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Collaborative Study to Evaluate the 1st WHO International Standard for Herpes Simplex Virus (HSV) DNA for Nucleic Acid Amplification Technology (NAT)-Based Assays

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **5 October 2020** 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 Ivana Knezevic** at email: knezevici@who.int.

Summary

This report describes the preparation and collaborative study evaluation of the 1st WHO International Standard(s) for Human Simplex Virus (HSV) for use in the standardization of NAT based assays. The candidate preparations comprise of lyophilised cultured whole virus: HSV1 (Strain 17) and HSV2 (Strain HG52), formulated in universal buffer (Tris-HCl, Human serum albumin and trehalose) to provide single stable reference materials, suitable for different sample matrix types and lyophilised for long term stability. Thirty laboratories from 14 countries were recruited for participation in this multi-center collaborative study. Data was returned by 25 laboratories using a diverse range of assay methods, comprising of both qualitative (n=12) and quantitative assays (n=20). All the qualitative assays were commercial and 9 of the 20 quantitative assays were laboratory developed. The candidate standards for HSV-1 16/368 (sample C) and HSV-2 17/122 (Sample D) were evaluated alongside their liquid bulks (samples A and B) and a HSV-1 positive swab in universal transport medium (UTM, Sample E) and a HSV-2 positive serum specimen (Sample F). The overall combined reported mean potency from qualitative and quantitative assays for HSV-1 candidate 16/368 was 7.19 and 7.31 Log_{10} units/mL for HSV-2 candidate 17/122. The overall mean reported potency of candidates C and D from qualitative assays was 6.39 and 6.74 Log_{10} NAT detectable units/mL, respectively and 7.58 and 7.65 Log_{10} copies/mL for quantitative assays. Higher interlaboratory variability was evident across qualitative assays compared to quantitative assays particularly for candidate sample C. Agreement between laboratories of the combined data set for estimating potencies of liquid bulk samples A and B, improved more when potency was expressed relative to independent candidate standards C and D respective of HSV type, as indicted by the reduction in standard deviation (SD). However, the SD of potencies for lower titre clinical samples largely increases when calculated relative to both candidates. Further evaluation of candidate standards alongside a larger number of clinical samples and using wider range of asays within a EQA study, demonstrated a moderate improvement in harmonisation (less bias) across the participating laboratories and methods when clinical sample results were expressed relative to each candidate standard. Overall the reported potencies indicated minimal loss upon freeze drying. Ongoing stability assessment indicates the candidates are stable and suitable for long term storage. Sequencing data indicates minimal single nucleotide poly morphisms (SNP's) and no large-scale re-arrangements.

The results of this study indicate suitability of both candidates as independent calibrators for HSV type 1 and 2 NAT based assays. Therefore, it is proposed that the candidates for HSV-1 (NIBSC code: 16/368) is established as the 1st WHO International Standard for HSV-1 NAT, with assigned potency of 7.19 Log_{10} IU/vial and HSV-2 (NIBSC code: 17/122) is established as the 1st WHO International Standard for HSV-2 NAT, with assigned potency of 7.31 Log_{10} IU/viaL.

Introduction

Herpes simplex virus (HSV) 1 and 2 are large enveloped linear double stranded DNA viruses belonging to the Alphaherpesvirinae, a subfamily of Herpesviridae. They are common widely distributed and highly contagious self-limiting lifelong recurrent chronic sexually transmitted infections (STI) [1,2]. High global seroprevalence is variable for each type, HSV-1 is spread through contact with contaminated oral secretions, where incidence increases rapidly from early age. For the 60-70 age group of Americans and Europeans seroprevalence is ~70 to 80% and for adults in Africa and Asia more than 90%. HSV-2 incidence increases rapidly from adolescence, peaking at 20-30% in Europe and America. However, estimates are underrepresented due to limited incidence data because most infections are asymptomatic and unrecognized [3].

HSV-1 and HSV-2 cause a variety of illnesses, including mucocutaneous, central nervous system (CNS) and occasionally visceral organ infections. In the newborns, severely malnourished and immune compromised hosts infections can be severe and life-threatening, and can trigger recurring erythema multiforme, can cause blindness, can result in severe neurological impairment and can increase the risk of acquisition of human immunodeficiency virus (HIV). HSV-1 predominately causes facial lesions in the form of cold sores and HSV-2 predominantly causes genital ulcers, however prevalence differs across population demographics and over time for example HSV-2 is the most common cause of genital herpes in most developed countries whereas in Japan HSV-1 is relatively more commonly attributed cause of genital infections [4]. In addition to visible lesions, HSV is an important pathogen of the central nervous system (CNS) manifesting either as meningitis (HSVM) or encephalitis (HSVE). Untreated HSVE can lead to focal necrotizing encephalitis which has high mortality rates in the region of 70%. Episodic and suppressive treatment with nucleoside analogues that inhibit viral DNA, reduce the severity duration recurrence and transmission, however viral shedding and latent virus reservoirs are not eliminated and remain risks to recurrence in transmission [5].

Treatment guidelines recommend clinical diagnosis should be confirmed by type specific laboratory testing, as a patient's prognosis and counselling needs will depend on the type of HSV infection. NAT PCR tests are the preferred test for patients seeking treatment for genital ulcers or other mucocutaneous lesions, central nervous system and systemic infections (e.g., meningitis, encephalitis, and neonatal herpes), providing greater sensitivity over other virologic tests such as cell culture-based methods. Early and accurate diagnosis and treatment of viral CNS infections caused by HSV decrease morbidity and mortality rates, which is especially important in immunocompromised patients. The use of molecular techniques for the diagnosis of patient infection is now recognized as the reference method for HSV detection, predominately for the specific diagnosis of CNS infections, where molecular assays are increasingly employed. HSV-2 infections typically give a low titre viral load in the CSF, however specimens isolated from primary lesions are often of high titre, thus requiring a wide dynamic assay range [6,7].

Many diagnostic assays allow for the simultaneous detection of HSV-1 and 2, however the majority of assays run by diagnostic laboratories report results in a qualitative format and vary greatly in sensitivity. Lower limits of detection for each specific virus type may vary in each test but currently there is no means of accurately assessing this. An increasing range of commercial assays are available for different sample matrices, comprising kits for closed systems for extraction and amplification and kits specific for different amplification platforms. The huge diversity of laboratory developed and commercial assays, along with the heterogeneity of possible combinations of reagents methodology and instrumentation without traceability to primary calibrant adds to the interlaboratory variability in viral load measurements. This makes

it difficult to compare results between different laboratories and develop treatment thresholds. Also, UK and European EQA studies for HSV NAT assays show great variability in assay detection ability, indicating greater standardisation of assays is clearly needed. There is a need for an international standard(s) to allow for accurate determination of assay sensitivity for both HSV-1 and HSV-2, which will ensure correct and timely patient treatment management.

This proposal was discussed at the Clinical Diagnostic Standardisation of Genomic Amplification Techniques (SoGAT) meeting in June 2014, where delegates agreed HSV DNA detection assays require standardisation. It was agreed that the candidate standard would comprise of characterized cell cultured virus formulated in universal buffer, for dilution in different sample matrices, to enable standardisation of both extraction and amplification steps for different clinical matrices. The World Health Organization, Expert Committee on Biological Standardisation (ECBS) which establishes primary higher order reference standards for biological medicines in October 2014 endorsed the proposal for the establishment of the first WHO International Standard (s) for HSV-1 and HSV-2 for NAT with arbitrary international units (IU) assigned [8].

However it was not know at this stage whether there was a need for one standard, where one strain had the ability to cross harmonise the other, or for two strains where indivudal harmonisation was required. Study data was presented and discussed at the SoGAT meeting in 2018. Delegates of this meeting comprised representatives from industry, regulatory, academic and the clinical diagnostic community. On presentation of the data, it was the view of community that two indivudal materials would be required, however as will be demonstrated in in this report, the study data did not fully support the commutability of both candidate materials. Therefore it was agreed at this meeting that additional work would be carried out by material inclusion in an external Quality assurance scheme (EQA).

Aims

The aim of this collaborative study is to evaluate the suitability of the candidates for the calibration of secondary reference materials for the standardization of NAT assays for one or both strain types of HSV, along with a limited assessment of commutability. The study will also determine the potency of each candidate standard(s) using a range of NAT based assays for HSV-1 and HSV-2.

Materials

Candidate standards

The two candidate materials prepared and taken forward for evaluation comprise lyophilised preparations of whole virus of laboratory cultured strains of HSV-1 (NIBSC code: 16/368) and HSV-2 (NIBSC code: 17/122) diluted to ~ $1x10^7$ genomes/mL in universal buffer (10mM Tris buffer, 0.5 % human serum albumin, 0.1% trehalose). Formulation in universal buffer enables a single reference material to standardize across different sample matrices, by further dilution in appropriate sample matrices specific to diagnostic clinical assays. Source material for both candidates HSV1 (Strain 17) and HSV2 (Strain HG52) were kindly donated by Dr Preston MRC Virology Unit, University of Glasgow.

Preparation of bulk materials
HSV-1 strain 17 and HSV-2 strain HG52 were propagated at NIBSC in confluent WHO Vero cells at 37 °C with 5% CO_2 using DMEM and 1% FCS, 2% 2mM Glutamine and P/S. Total culture was harvested when maximal CPE was visible, approximately after ~2 weeks. The total culture was snap frozen by soaking in methanol and dry ice bath for 3 min before being stored at -80 °C until preparation of the final bulk. The virus stock used for formulating bulk was derived from cell passage 7 for HSV-1 and passage 8 for HSV-2.

The concentration of the HSV-1 and HSV-2 stocks were determined using a commercially sourced quantitative real time PCR CE marked IVD kit (HSV1, HSV2, VZV R-GENE®, bioMérieux), which was performed on the Stratagene Mx3005P (Agilent Genomics). Extractions were performed using the CE marked IVD kit Cobas® AmpliPrep Total Nucleic Acid Isolation Kit (TNAi, Roche) on a Cobas Ampliprep instrument using 1 mL protocol. Laboratory developed quantitative assays for HSV-1 and HSV-2 [9] were also utilized in measuring concentration prior to filling.

Bulk preparations were formulated to contain approximately $1x10^7$ copies/mL of HSV-1 and HSV-2 respectively in a final volume of 7.2 L of universal buffer: 10mM tris HCl pH 7.4, 0.5% HSA, 0.1% D-(+)-Trehalose dehydrate. The human serum albumin (HSA) was derived from licensed products (Zenalb, BPL). Aliquots of viral supernatants stored at -80 °C were thawed in a water bath set at 37 °C prior to a 100-fold dilution in universal buffer to achieve the desired concentration. Approximately 200 mL of each bulk was aliquoted into 2ml screw cap Sarstedt tubes and stored at -20 °C for inclusion in the collaborative study. The remaining volume of bulks for |HSV-1 and HSV-2 were processed for freeze drying and assigned NIBSC product codes 16/368 and 17/122 respectively.

