First WHO International Standard for antibodies to Crimean-Congo haemorrhagic fever virus (CCHFV). The study is organized by the MHRA on behalf of the World Health Organization (WHO) and is facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI).

International Standards (IS) are recognized as the highest order of reference material for biological substances and are assigned a potency in International Units (IU). They are intended to be used to calibrate assays to report the relative biological activity of samples in terms of IU. This allows for comparability between assays/laboratories and enables parameters, such as analytical sensitivity of tests, or clinical measures, such as protective levels of antibody, to be better defined. CCHFV is specified as a priority and prototype pathogen for the Nairoviridae family in the recently published WHO pathogens prioritization framework [1]. The availability of an IS for CCHFV antibody will facilitate the standardization of serological assays, including supporting the evaluation of epidemiological and clinical trial data during vaccine development, as outlined in the R&D Roadmap for CCHF [2]. The study will follow published WHO guidelines [3] and be submitted for formal establishment by the WHO.

The aims of this study are to:

- Assess the suitability of the candidate to serve as a WHO International Standard and harmonise data in a range of typical assays performed in different laboratories.
- Characterise the candidate's reactivity/specificity in different assay systems.
- Assess commutability, i.e. establish the extent to which the candidate is suitable to serve as a standard for a variety of different samples.
- Recommend to the WHO ECBS the suitability of the candidate to serve as a WHO International Standard and propose an assigned International Unit.

Study Samples

All study samples are provided coded and blinded to participants (Table 1.). Each participant is provided with 4 sets of study samples C-01 to C-10 and C-12 to C-15 per method, which allows for 3

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independent assays and includes a spare sample set. Participants with more than one method will only receive one spare sample of C-11.

The samples comprise plasma or sera of human origin which have been collected from healthy donors who were previously exposed to CCHFV. The samples were donated by Sivas Cumhuriyet University Hospital (SCUH), Turkey and Integrum Scientific, LLC, in partnership with the Uganda Virus Research Institute (UVRI) and National Center for Disease Control and Public Health (NCDC), Georgia. The samples were collected under the approval of local Ethics Committees.

All samples have tested negative for the presence of CCHFV via real-time RT-PCR and confirmatory testing for any indeterminate results via culture on permissive cells, examined for cytopathic effect (CPE) and viral RNA amplification by real-time RT-PCR. The samples have tested negative for blood borne pathogen markers HBsAg, anti-HIV Ab and HCV RNA, except sample C-12 which tested positive for HBsAg. Further, all samples have undergone a precautionary inactivation treatment using a validated Solvent-Detergent method to inactivate enveloped viruses and the samples are provided as non-infectious.

Table 1. CS751 Study Sample Panel.

All samples should be stored at -20°C or below

Sample Code	Formulation	Volume (mL)	Vial
C-01	Freeze-dried	0.25	DIN Ampoule
C-02	Liquid frozen	0.25	Screw Cap Vial
C-03			
C-04			
C-05	Liquid frozen	0.1	Screw Cap Vial
C-06			
C-07			
C-08			
C-09			
C-10			
C-11			
C-12			
C-13			
C-14			
C-15			

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CAUTION: As with all materials of biological origin, the material should be regarded as potentially hazardous to health.

Study Protocol

As per the Instructions for Use (IFU), freeze-dried sample C-01 requires reconstitution in 0.25mL of sterile ultra-pure H_2O. Allow 10 minutes for complete dissolution and mix gently. All other samples should be thawed at room temperature prior to assay.

Participants are requested to:

- Perform 3 independent tests on different days or with different operators.
- Use a fresh set of samples for each independent test.
- Prepare dilutions of the samples in the sample matrix routinely used in the test method (e.g. plasma/media/buffer). The optimal dilution range should cover at least 5 points and include at least one point beyond the endpoint dilution. If needed, dilutions can be adjusted for subsequent assays and recorded clearly in the results reporting sheet.
- Test samples at least in duplicate, ideally from independent dilution series.
- If feasible, test all samples (C-01 to C-15) within the same assay so that sample potencies relative to each other can be calculated. Please indicate in the results reporting sheet if samples have not been tested concurrently.