Filling and lyophilization of candidate standards
The liquid bulks of both HSV-1 (16/368) and HSV-2 (17/122) candidate materials were filled and lyophilized at NIBSC, using a negative pressure isolator (Metal & Plastic GmbH, Radolfzell, Germany) that contains the filling line (FVF5060, Bausch & Strobel, Ilfshofen, Germany) and interfaced freeze dryer (CS150 $12m^2$, Serail, Le Coudray Saint Germer, France), summary of production is detailed in Table 1. During the filling process the bulk was constantly stirred using a magnetic stirrer. The bulk was dispensed into 5 mL screw cap glass vials in 1 mL volumes. Online check weighting of the wet weight was used to determine the homogeneity of the fill, any vials outside of the defined specifications were discarded. Vials were loaded onto shelves at 4 °C, the temperature was lowered to -50 °C over 90 minutes and held for a further 4 hours. For primary drying a vacuum was applied to 100 µb over 2 hours, the shelf temperature raised to -15°C over 1 hour and maintained for a further 40 hours. For secondary drying the shelf temperature was ramped to 25 °C over 15 hours then held at 25°C for 20 hours at 30 µb vacuum. The vials were back filled with dry nitrogen and stoppered in the isolator before removal. Vials were capped in the isolator which was then decontaminated with formaldehyde prior to removing product. The sealed vials are stored at -20 °C under continuous temperature monitoring at NIBSC for the products lifetime.

Post-fill testing
Tests were performed for residual moisture determination and oxygen content, to indicate integrity of the vial contents after sealing. Samples of each freeze-dried candidate (n=12) were assessed using non-invasive near-infrared (NIR) spectroscopy (MCT 600P, Process Sensors, Corby, UK) to determine residual moisture. NIR are correlated to the coulometric Karl Fischer method using calibration samples (the same excipient dried using the same cycle at laboratory

scale and tested using both NIR and Karl Fischer methods) to give readings in % w/w moisture. Frequency modulated spectroscopy (FMS-760, Lighthouse Instruments, Charlottesville, VA, USA) was used to determine headspace oxygen content, calibrating against NIST-traceable certified gases in identically sealed container standards. Sterility testing at NIBSC by an internal microbiologist confirmed pre and post fill materials for bacterial and fungal colony counts, which were in compliance to predetermined limits.

Sample preparation for sequencing involved ultra-centrifuging at 15000rpm for 2 hours viral master-pool stocks used to prepare candidate bulk materials. The resultant pellet was incubated overnight at +4 ºC and resuspended in residual supernatant and DNA was extracted using SDS proteinase K digestion prior to elution with spin columns using Zumo Genomic DNA clean and concentrator kit. The concentration was confirmed using nanodrop reader.

Whole-genome sequencing for both candidates was performed at the NIBSC NGS core facility. Sequencing libraries were constructed PCR-free with the KAPA Hyper Plus Kit (Roche, 07962436001). Samples were processed in duplicate with initial enzymatic fragmentation times of 5 min and 10 min respectively. Individual samples were single-indexed during library preparation using the KAPA Single-Indexed Adapter Kit (Roche, 08005699001) and pooled for sequencing. Libraries were sequenced paired-end on the Illumina MiSeq platform for 2x 250 cycles. Additionally, HSV-1 candidate sample C was assessed using long read whole-genome sequencing on the Oxford Nanopore Technologies (ONT) MinION sequencer. An individual sample was prepared for sequencing using the 1D Ligation Sequencing Kit (ONT, SQK-LSK108) and sequenced for 3 h on a SpotON Flow Cell Mk I (ONT, FLO-MIN106).

Stability of the freeze-dried candidates

Accelerated degradation studies to predict stability upon long term storage at -20 °C for candidates 16/368 and 17/122 were initiated following filling and lyophilization. Sufficient vials of freeze dried product were placed in storage at -20 °C, +4 °C, +20 °C, +37 °C, and +45 °C, to enable retrieval of three vials at each temperature at specified time points during the life of the product to quantify HSV-1 and HSV-2 DNA using NAT assays described in post fill testing.

Study samples

The freeze-dried International standard candidates for HSV-1 16/368 (sample C) and HSV-2 17/122 (Sample D) and their liquid bulk equivalents (samples A and B) were evaluated alongside two clinical specimens a HSV-1 positive swab UTM (Sample E) and HSV-2 positive serum (Sample F). Samples E was kindly donated to NIBSC by a staff member, and sample F was commercially sourced from Cerba specimen services. Both samples were further diluted in relevant matrices, to yield sufficient volume and titer for distribution in multicenter collaborative study. The concentrations of HSV-1 and HSV-2 in study panel samples were determined using HSV-1 and HSV-2 NAT assays described in preparation of bilk materials. Samples C, D, E and F were aliquoted in 1 mL volumes into 2 mL sarstedt screw cap tubes and stored at -80°C prior to distribution to participants.

Study panel distributed to participants was coded as samples A-F and was as follows:

- Sample A (SA) - Liquid frozen preparation HSV-1 16/368 in a 2 mL Sarstedt tube.
- Sample B (SB) - Liquid frozen preparation HSV-2 17/122 in a 2 mL Sarstedt tube.
- Sample C (SC) - Lyophilized preparation HSV-1 16/368 in a 3 mL screw cap glass vial.
- Sample D (SD) - Lyophilized preparation HSV-2 17/122 in a 3 mL crimp cap glass vial.
- Sample E (SE) - Liquid frozen preparation HSV-1 positive UTM clinical sample in a 2 mL Sarstedt tube.

– Sample F (SF) - Liquid frozen preparation HSV-2 positive serum clinical sample in a 2 mL Sarstedt tube.

Study design
The aim of this multicentre collaborative study was to evaluate the suitability and potency of the two candidate preparations to calibrate secondary references for HSV-1 and HSV-2 in parallel with clinical specimens, using a range of NAT based assays. Study panels were shipped to participating laboratories, on dry ice by courier, along with study documentation including instructions for storage and reconstitution.

Study protocol
Participating laboratories were requested to test dilutions of each study sample using their routine HSV-1 and HSV-2 NAT assay on four separate occasions. Sufficient material was provided to test a fresh vial of each sample in each independent assay (Appendix 2). Lyophilised samples C and D were to be reconstituted in 1 mL of deionized, nuclease-free molecular-grade water and left for a minimum of 20 minutes with occasional agitation before use. Liquid frozen samples A B E and F were to be thawed and vortexed prior to use. Samples E and F were to be tested neat. All samples were to be extracted prior to amplification. The first of the four assays were intended to be used by the participating laboratories to assess whether dilutions were within the linear range of the assay and to determine the correct dilutions for the method.

For quantitative methods participants were requested to test, a single dilution of samples A & B, three tenfold serial dilutions of samples C & D and undiluted samples E & F. For qualitative assays participants were requested to test in the first assay serial tenfold dilutions of samples A-F to determine end-point and in subsequent assays to test the end-point and two serial half log dilutions either side.

Participating laboratories were requested to return details on methodology used and raw crossing point or cycle threshold values. Results reporting units requested were copies/mL for quantitative assays and positive or negative for qualitative assays.

Participants
In this study 30 laboratories were recruited for participation from 14 countries, comprising IVD manufacturers, reference, academic and control clinical laboratories. Participants were selected on the basis of their experience with HSV NAT and geographical location. All laboratories that accepted invitation for voluntary participation, were assigned a Laboratory code for reference, these laboratory codes were allocated at random and do not represent listing order of participants in Appendix 1. Laboratories returning data from more than one assay (or different extraction and amplification methods or platforms), or multiplexed assays were assigned different codes e.g. 23a 23b 23c.a-b respectively.

Statistical methods
Qualitative and quantitative assay results were evaluated separately. In the case of qualitative assays, for each laboratory and assay method, data from all assays were pooled to give a number positive out of number tested at each dilution step. A single ‘end-point’ for each dilution series was calculated, to give an estimate of ‘Log_{10} NAT detectable units/mL. It should be noted that these estimates are not necessarily directly equivalent to a genuine genome equivalent number/mL [10]. Relative potencies were then calculated as the difference of Log_{10} NAT detectable units/mL between samples.

In the case of quantitative assays, reported values (Log_{10} copies/mL) have been presented using reported data, corrected for dilution. Relative potencies were estimated using a parallel line model with untransformed Ct values or log-transformed copies/mL responses [11]. Calculations were performed using the EDQM software CombiStats Version 5.0 [12]. 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 reported in these cases. Potency estimates were expressed as Log_{10} units and these estimates from all valid assays were combined to generate an arithmetic mean in Log_{10} units for each laboratory and assay type.

Overall analysis was based on the Log_{10} estimates of copies/mL, 'Log_{10} NAT detectable units/mL' or relative potency, as required. Overall mean estimates were calculated as the means of all individual laboratories. Variation between laboratories (inter-laboratory) was expressed as standard deviations (SD) of the Log_{10} estimates and % geometric coefficient of variation (GCV = $\{10^{s}-1\}\times100\%$ where s is the standard deviation of the Log10 transformed estimates) of the actual estimates. Variation within laboratories and between assays (intra-laboratory) was expressed as standard deviations of the Log_{10} estimates and %GCVs of the individual assay mean estimates.

For the additional assessment of commutability, EQA estimates for the clinical samples from quantitative assays were used as reported by the participating laboratories and also expressed relative to the candidate standards within each laboratory. Laboratories that reported a negative value for the reference, and thus no relative values, were excluded from further analysis. For both reported and relative values, median values were calculated using log_{10} transformed estimates and were used as the study consensus values for each sample in the analysis. Bias values were calculated as the laboratory reported estimate log_{10} value difference from the study consensus value for the sample. Plots showing all bias estimates are given in Figures 4a-b and 5a-b along with tables of summary statistics for the data shown.

Results and data analysis

Validation of study samples and stability assessment
Production data summary for candidate standards sample C (16/368, HSV-1) and sample D (17/122, HSV-2) in table 1 shows fill mass mean coefficience of variance (CV) and mean residual moisture are acceptably within the limits for WHO International Standards [13]. Residual oxygen content for both candidates was within NIBSC working limit of 1.1%. Accelerated degradation studies for both candidate standards 16/368 and 17/122 are ongoing. Samples stored at elevated temperatures of +4, +20, +37 and +45 °C were evaluated in parallel with base line samples stored at -20 °C. Following 4 and 12 months of storage for sample C and 2 and 9 months of storage for sample D, 3 vials of each storage temperature sample were extracted and amplified, the mean Log_{10} copies/mL, difference from base line is shown in tables 2a-b. Results show minimal loss in potency for both candidates upon storage at elevated temperatures. There is no evidence of instability upon storage at -20 °C after 24 months storage for sample C and 21 months for sample D, the available data indicates adequate stability. Further tests at 2, 3, 4, and 5-year time points are planned for both candidates.

Sequence alignment assessment of candidate standards
A total of 18,167,700 Illumina paired sequence reads were obtained. Trimmed reads were aligned to HSV1-strain17 (NCBI reference: NC_001806.2) and HSV2-HG52 (NCBI reference:

820945149) reference genomes (Figure 4a-b). SNVs were called where variants were supported by at least 100 reads and annotated by overlap with known genes and repeat regions. Three of the variants identified for the HSV-2 candidate were only called in the 5min sample. For two of these, the genomic context was highly repetitive, and mapping quality was low, leading to limited accuracy in calling variants in these repetitive regions at the ends of the genome Long-read alignment indicates structurally variable tandem repeat region at position 143,716-143,868, shown in figure 4c.

Data received

Data was returned by 25 of the 30 laboratories recruited to the study. A wide variety of different assay methods were performed by participants, with some laboratories reporting data using more than one assay and some using multiplexed assays. A total of 12 qualitative and 20 quantitative assays were analysed. All of the qualitative assays were commercial and 9 of the 20 quantitative assays were laboratory developed.