Results Reporting

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

- Within the reporting sheet, under the 'Result Summary' record the qualitative (+/-) and where applicable quantitative (endpoint titre/IC50 etc.) result for each sample as per analysis in your laboratory. It is important to know whether the samples are considered positive, as per analysis in your laboratory.
- Under the 'Raw data' section of the reporting sheet record the raw assay readout for each dilution tested (e.g. absorbance OD, RLU, plaques etc.). Ensure that the dilutions tested are correctly recorded. Include the assay cut-off value indicating sero-reactivity for each assay. Our statistician will use the raw data readouts to perform statistical analysis.
- Where multiple methods have been used, complete one reporting sheet per method by adding a new tab to the template excel file.
- Record in the Excel reporting sheet any deviations from the study protocol and complete all fields requesting additional method details.

Deadline for return of results is 10th October 2025

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All completed results spreadsheets should be returned electronically to:

Project Leader: Emma Bentley Emma.Bentley@mhra.gov.uk
Deputy: Giada Mattiuzzo Giada.Mattiuzzo@mhra.gov.uk

Data Analysis

Analysis of the study data will assess the potencies of each sample relative to each other, and their performance within the different assay methods. The anonymity of each laboratory is maintained by reporting datasets with a blind code, details of the assay performed will be included.

A draft study report will be sent to participants for comment. The report will include data analysis, proposed conclusions and recommendations on the selection, use and unitage of the candidate WHO IS for antibodies to CCHFV. Participants' comments will be included in the report prior to submission to the WHO in January 2026. Study participants will be notified of the outcome of the study after the WHO ECBS meeting due to be held in April 2026.

References

[1] Pathogens prioritization: a scientific framework for epidemic and pandemic research preparedness

[2] A Research and Development Roadmap for CCHF: 2024 Update

[3] Recommendations for the preparation, characterization and establishment of international and other biological reference standards, Annex 2, TRS No 932

As outlined in the study questionnaire, participation in the collaborative study is conducted under the following WHO Collaborative Study Terms and Conditions:

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

MHRA, as the Collaborative Study coordinator, notes that:

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

Appendix 3. Proposed Instruction for Use

WHO International Standard
First International Standard for antibodies to
Crimean-Congo haemorrhagic fever virus for neutralisation assays
NIBSC code: 24/228_NT
Instructions for use

1. INTENDED USE

The First WHO International Standard for antibodies to Crimean-Congo haemorrhagic fever (CCHF) virus is the freeze-dried equivalent of 0.25 mL of pooled plasma obtained from five individuals recovered from CCHF. The preparation has been evaluated in a WHO International collaborative study (1). The intended use of the International Standard is for the calibration and harmonisation of serological assays detecting anti-CCHF virus neutralising antibodies. The preparation has been solvent detergent treated to minimise the risk of the presence of enveloped viruses (2).

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. This preparation is not for administration to humans or animals. As for all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The assigned potency of the WHO International Standard for antibodies to CCHF virus is 25 IU/ampoule for neutralising antibodies. After reconstitution of the lyophilised cake in 0.25 mL of distilled water or other matrix, the final concentration will be 100 IU/mL.

International Units are arbitrary assigned to a WHO International Standard to express the biological activity of the standard, and as such there is no conversion factor between IU and mass (e.g. ng of antibody)

4. CONTENTS

Country of origin of biological material: Turkey.
Each ampoule contains the freeze-dried equivalent of 0.25 mL of pooled human plasma.

5. STORAGE

Ampoules should be stored at -20°C or below until use.
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.

The contents of each ampoule should be reconstituted in 0.25mL distilled water. Following addition of the distilled water, the material must be allowed to become fully reconstituted before use.

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.

It is the policy of WHO not to assign an expiry date to International Standards. They remain valid with the assigned potency and status until withdrawn or amended. Please note that the stability of International Standard when reconstituted has not been specifically determined. Therefore, it is recommended that the reconstituted material is for single use only. Should users wish to store reconstituted material, they should determine the stability of reconstituted material according to their own method of preparation, storage and use

9. REFERENCES

(1) Bentley et al Collaborative Study for the Establishment of the First WHO International Standard for antibodies to Crimean-Congo haemorrhagic fever virus. 2026 WHO Expert Committee on Biological Standardization. WHO/BS/2026.2504
(2) Dichtelmuller, H.O., et al., Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion, 2009. 49(9): p. 1931-43