Summary of assay methodologies

A wide variety of extraction and amplification assay methodologies and platforms were used by participants to report results. The variations in diluents and PCR gene targets adds to the complexity in diversity, Tables 3a-c summarises the extraction and amplification kits used, as well as diluents and gene targets selected. The vast majority of participants prepared dilutions using Transport medium (Universal, viral, swab), some laboratories used water (deionised, nuclease-free, molecular PCR-grade) or Phosphate buffered saline (PBS). Clinically relevant sample matrices of cerebrospinal fluid (CSF), plasma, serum were also represented. Extraction assays were largely performed using automated platforms, six participants returned data sets utilising manual extraction methods. The majority of the participants used NucliSENS® easyMag® (bioMerieux) for extraction, this was followed by Magnapure 96 DNA and Viral NA Small volume kit (Qiagen), there were also closed systems including the Cobas® HSV 1 and 2 test on the cobas 4800 (Roche). The commercial real-time PCR NAT assays comprised 8 different qualitative and 10 quantitative assays, additionally 9 quantitative laboratory developed assays were also used. The vast majority of participants used ABI 7500 fast (Applied Biosystems) followed by LightCycler 480 (Roche) and Rotor gene (Qiagen). Due to the diversity of assay methods, it has not been possible to group methods and to analyse based on such groupings.

Estimated IU/mL or 'NAT detectable units/mL'

Mean laboratory potency reported for each sample by participants using qualitative (Log_{10} NAT detectable units) and quantitative (Log_{10} copies/mL) assays with different sample matrix diluents, are shown in table 4, with qualitative assays shaded grey. Mean laboratory potencies reported for samples C to F are shown in histograms in figures 1a-b, each box represents a mean estimate from a laboratory labelled with the corresponding laboratory code. Boxes representing qualitative assays have been coloured to distinguish from quantitative assays. NAT detectable and IU/mL units are not interchangeable. Qualitative assays reported lower potencies and were more variable compared to quantitative assays, this is consistent with previous experience. The range for mean laboratory potency reported using quantitative assays for sample C and D was 2.12 and 2.26 Log_{10} copies/mL compared to qualitative assay ranges of 1.80 and 2.25 Log_{10} NAT detectable units/mL the ranges are lower and the potencies were ~1 Log_{10} (10-fold) higher.

Overall mean laboratory reported potency estimates from qualitative quantitative and combined, along with mean range and standard deviation, are all shown in table 5. The higher interlaboratory variability in qualitative assays is demonstrated by higher standard deviations of

reported mean potencies for the candidates, sample C & D 0.64 and 0.67 Log_{10} NAT detectable units/mL compared to standard deviations for samples C and D from quantitative assays of 0.44 and 0.52 Log_{10} copies/mL. The overall mean reported potency of candidates C and D from qualitative assays was 6.39 and 6.74 Log_{10} NAT detectable units/mL, and from quantitative assays was 7.58 and 7.65 Log_{10} copies/mL. The range for sample C was lower in qualitative assays at 1.80 Log_{10} NAT detectable units/mL compared to quantitative assays at 2.12 Log_{10} copies/mL, the ranges of reported potencies from qualitative and quantitative assays for sample D were similar around ~2 Log_{10}.

Comparison of overall mean laboratory reported potency estimates from qualitative and quantitative assays combined for freeze dried candidate samples C and D with liquid frozen preparations samples A and B indicates a mean gain in potency of 0.04 and 0.03 Log10 units/mL respectively for sample C and D neither significant.

Potencies relative to the Candidate for HSV-1, 16/368 (Sample C)
Mean laboratory reported potencies of liquid frozen preparations of candidate sample A and clinical specimens sample E & F expressed relative to candidate standard sample C as described in statistical methods section enables assessment of suitability of the material for standardization of HSV NAT assays for both types 1 and 2. Potencies of samples A, E and F calculated relative to candidate standard sample C with a potency of 7.15 Log_{10} proposed IU/ml are shown in table 6, with qualitative assays shaded in grey. Relative potencies of sample A are also shown in histograms in figure 2. Figures 2a-b shows a marked improvement in potency estimate agreement between laboratories when potencies reported by laboratories for liquid frozen preparation of the standard (sample A) are expressed relative the freeze-dried preparation of the candidate standard (sample C) compared to reported potencies shown in figure 1a, the range is reduced and outlier labs are centered into the consensus group.

Overall mean potency estimates for samples A, E and F relative to sample C along with number of datasets, potency range and standard deviations for qualitative, quantitative and both assays combined with units expressed in Log_{10} proposed IU/ml are shown in Table 7. A reduction in standard deviations is observed for combined qualitative and quantitative assay potencies of samples A when potencies are expressed relative to candidate standard sample C from 0.76 to 0.58 Log_{10} units/mL, demonstrating its use would lead to a reduction in interlaboratory variability for a similar sample and harmonization of diverse variety of methodologies used to HSV NAT diagnostics. Standard deviations of potencies calculated relative to sample C, combining qualitative and quantitative assays for low titre samples E and F show an increase from 0.47 and 0.44 to 0.58, 0.60 Log_{10} units/ml respectively.

Potencies relative to the Candidate for HSV-2, 17/122 (Sample D)
Mean potencies reported by laboratories for samples B, E and F expressed relative to candidate standard sample D combined assay potency of 7.31 Log10 proposed IU/ml are shown in table 8, qualitative assays shaded in grey. The relative potencies of sample B are also shown in histogram in figure 3a-b. Using sample D as a standard to calculate relative potencies reduces the range, standard deviation and interlaboratory variability, figures 3 show a reduced distribution of potencies compared to reported potencies for samples B, E and F, and brings distinguishable qualitative and quantitative groups and outlier into a centered consensus group.

Overall mean potency estimates for samples B, E and F relative to sample D are shown in table 9, standard deviations units expressed in Log_{10} proposed IU/ml. Standard deviations of potencies for samples B, are reduced for qualitative quantitative and both assays combined when potency

is calculated relative candidate standard sample D. This reduction in standard deviation of reported potencies from expressing potency relatively is markedly greater for candidate standard sample D. Standard deviations of potencies calculated relative sample D combining qualitative and quantitative assays for sample E a HSV-type 1 strain shows increase from 0.47 to 0.63 Log_{10} unit/mL whereas potencies of sample F a HSV-type 2 strain shows no difference.

Reported and relative inter laboratory variation
Table 10 summarizes standard deviation of potencies reported, relative to candidate standard HSV-1 sample C and relative to candidate standard HSV-2 sample D, for liquid frozen preparations of candidate standards HSV-1 samples A and HSV-2 sample B and liquid frozen clinical specimens of HSV-1 and HSV-2 samples E and F, along with the number of data sets. A clear reduction in standard deviation of samples A and B is seen when potencies are calculated relative to candidate standards samples C and D respectively compared to laboratory reported mean potencies. However the standard deviations of potencies for clinical samples increases when potencies are calculated relative to candidate standards samples C and D, with the exception of sample F. These results show that when candidate standard samples C and D are used as a standard, standard deviations of potencies for both HSV-1 and HSV-2 liquid frozen preparations and interlaboratory variability are reduced.

Reported and relative EQA panel bias in inter laboratory variation
The candidates were entered into an external quality assessment scheme run by QCMD, to be tested alongside a larger number of clinical isolates and by a larger number and range of assays. The HSV-1 candidate was distributed alongside 5 clinical isolates and tested using 237 assays of which 35 quantitative assay reported potencies were analysed, the HSV-2 candidate was distributed alongside 8 clinical isolates and tested using 195 assays of which 28 quantitative assays were analysed. Qualitative assay outputs were not included in the commutability assessment. Plots showing all bias estimates are given in Figure 4a-b and 5a-b, together with summaray statistics for the data shown. The plots and the reduction in inter-quartile range values for estimates expressed relative to the candidate standards (0.783 to 0.707 for HSV-1 candidate and 0.918 to 0.782 for HSV-2 candidate) illustrate an overall moderate improvement in harmonisation (less bias) across the participating laboratories and methods when each candidate is used as a standard.

Discussion

In this international multicenter collaborative study, a diverse range of commercial and laboratory developed NAT based assays for HSV-1 and HSV-2 have been used by laboratories across industry sectors to evaluate the suitability and potency of the two candidate standard preparations for establishment as the 1st WHO International Standard(s) for HSV NAT. The candidate standard lyophilised preparations 16/368 (sample C) and 17/122 (sample D) comprise tissue cell culture propagated HSV-1 strain 17 and HSV-2 strain HG52 respectively. Strains were selected because they are well characterized and both candidate preparations were formulated at high titer in universal buffer to allow subsequent dilution with sufficient range in appropriate sample matrix for this diagnostic clinical marker.

The panel comprised also of the liquid frozen preparations of the bulk materials used to prepare candidates 16/368 (sample A) and 17/122 (sample B) and clinical samples prepared by diluting a donated HSV-1 positive specimen of oral swab in UTM in negative UTM (sample E) and a

commercially sourced HSV-2 positive human serum specimen diluted in negative human serum (sample F). The overall mean estimate for the candidate 16/368 (sample C) reported by laboratories using qualitative assays was 6.39 Log_{10} NAT detectable units/mL and using quantitative assays was 7.58 Log_{10} copies/mL. Corresponding overall mean estimates for the candidate 17/122 (sample D) reported by laboratories using qualitative assays was 6.74 Log_{10} NAT detectable units/mL and using quantitative assays was 7.65 Log_{10} copies/mL. There is a discrepancy between qualitative assays and quantitative assays for high titre candidate standards of 1.19 and 0.91 Logs and for low titer clinical samples of 0.30 and 0.05 Logs, quantitative assay estimates are higher than qualitative assay estimates the difference is larger for higher potency samples.

Agreement between laboratories of reported mean potency estimates for high titer liquid frozen preparations (samples A and B) from qualitative and quantitative assays combined is improved when potencies are expressed relative to the candidates 16/368 and 17/122 (sample C and D). A wide variety of diverse commercial and laboratory developed diagnostic solutions in market with complex combinations of variable parameters, makes grouping assays for comparison difficult and meaningless, for example two labs may report a HSV-1 result using the same commercial amplification assay but different extraction assays, their measurements of potencies may be very different due to the different extraction assays. The reduction in standard deviation, a measure for inter-lab variability is similar for the high titer samples A and B, when potency is expressed relative to either candidate. The agreement between laboratories for low titer samples E and F for both qualitative and quantitative assay potency estimates is reduced or is similar when potency is expressed relative to the candidate sample C and D, this may be due to the assay, method and protocol specific variables and may not reflect on controllable suitability characteristics of this reference material preparation.

The ongoing accelerated thermal degradation study at 21 months of storage for HSV-1 candidate sample C and 24 months of storage for HSV-2 candidate sample D indicate minimal loss in potency on storage at recommended temperature of -20 ºC. There is a minimal drop or increase in titre observed for samples stored at +4 +20 +37 and +45 ºC compared to the -20 ºC baseline sample, the fluctuations in titer are within or close to standard deviations for sample replicates and may well represent vial to vial variation rather than reflect on inherent stability. The stability predictions indicate long term stability for both candidates when stored at recommended temperature of -20 ºC, however the reliability of the Arrhenius model used for prediction depends on the scale of observed trend for loss in titer with increasing temperature and time, with little to no loss observed this serves indication and comparison purposes.

Commutability, assessment within the confines of a multicenter collaboration has been limited to assessing variability between laboratories for a HSV-1 and a HSV-2 low titre pseudo samples, testing a more comprehensive panel of samples, evenly distributed across the range of quantitation for the different matrices using the different methodologies available hasn’t been possible, because significant additional resources would be required that are not always available. The candidates have been prepared in universal buffer to pragmatically control for matrix effect of commutability however this approach doesn’t consider genetic and biological differences of the analyte within the different bodily tissue compartments that are routinely tested. As previously commented the potencies reported for low titer clinical samples E and F are both are more variable or only marginally improved, when potency estimates from qualitative and quantitative assays combined are expressed relative to candidate samples C or D.

The collaborative study evaluations findings were presented to SOGAT in 2018 to seek input from a wide range of stakeholders, the community requested the establishment of both candidates, as independent calibrators of NAT assays for HSV-1 & 2, and requested further assessment of commutability for both candidates. . For the commutability assessment the candidates were entered into an external quality assessment scheme and tested alongside a large number of clinical isolates and in a range of assays. Laboratory reported and relative potency estimates for each sample from quantitative assays were compared for bias from study consensus values. An overall reduction in bias was observed when potencies were expressed as relative for both candidates.

Our sequencing analysis pipeline for the Illumina short read data called 15 SNVs in the HSV-1 candidate 16/368 (sample C) along with 3 indels at reference positions 40797, 50769 and 62142 in genes (US5:US6:US7), (UL24:UL25) and (intergenic) respectively. For the HSV-2 candidate 17/122 (sample D) 6 SNVs were identified, 3 were in repeat regions and 3 in gene regions. In addition to these an indel was called at position 48800, overlapping with gene UL24.
Long read MinION sequencing data for HSV-1 candidate (sample C) alignment resulted in complete coverage of the genome and no large-scale re-arrangements were detected. However, a structurally diverse tandem repeat region was identified with 3-4 of 10 repeats (10x TGGGTGGGTGGGGAG) deleted compared to the annotated NCBI reference JN555585.1 at position 143,716-143,868.

Proposal

It is proposed that the candidates for HSV-1 (NIBSC code: 16/368) and HSV-2 (NIBSC code: 17/122) are established as independent International Standards for HSV-1 and 2 NAT, with assigned potencies of 7.19 and 7.31 Log_{10} IU/vial respectively. The proposed standards are intended to be used by IVD manufacturers, regulatory, reference, clinical and laboratories to calibrate secondary reference materials to harmonize potency estimates from pan and differentiating HSV NAT based assays.

Comments from participants
This report has been circulated to all participants of the study for comment. Comments, corrections and minor editorial changes received were all addressed, implemented and included in this report. One participant commented that their laboratories data was not included for some samples, once outstanding queries were resolved, report was updated with reanalysis inclusive of this data.

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Table 1. Production summary for Candidates 16/368 and 17/122.

NIBSC code	**16/368**	**17/122**
Product name	HSV-1 DNA	HSV-2 DNA
Dates of production	12-May-17	27-Jul-17
Presentation	Freeze-dried preparation in 3 mL screw-cap glass vial	Freeze-dried preparation in 3 mL screw-cap glass vial
Appearance	Robust opaque cake	Robust opaque cake
No. of vials filled	6775	6941
Mean fill weight (g)	1.0068 (n=226)	1.0063 (n=232)
CV of fill weight (%)	0.243	0.266
Mean residual moisture (%)	0.24 (n=12)	0.10 (n=11)
CV of residual moisture (%)	37.00	26.60
Mean oxygen content (%)	0.95 (n=12)	0.90 (n=12)
CV of oxygen content (%)	11.30	6.46
No. of vials available to WHO	6663	6699

Table 2a. Stability of 16/368 at 21 months.

Temperature (°C)	Mean Log_{10} units/mL 21 months	Difference in Log_{10} units/mL from -20 °C baseline
-20	7.270	
4	7.296	0.026
20	7.285	0.015
37	7.270	0.000
45	7.475	0.205

Table 2b. Stability of 17/122 at 24 months.

Temperature (°C)	Mean Log_{10} units/mL 24 months	Difference in Log_{10} units/mL from -20 °C baseline
-20	7.003	
4	7.012	0.009
20	7.030	0.027
37	7.333	0.330
45	7.346	0.343

Table 3a. Collaborative study NAT assay methods.

Assay code	**NAT method**	**HSV gene target**	**No data sets**	**Lab codes**
Qualitative				
RSH	AltoStar HSV PCR Kit 1.5 (altona Diagnostics GmbH)	NA	1	5
BPH	Bosphore® HSV Quantification Kit v1 (Anatolia Geneworks)	gD gene	1	24
FTD	FTD-7 Vesicular rash (Multiplex detection Taqman technology, Fast track diagnostics)	HSV1: Glycoprotein B (gB) gene, HSV2: Glycoprotein D gene	1	7
GPR	GeneProof Herpes Simplex Virus (HSV-1/2) PCR Kit	Glycoprotein B (gB) gene	2	4, 14
DHD	H-DiaHSVTM (Diagenode Diagnostics)	Glycoprotein B (UL 27) gene	1	19
SBP	Herpes Simplex Virus Type 1/2 DNA Fluorescence Diagnostic Kit (PCR-Fluorescence Probing, Sansure)	US5	1	8c

SSB	HSV 1 and 2 DNA qPCR Detection Kit (Promega)	DNA polymerase gene	1	8b
SBT	HSV I & II Typing Real Time PCR Kit (Liferiver)	UL27	2	6, 8a
Quantitative				
RGB	HSV1 HSV2 VZV R-gene® (assay under development, ref.: 69-014B – bioMérieux)	HSV1: US7 gene, HSV2: US27 gene	1	11b.a-f
RGA	HSV1 HSV2 VZV R-gene® (ref.: 69-004B – bioMérieux)	HSV1: US7 gene, HSV2: US2 gene	1	11a.a-d
KPX	kPCR PLX Herpes Simplex Virus 1 and 2 (HSV) DNA Assay (Siemen's)		1	26
LD	Laboratory developed assay.	UL4, UL27, UL30, gG, gD, gB, Pol genes	9	2, 9, 12, 13, 16, 21, 22, 25, 27, 29
LM1	LightMix® Kit Modular HSV-1 Cat.-No. 53-0135-96 Lot No: 42011701	Glycoprotein G gene	1	23c.a
LM2	LightMix® Kit Modular HSV-2 Cat.-No. 64-0136-96 Lot No: 42021701	Glycoprotein D gene	1	23c.b
LME	LightMix® Kit for the detection of HSV-1/2 (EC), Ref No: 40-0378-32 (Roche)	DNA polymerase gene	1	23a
LMM	LightMix® Modular Herpes-simplex Virus-1/2 Cat.-No. 53-0133-96 Lot No.: 35711702	DNA Polymerase gene	1	23b
NQ12	Q-Alert Real time kit (Nanogen)	HSV1: gD gene, HSV2: gG gene	1	18
RSA	RealStar® alpha Herpesvirus PCR (Altona Diagnostics GmbH)	5' UTR	1	3
STR	Sacace HSV I/II Typing Real-TM (ref. V38-100FRT)	Glycoprotein B (gB) gene	1	10
C48	The cobas® HSV 1 and 2 test on the cobas 4800		1	15

Table 3b. Collaborative study Extraction assay methods.

Assay code	**Extraction method**	**Frequency**	**Lab codes**
ASP	AltoStar Purification Kit 1.5 (altona Diagnostics GmbH)	1	5
QCM	Customized midi kit (Qiagen)	1	13
DSB	DNA Sorb B, Ref K-1-1/B (Sacace)	1	10
BNG	Generic (bioMerieux)	1	27
KBL	HSV I & II Typing Real Time PCR Kit (Liferiver)	2	6, 8a
IIP	iPrep Pure Link Virus Kit (Invitrogen)	1	21

GPP	Magnapure 96 DNA and Viral NA Large volume kit (Qiagen; 06374891001)	2	4, 14
RMP	Magnapure 96 DNA and Viral NA Small volume kit (Qiagen)	5	18, 19, 23a, 23b, 23c.a-b
MRV	Magrev® Viral DNA/RNA Extraction Kit (Anatolia Geneworks)	1	24
KBS	Multi-type Sample DNA/RNA Extraction-Purification Kit (Sansure)	1	8c
BNE	NucliSENS® (bioMerieux)	6	2, 3, 7, 11a.a-d, 11b.a-f, 12
QAM	QIAamp MinElute Virus Spin Kit (Qiagen)	1	8b
QAB	QiAmp DNA Blood Mini Kit (Qiagen)	3	9, 16, 25
QDS	QIAsymphony DSP Virus/pathogen kit (Qiagen)	1	22
SKP	Siemens' nucleic acid extraction technology	1	26
BNS	Specific A 3.0.4 (bioMerieux)	1	29
C48	The cobas® HSV 1 and 2 test on the cobas 4800 (Roche)	1	15

Table 3c. Collaborative study Extraction assay diluents used.

Diluent	**Frequency**	**Lab codes**
Cerebrospinal Fluid (CSF)	2	11a.a-d, 11b.a-c
Phosphate buffered saline (PBS)	4	14, 16, 18, 19
Plasma	4	5, 7, 11b.d-f, 25
Serum	3	6, 9, 24
Transport Medium (Universal, Viral, Swab)	16	2, 3, 4, 6, 7, 10, 12, 15, 21, 22, 23a, 23b, 23c.a-b, 25, 27, 29
Water (Deionised, nuclease-free, molecular PCR-grade)	5	8a, 8b, 8c, 13, 26

Table 4. Laboratory mean estimates from quantitative assays (Log_{10} copies/mL) and qualitative assays (Log_{10} NAT detectable units/mL). Qualitative results are shaded in grey.

Lab code	**Method**	**Diluent**	**Sample code**					
			A	**B**	**C**	**D**	**E**	**F**
02	Qualitative	Transport Medium	5.80	6.53	5.88	6.72	3.98	3.98
03	Qualitative		7.76	6.76	6.76	7.37	4.76	4.76
07	Qualitative	Transport Medium, Plasma	6.53	7.49	6.62	7.14	4.73	4.45
08b	Qualitative	Water	6.46	6.46	6.46	6.46	4.46	3.82
08c	Qualitative	Water	5.85	5.00	5.59	6.00	4.05	3.94
10	Qualitative	Transport Medium	6.82	7.42	7.14	7.14	3.76	3.91
12	Qualitative	Transport Medium	7.30	6.70	5.34	7.59		

15a	Qualitative	Transport Medium	7.79	4.29		5.34	5.02	
15b	Qualitative	Transport Medium	5.29			6.82		4.12
18	Qualitative	PBS	6.53	6.68	6.07	5.97		
19	Qualitative	PBS	7.30	7.76	7.02	7.15	5.11	4.08
22	Qualitative	Transport Medium	6.75	7.28	7.04	7.19		
04	Quantitative	Transport Medium	7.88	8.13	8.32	8.22	4.15	4.08
05	Quantitative	Plasma	6.90	7.14	7.05	7.34	4.92	4.30
06	Quantitative	Transport Medium, Serum	6.25	6.67	6.20	5.99	3.97	3.16
08a	Quantitative	Water	7.76	8.28	7.77	8.25	4.78	4.28
09	Quantitative	Serum	7.05	6.83	7.19	7.66	4.28	4.07
11a	Quantitative	Plasma	7.70	7.87	7.97	7.66	4.39	4.28
11b	Quantitative	Plasma	7.50	7.47	7.69	7.73	5.01	4.42
11c	Quantitative	Plasma	7.61	7.64	7.69	7.61	5.02	4.39
13	Quantitative	Water	8.12	8.05	7.76	8.07	5.14	4.70
14	Quantitative	PBS	7.01	7.29	7.29	7.27	4.41	3.17
16	Quantitative	PBS	7.35	6.61	7.42	6.83	4.08	3.30
21	Quantitative	Transport Medium	7.40	7.67	7.75	7.66	5.32	4.16
23a	Quantitative	Transport Medium	6.77	7.76	7.39	7.58	4.87	3.81
23b	Quantitative	Transport Medium	7.28	8.01	7.58	7.84	5.18	4.47
23c	Quantitative	Transport Medium	7.71	8.45	7.98	8.05	5.39	4.73
24	Quantitative	Serum	7.54	7.92	7.64	7.66	4.86	4.26
25	Quantitative	Transport Medium	7.89	7.87	7.91	7.80	4.62	4.14
26	Quantitative	Water	7.82	8.32	7.95	8.22	5.31	4.84
27	Quantitative	Transport Medium	7.62	7.68	7.65	7.87	5.07	4.56
29	Quantitative	Transport Medium	7.39	7.60	7.46	7.76	4.87	4.61

Table 5. Overall mean estimates and inter-laboratory variation (Log_{10} copies/mL) for quantitative assays (Log_{10} NAT detectable units/mL) for qualitative assays.