10. ACKNOWLEDGEMENTS

We would like to wholeheartedly thank the anonymous donors of the serum and plasma samples for their consent which has allowed this study to be undertaken. We gratefully acknowledge the important contributions of the collaborative study participants.
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The project has been funded by the Coalition for Epidemic Preparedness Innovations

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.orgWHO Biological Standards: http://www.who.int/biologicals/en/JCTLM Higher order reference materials:http://www.bipm.org/en/committees/jc/jctlm/Derivation of International Units:http://www.nibsc.org/standardisation/international_standards.aspxOrdering standards from NIBSC:http://www.nibsc.org/products/ordering.aspxNIBSC Terms & Conditions:http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

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

13. CITATION

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

14. MATERIAL SAFETY SHEET

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

Physical and Chemical properties	
Physical appearance: Freeze dried	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): human origin	
Toxicological properties	
Effects of inhalation: Not established, avoid inhalation	
Effects of ingestion: Not established, avoid ingestion	
Effects of skin absorption: Not established, avoid contact with skin	
Suggested First Aid	
Inhalation: Seek medical advice	
Ingestion: Seek medical advice	
Contact with eyes: Wash with copious amounts of water. Seek medical advice	
Contact with skin: Wash thoroughly with water.	
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

15. LIABILITY AND LOSS

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

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.25 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached: Not Applicable**

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

Appendix 4. Proposed Instruction for Use

WHO International Standard
First International Standard for antibodies to
Crimean-Congo haemorrhagic fever virus for binding assay
NIBSC code: 24/228_BA
Instructions for use

1. INTENDED USE

The First WHO International Standard for antibodies to Crimean-Congo haemorrhagic fever (CCHF) virus is the freeze-dried equivalent of 0.25 mL of pooled plasma obtained from five individuals recovered from CCHF. The preparation has been evaluated in a WHO International collaborative study (1). The intended use of the International Standard is for the calibration and harmonisation of serological assays detecting binding immunoglobulin G to the CCHF virus Gc protein or nucleoprotein (NP). The preparation has been solvent detergent treated to minimise the risk of the presence of enveloped viruses (2).

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. This preparation is not for administration to humans or animals. As for all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The assigned potency of the WHO International Standard for antibodies to CCHF virus is 250 IU/ampoule for binding immunoglobulin G (igG) to the Gc protein and 250 IU/ampoule for binding IgG to the NP protein. These values have been arbitrarily chosen and do not reflect the proportion of the antibody in the preparation. After reconstitution of the lyophilised cake in 0.25 mL of distilled water or other matrix, the final concentration will be 1000 IU/mL for anti-Gc IgG and 1000 IU/mL for anti-NP IgG.

International Units are arbitrary assigned to a WHO International Standard to express the biological activity of the standard, and as such there is no conversion factor between IU and mass (e.g. ng of antibody).
Activities against other CCHF virus proteins (e.g. Gn, GP38, etc.) are likely to be present. However, as part of the collaborative study [1] there were not enough data to support the assignment of an unitage against other viral targets.

4. CONTENTS

Country of origin of biological material: Turkey.
Each ampoule contains the freeze-dried equivalent of 0.25 mL of pooled human plasma.

5. STORAGE

Ampoules should be stored at -20°C or below until use.
Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

11. 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.

12. USE OF MATERIAL

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

The contents of each ampoule should be reconstituted in 0.25mL distilled water. Following addition of the distilled water, the material must be allowed to become fully reconstituted before use.

13. 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.

It is the policy of WHO not to assign an expiry date to International Standards. They remain valid with the assigned potency and status until withdrawn or amended. Please note that the stability of International Standard when reconstituted has not been specifically determined. Therefore, it is recommended that the reconstituted material is for single use only. Should users wish to store reconstituted material, they should determine the stability of reconstituted material according to their own method of preparation, storage and use

14. REFERENCES

(1) Bentley et al Collaborative Study for the Establishment of the First WHO International Standard for antibodies to Crimean-Congo haemorrhagic fever virus. 2026 WHO Expert Committee on Biological Standardization. WHO/BS/2026.2504
(2) Dichtelmuller, H.O., et al., Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion, 2009. 49(9): p. 1931-43

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