Sample	No. of datasets	Mean	Range	SD
Qualitative				
A: 16/368 Candidate HSV1 Liquid Bulk	12	6.68	6.77 to 7.89	0.78
B: 17/122 Candidate HSV2 Liquid Bulk	12	6.58	7.46 to 8.45	1.06
C: 16/368 Candidate HSV1 Freeze dried	12	6.39	7.39 to 7.98	0.64
D: 17/122 Candidate HSV2 Freeze dried	12	6.74	7.58 to 8.25	0.67
E: Clinical Sample UTM HSV-1	12	4.48	4.39 to 5.39	0.50
F: Clinical Sample Serum HSV-2	12	4.13	3.81 to 4.84	0.32
Quantitative				
A: 16/368 Candidate HSV1 Liquid Bulk	20	7.43	5.29 to 8.12	0.45
B: 17/122 Candidate HSV2 Liquid Bulk	19	7.66	4.29 to 8.13	0.53
C: 16/368 Candidate HSV1 Freeze dried	18	7.58	5.34 to 8.32	0.44

D: 17/122 Candidate HSV2 Freeze dried	20	7.65	5.34 to 8.22	0.52
E: Clinical Sample UTM HSV-1	16	4.78	3.76 to 5.32	0.43
F: Clinical Sample Serum HSV-2	16	4.19	3.16 to 4.76	0.49
Combined				
A: 16/368 Candidate HSV1 Liquid Bulk	32	7.15	5.29 to 8.12	0.69
B: 17/122 Candidate HSV2 Liquid Bulk	31	7.28	4.29 to 8.45	0.91
C: 16/368 Candidate HSV1 Freeze dried	30	7.19	5.34 to 8.32	0.76
D: 17/122 Candidate HSV2 Freeze dried	32	7.31	5.34 to 8.25	0.73
E: Clinical Sample UTM HSV-1	28	4.70	3.76 to 5.39	0.47
F: Clinical Sample Serum HSV-2	28	4.17	3.16 to 4.84	0.44

Table 6. Laboratory estimates of potency for samples A, E and F expressed relative to HSV-1, 16/368 (Sample C), Log_{10} proposed IU/ml. Results from qualitative assays shaded in grey.

Lab code	Method	Diluent	Sample code		
			A	E	F
02	Qualitative	Transport Medium	7.11	5.29	5.29
03	Qualitative		8.19	5.19	5.19
07	Qualitative	Transport Medium, Plasma	7.10	5.30	5.02
08b	Qualitative	Water	7.19	5.19	4.55
08c	Qualitative	Water	7.45	5.65	5.54
10	Qualitative	Transport Medium	6.87	3.81	3.96
12	Qualitative	Transport Medium	9.15		
15a	Qualitative	Transport Medium			
15b	Qualitative	Transport Medium			
18	Qualitative	PBS	7.65		
19	Qualitative	PBS	7.47	5.28	4.25
22	Qualitative	Transport Medium	6.90		
04	Quantitative	Transport Medium	6.97	3.34	3.28
05	Quantitative	Plasma	7.04	5.04	4.43
06	Quantitative	Transport Medium, Serum	7.05	4.78	4.23
08a	Quantitative	Water	7.17	4.20	3.75
09	Quantitative	Serum	6.65	4.28	3.72
11a	Quantitative	Plasma	6.97	3.74	3.65
11b	Quantitative	Plasma	7.05	4.63	4.14
11c	Quantitative	Plasma	7.10	4.58	4.08
13	Quantitative	Water	7.72	4.70	4.47
14	Quantitative	PBS	6.83	4.28	3.35
16	Quantitative	PBS	7.14	3.91	3.34
21	Quantitative	Transport Medium	6.97	4.56	3.82
23a	Quantitative	Transport Medium	6.97	4.67	3.99
23b	Quantitative	Transport Medium	7.01	4.62	4.12
23c	Quantitative	Transport Medium	7.01	5.53	4.16
24	Quantitative	Serum	7.07	4.48	3.94
25	Quantitative	Transport Medium	7.12	3.74	3.20
26	Quantitative	Water	6.82	4.55	4.09

27	Quantitative	Transport Medium	7.20	4.59	4.08
29	Quantitative	Transport Medium	7.13	4.59	4.59

Table 7. Overall mean estimates and inter-laboratory variation of potency for samples A, E and F relative to HSV-1, 16/368 (Sample C), Log_{10} proposed IU/ml.

Sample	**No. of datasets**	**Mean**	**Range**	**SD**
Qualitative				
A: 16/368 Candidate HSV1 Liquid Bulk	10	7.51	6.87 to 9.15	0.70
E: Clinical Sample UTM HSV-1	7	5.10	3.81 to 5.65	0.59
F: Clinical Sample Serum HSV-2	7	4.83	3.96 to 5.54	0.58
Quantitative				
A: 16/368 Candidate HSV1 Liquid Bulk	20	7.05	6.65 to 7.72	0.20
E: Clinical Sample UTM HSV-1	20	4.44	3.34 to 5.53	0.49
F: Clinical Sample Serum HSV-2	20	3.92	3.20 to 4.59	0.40
Combined				
A: 16/368 Candidate HSV1 Liquid Bulk	30	7.20	6.65 to 9.15	0.48
E: Clinical Sample UTM HSV-1	27	4.61	3.34 to 5.65	0.58
F: Clinical Sample Serum HSV-2	27	4.16	3.20 to 5.54	0.60

Table 8. Laboratory estimates of potency for samples B, E and F relative to HSV-2, 17/122 (Sample D), Log_{10} proposed IU/ml. Results from qualitative assays shaded in grey.

Lab code	**Method**	**Diluent**	**Sample code**		
			B	**E**	**F**
02	Qualitative	Transport Medium	7.12	4.57	4.57
03	Qualitative		6.70	4.70	4.70
07	Qualitative	Transport Medium, Plasma	6.70	4.90	4.62
08b	Qualitative	Water	7.31	5.31	4.67
08c	Qualitative	Water	6.31	5.36	5.25
10	Qualitative	Transport Medium	7.59	3.93	4.08
12	Qualitative	Transport Medium	6.26	6.99	
15a	Qualitative	Transport Medium			4.61
15b	Qualitative	Transport Medium	6.42		
18	Qualitative	PBS	8.02		
19	Qualitative	PBS	7.92	5.27	4.24
22	Qualitative	Transport Medium	7.40		
04	Quantitative	Transport Medium	7.25	3.50	3.46
05	Quantitative	Plasma	7.06	5.00	4.25
06	Quantitative	Transport Medium, Serum	7.74	5.04	4.48
08a	Quantitative	Water	7.37	4.05	3.60
09	Quantitative	Serum	6.61	3.88	4.01
11a	Quantitative	Plasma	7.46	4.10	4.01
11b	Quantitative	Plasma	7.10	4.52	4.06

11c	Quantitative	Plasma	7.35	4.61	4.15
13	Quantitative	Water	7.27	4.34	4.04
14	Quantitative	PBS	7.39	4.39	3.27
16	Quantitative	PBS	7.17	4.47	3.99
21	Quantitative	Transport Medium	7.31	4.76	3.97
23a	Quantitative	Transport Medium	7.57	4.66	4.06
23b	Quantitative	Transport Medium	7.42	4.56	4.02
23c	Quantitative	Transport Medium	7.59	4.55	4.09
24	Quantitative	Serum	7.50	4.57	4.06
25	Quantitative	Transport Medium	7.27	4.51	4.13
26	Quantitative	Water	7.34	4.54	4.16
27	Quantitative	Transport Medium	6.96	4.33	3.87
29	Quantitative	Transport Medium	7.07	4.36	3.31

Table 9. Overall mean estimates and inter-laboratory variation of potency for samples B, E and F relative to HSV-2, 17/122 (Sample D), Log_{10} proposed IU/ml.

Sample	**No. of datasets**	**Mean**	**Range**	**SD**
Qualitative				
B: 17/122 Candidate HSV2 Liquid Bulk	11	7.07	6.26 to 8.02	0.63
E: Clinical Sample UTM HSV-1	8	5.13	3.93 to 6.99	0.89
F: Clinical Sample Serum HSV-2	8	4.59	4.08 to 5.25	0.35
Quantitative				
B: 17/122 Candidate HSV2 Liquid Bulk	20	7.29	6.61 to 7.74	0.25
E: Clinical Sample UTM HSV-1	20	4.44	3.50 to 5.04	0.36
F: Clinical Sample Serum HSV-2	20	3.95	3.27 to 4.48	0.31
Combined				
B: 17/122 Candidate HSV2 Liquid Bulk	31	7.21	6.26 to 8.02	0.43
E: Clinical Sample UTM HSV-1	28	4.63	3.50 to 6.99	0.63
F: Clinical Sample Serum HSV-2	28	4.13	3.27 to 5.25	0.43

Table 10. Overall mean estimates inter-laboratory variation for reported potency and relative to Sample C and D.

Sample	**SD Relative Sample C: 16/368**	**SD Relative Sample D: 17/122**	**SD Reported Raw data potency**	**No. of datasets (raw)**
Qualitative				
A: 16/368 Candidate HSV1 Liquid Bulk	0.70	0.63	0.78	12
B: 17/122 Candidate HSV2 Liquid Bulk	0.55	0.97	1.06	12
E: Clinical Sample UTM HSV-1	0.59	0.89	0.50	12
F: Clinical Sample Serum HSV-2	0.58	0.35	0.32	12
Quantitative				
A: 16/368 Candidate HSV1 Liquid Bulk	0.20	0.25	0.45	20

B: 17/122 Candidate HSV2 Liquid Bulk	0.41	0.38	0.53	19
E: Clinical Sample UTM HSV-1	0.49	0.36	0.43	16
F: Clinical Sample Serum HSV-2	0.40	0.31	0.49	16
Combined				
A: 16/368 Candidate HSV1 Liquid Bulk	0.48	0.43	0.69	32
B: 17/122 Candidate HSV2 Liquid Bulk	0.48	0.63	0.91	31
E: Clinical Sample UTM HSV-1	0.58	0.43	0.47	28
F: Clinical Sample Serum HSV-2	0.60	0.66	0.44	28

Figures 1a and 1b. Laboratory reported mean estimates of samples C and D from quantitative and qualitative assays (Log_{10} IU/mL for qualitative assays and Log_{10} NAT-detectable units for qualitative assays). Each box labeled with a laboratory code. Results from qualitative assays are coloured red.

Figures 2a-b. Laboratory mean estimate calculated relative to the candidate for HSV-1, 16/368 (Sample C) from quantitative and qualitative assay. Units are Log_{10} proposed IU/mL. Each box labeled with a laboratory code. Results from qualitative assays are coloured red.

Figures 3a-b. Laboratory mean estimate calculated relative to the candidate for HSV-2, 17/122 (Sample D) from quantitative and qualitative assay. Units are Log10 proposed IU/mL. Each box labeled with a laboratory code. Results from qualitative assays are coloured red.

Figures 4a-b. Individual value and box plots showing all bias estimates, and estimates of the inter-quartile range are summarized for HSV-1 candidate 16/368. All study bias estimates for samples (reported and relative, as difference in log values from study median shown as a) individual value plot and b) boxplot

Figures 5a-b. Individual value and box plots showing all bias estimates, and estimates of the inter-quartile range are summarized for HSV-2 candidate 17/122. All study bias estimates for samples (reported and relative, as difference in log values from study median shown as a) individual value plot and b) boxplot

Figures 6a-c. Sequence analysis data from short and long reading frames.

Figure 1a: Histogram of A raw (Log_{10} Units)

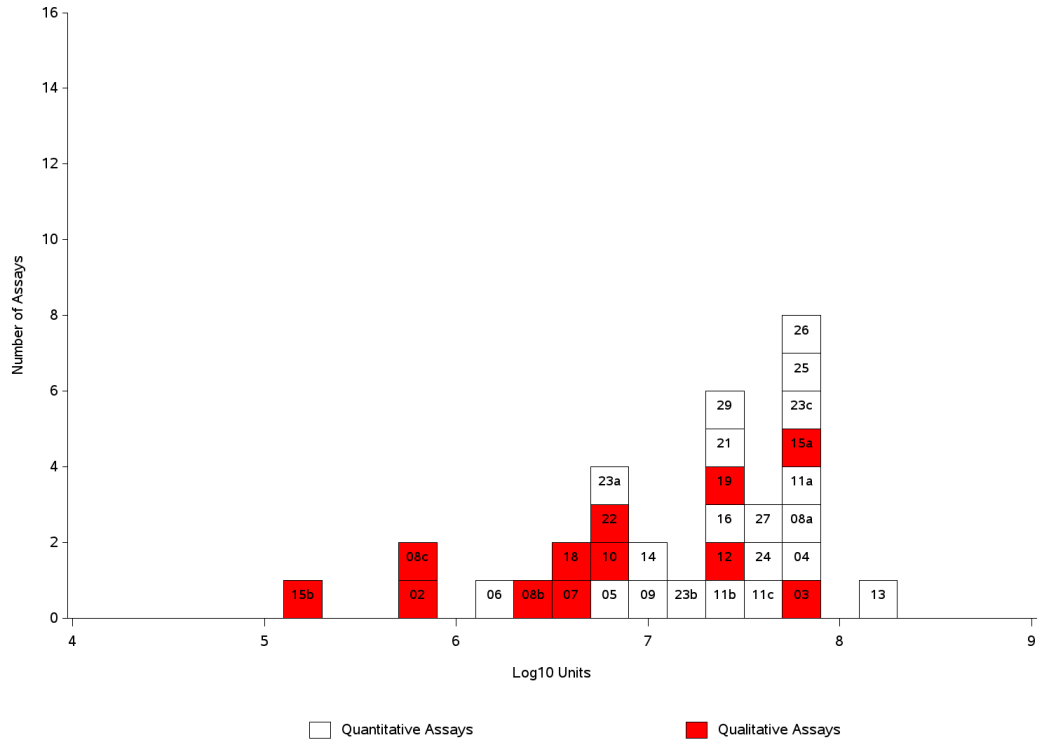


Figure 1b: Histogram of B raw (Log_{10} Units)

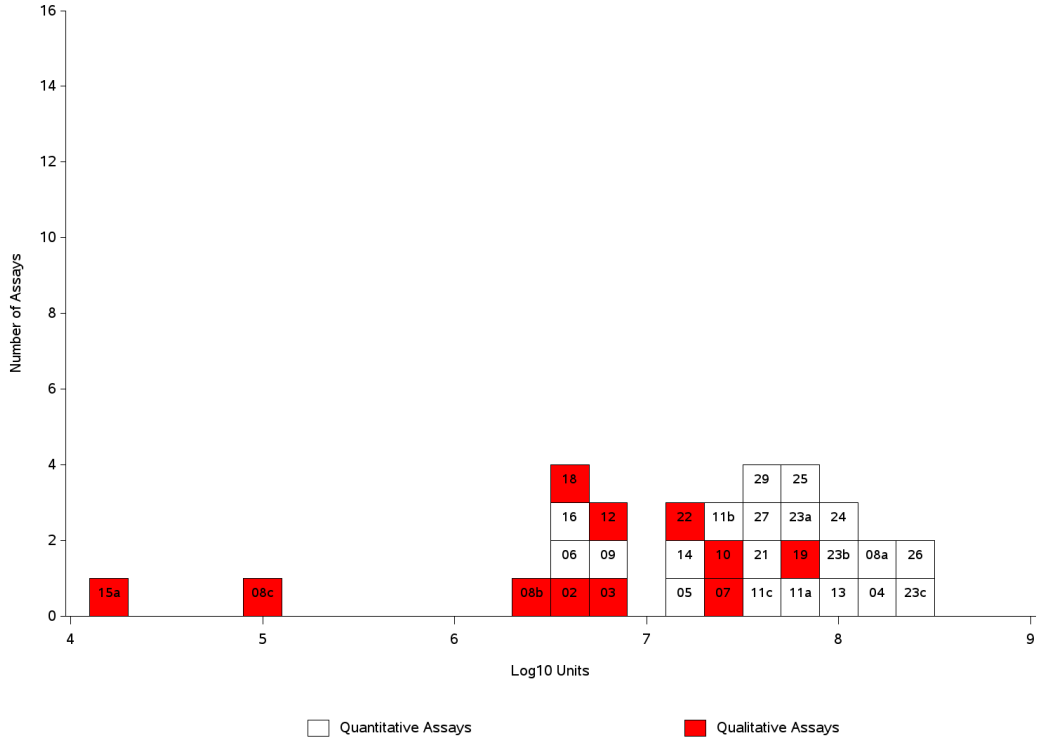


Figure 2a: Histogram of A relative to C (Log_{10} Units)

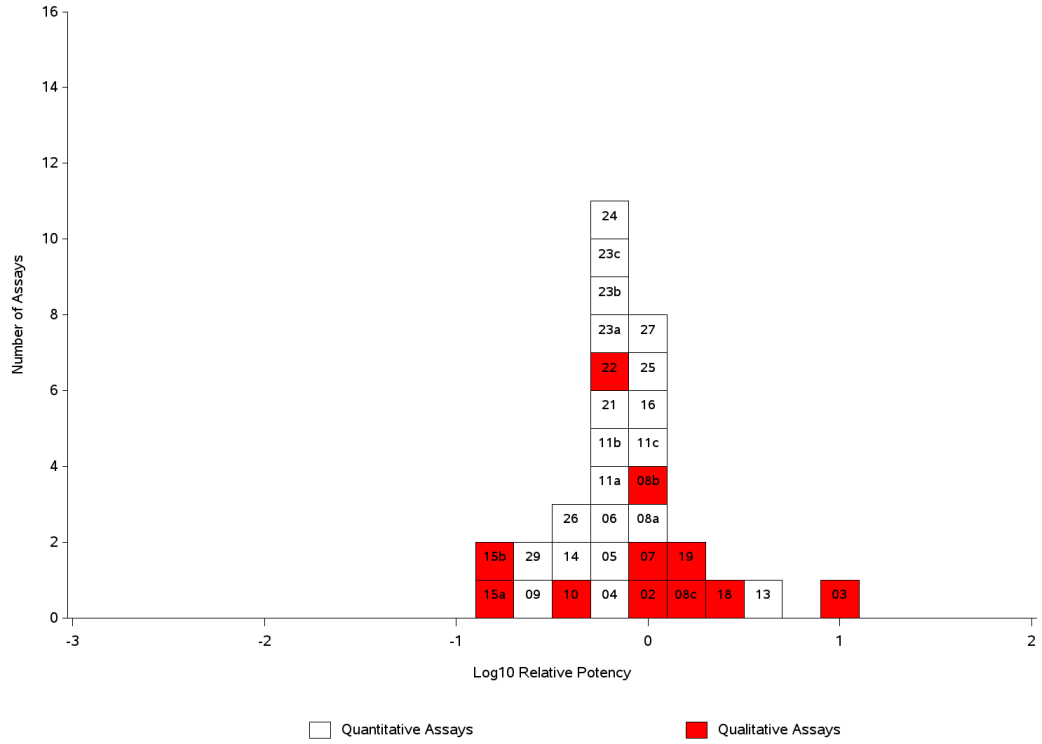


Figure 2b: Histogram of B relative to C (Log_{10} Units)

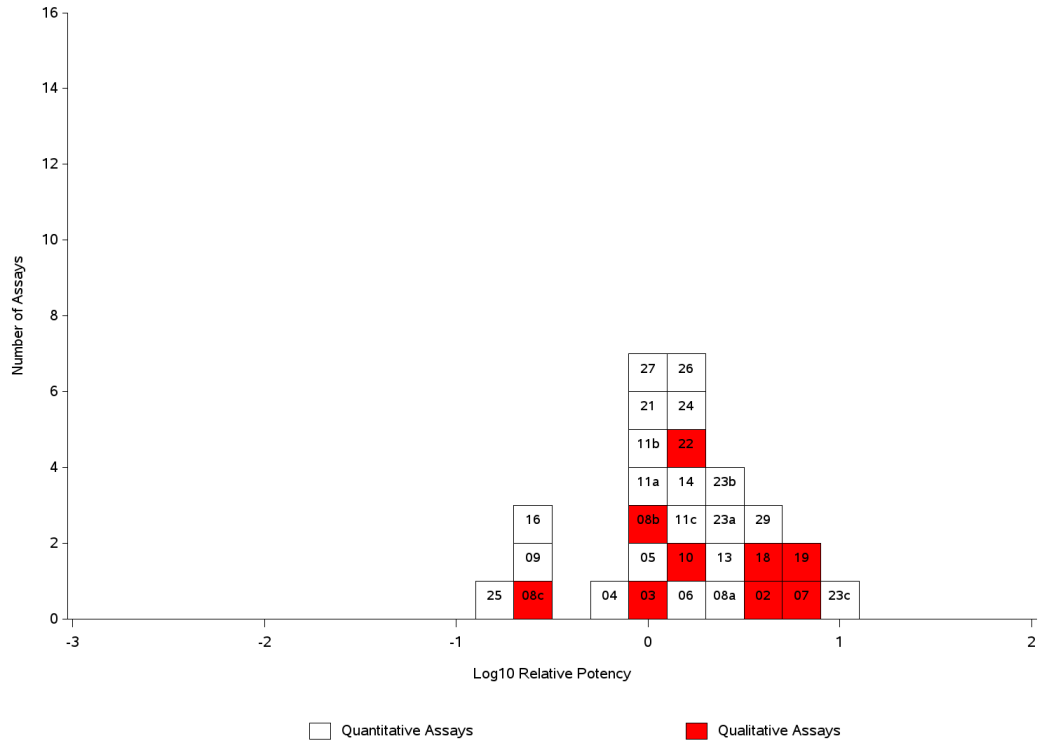


Figure 3a: Histogram of A relative to D (Log_{10} Units)

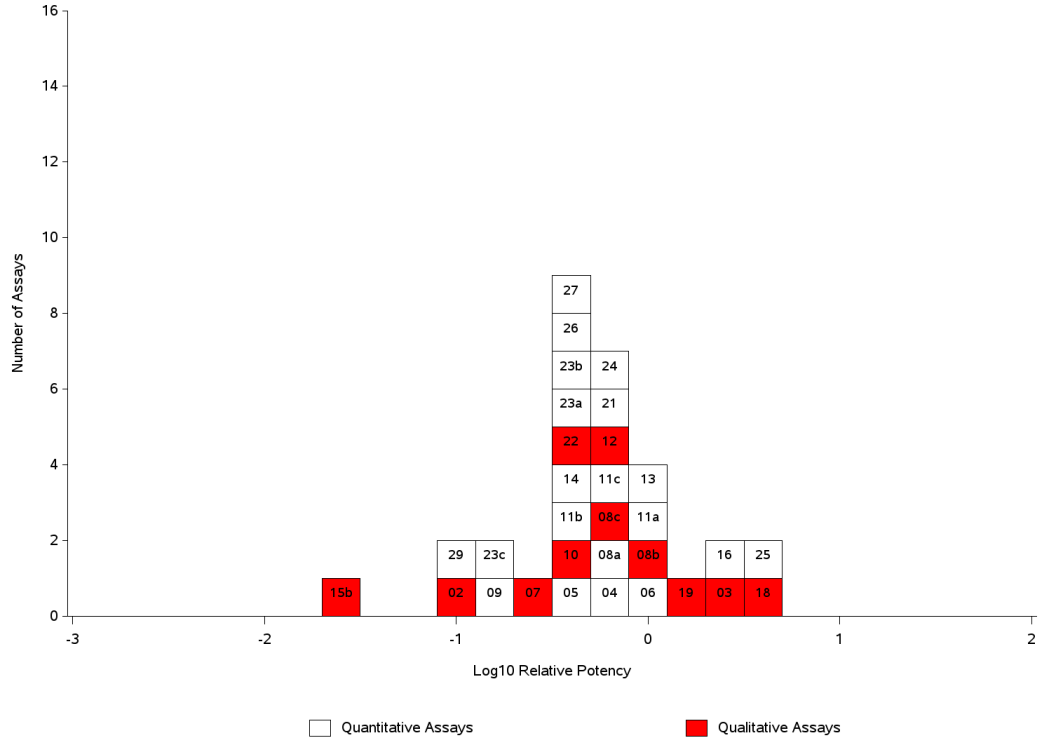


Figure 3b: Histogram of B relative to D (Log_{10} Units)

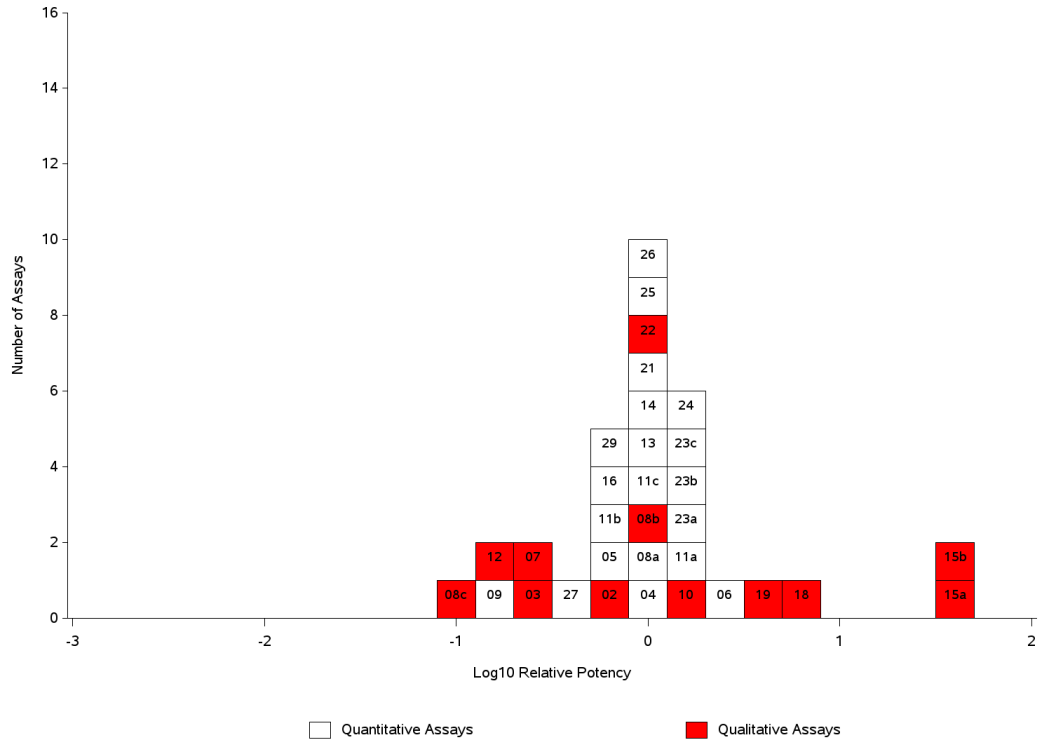
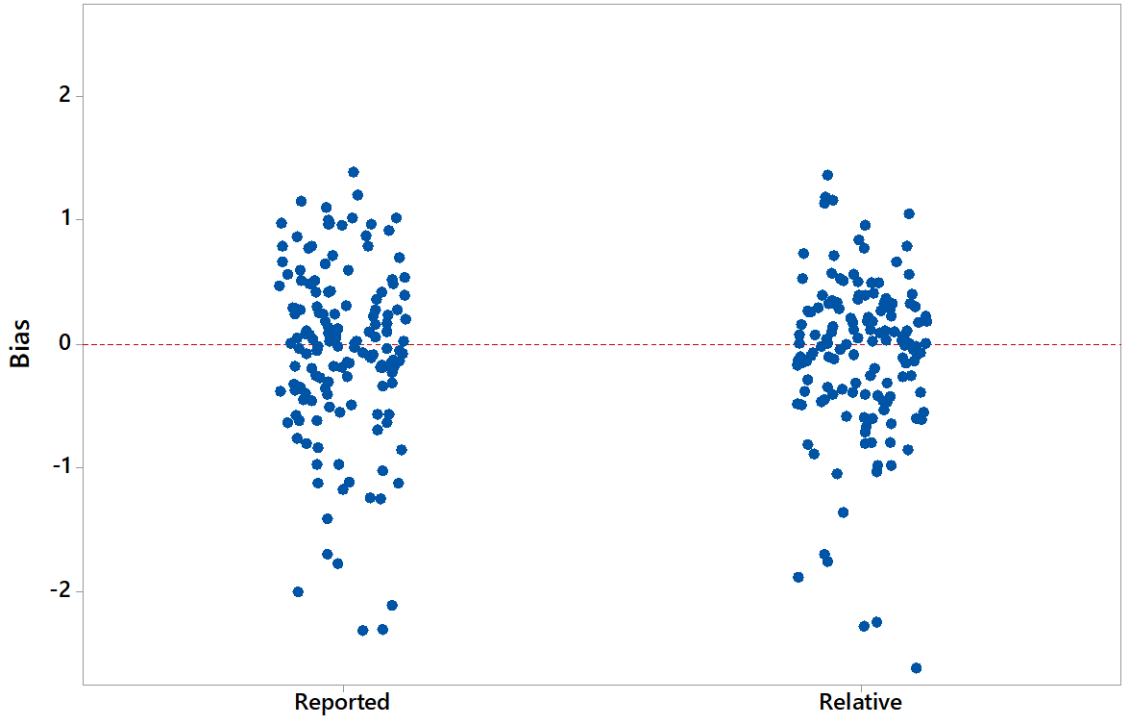
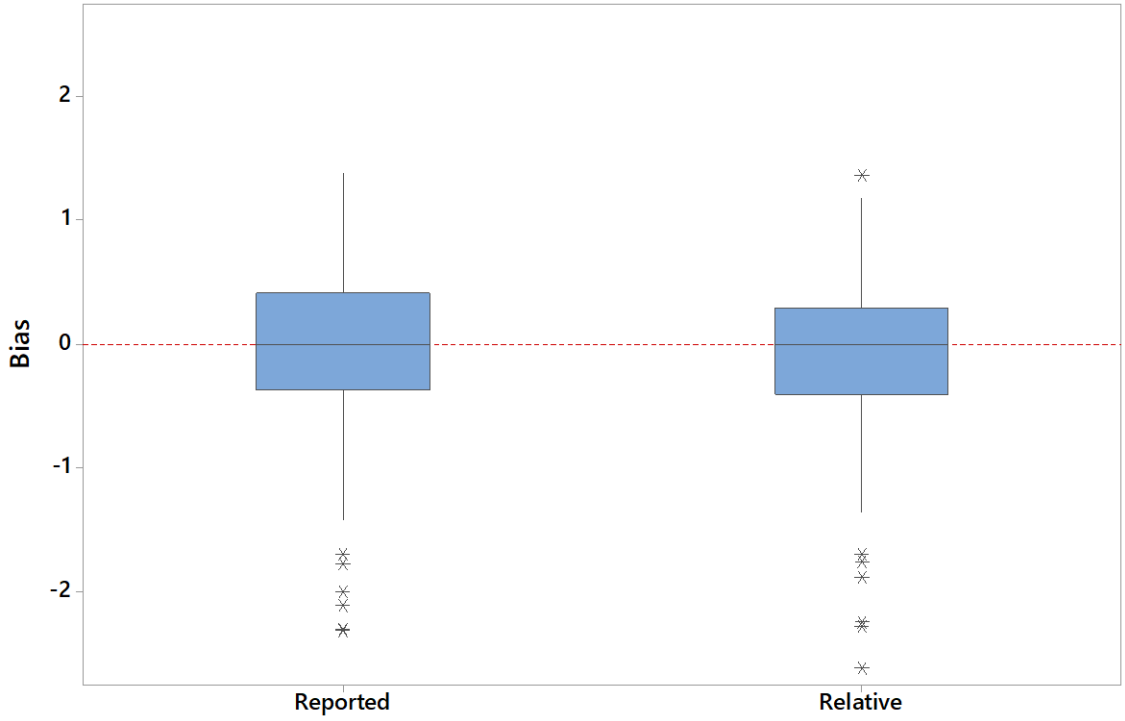


Figure 4a-b: Bias estimate reported and relative to HSV-1 candiadate, a) individual value plot and b) boxplot

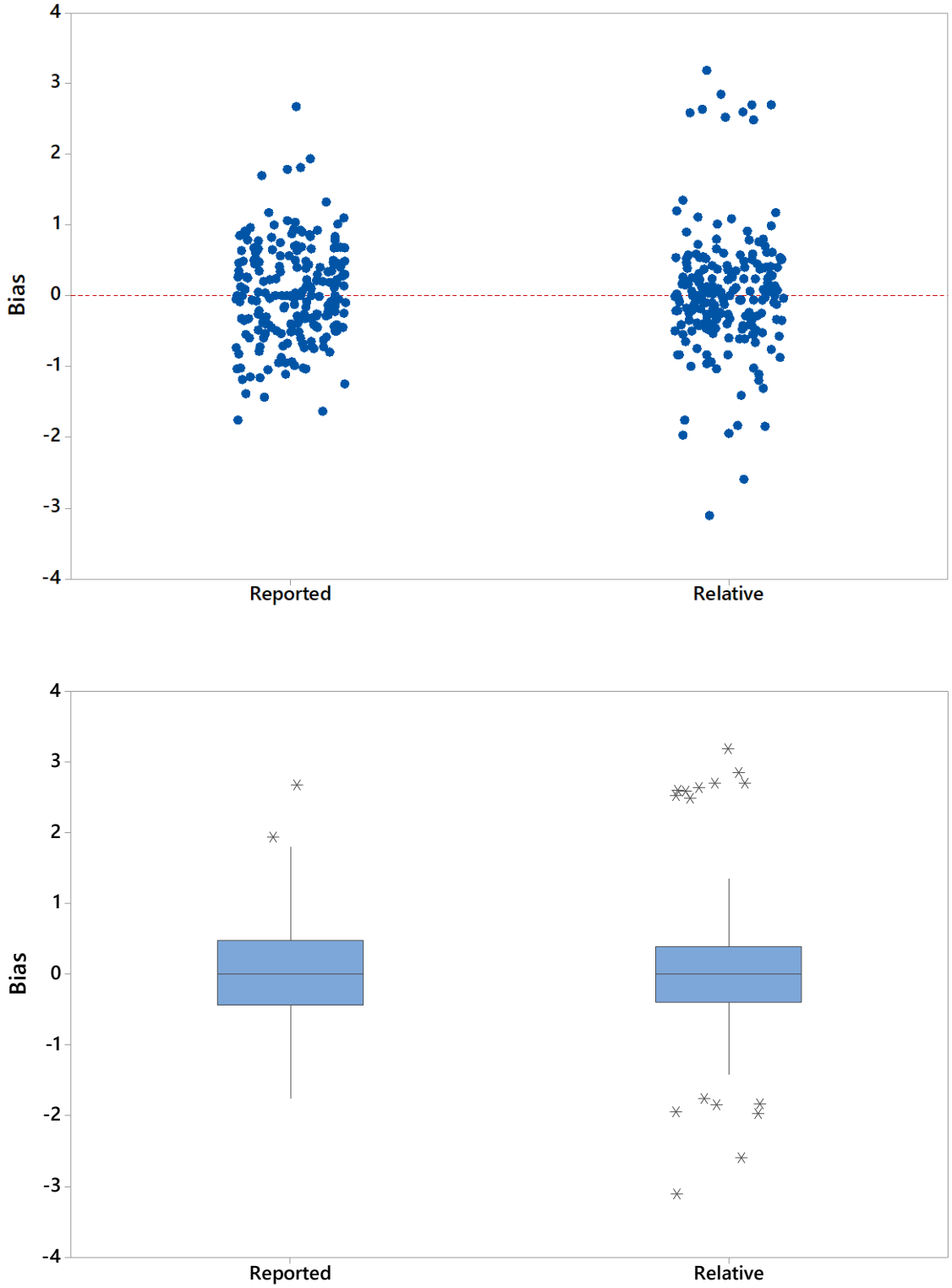




Summary Statistics for Bias

Method	Lower Quartile	Upper Quartile	Inter-Quartile
Reported in Log	-0.373	0.410	0.783
Relative in Log	-0.408	0.299	0.707

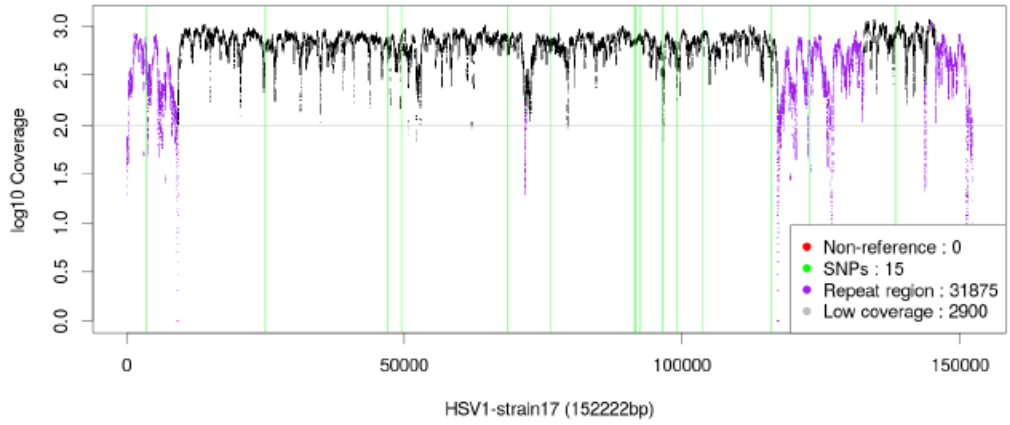
Figure 5a-b: Bias estimate reported and relative to HSV-2 candiadate, a) individual value plot and b) boxplot.



Summary Statistics for Bias

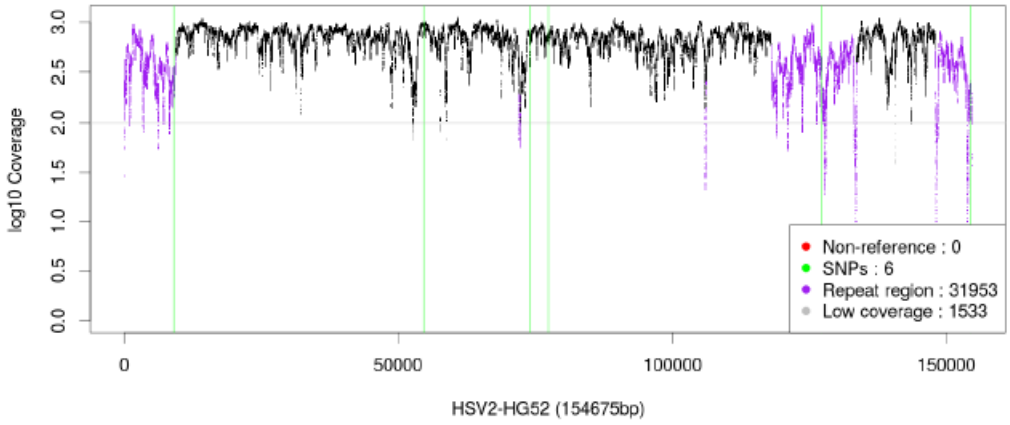
Method	Lower Quartile	Upper Quartile	Inter-Quartile
Reported	-0.436	0.482	0.918
Relative	-0.390	0.392	0.782

Figure 6a: Short-read alignment of HSV-1 strain 17 indicates 15 SNP variants.



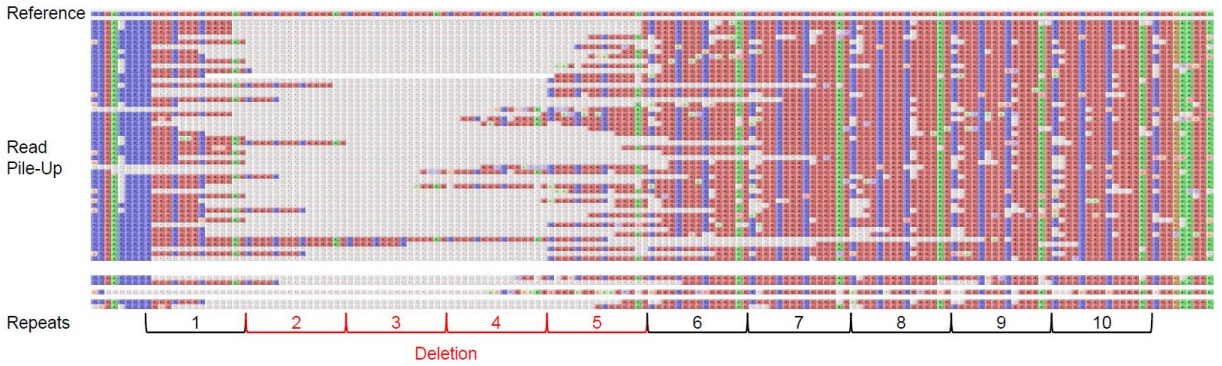
	chr	pos	ref	cov	pA	pC	pG	pT	repeats	genes
1	NC_001806.2	3497	C	732	37.40	62.60	0.00	0.00	repeat region	LAT,RL2
2	NC_001806.2	24978	C	621	0.00	80.00	0.00	20.00	-	UL14,UL13,UL12,UL11
3	NC_001806.2	46927	G	621	21.60	0.00	78.40	0.00	-	UL23
4	NC_001806.2	49496	A	747	74.20	0.00	25.70	0.10	-	UL24,UL25
5	NC_001806.2	68539	G	805	42.70	0.00	57.30	0.00	-	UL32
6	NC_001806.2	76283	G	546	22.20	0.00	77.80	0.00	-	UL36
7	NC_001806.2	91484	G	699	0.00	0.00	76.70	23.30	-	UL41
8	NC_001806.2	91722	C	682	0.00	80.90	0.00	19.10	-	UL41
9	NC_001806.2	92322	C	797	0.00	79.00	0.00	21.00	-	UL41
10	NC_001806.2	96481	G	324	50.00	0.00	50.00	0.00	-	UL44
11	NC_001806.2	99115	G	645	19.10	0.00	80.90	0.00	-	UL47,UL46
12	NC_001806.2	103824	G	647	22.10	0.00	77.90	0.00	-	UL48
13	NC_001806.2	115922	C	561	58.30	41.70	0.00	0.00	-	UL55
14	NC_001806.2	122877	G	710	0.00	0.00	58.00	42.00	repeat region	LAT,RL2
15	NC_001806.2	138345	C	790	0.00	80.60	0.00	19.40	-	US5,US6

Figure 6b: Short-read alignment of HSV-2 strain HG52 indicates 6 SNP variants.



	chr	pos	ref	cov	pA	pC	pG	pT	repeats	genes
1	NC_001798.2	9016	C	269	0.00	59.50	0.00	40.50	repeat region	-
2	NC_001798.2	54700	C	973	0.00	65.20	0.00	34.80	-	UL28,UL27
3	NC_001798.2	74138	T	485	0.00	25.20	0.00	74.80	-	UL36
4	NC_001798.2	77375	C	735	0.00	79.00	0.00	21.00	-	UL36
5	NC_001798.2	127310	A	423	39.70	0.00	60.30	0.00	repeat region	LAT
6	NC_001798.2	154432	T	260	0.00	60.00	0.00	40.00	repeat region	-

Figure 6c: Long-read alignment of HSV-1 strain 17, indicates structurally variable tandem repeat region at position 143,716-143,868.



Appendix 1

Main collaborative study participants

(In alphabetical order by country)

Name	Laboratory	Country
Dr. Cristina Videla, Dr. Alfredo Martinez	Clinical Virology laboratory, Clinical Analysis Department, CEMIC, Buenos Aires	Argentina
Dr. Chuntao Zhang	National Institutes for Food and Drug Control, China. Division II of In Vitro Diagnostic Reagents for Infectious Disease, Beijing	China
Dr. Walter Zhang	Shanghai ZJ Bio-Tech Co., Ltd., Shanghai	China
Dr. Christina Christodoulou	Molecular Virology Department, The Cyprus Institute of Neurology and Genetics, Ayios Dometios Nicosia	Cyprus
Dr. Petra Švástová	GeneProof a. s. Brno	Czech Republic
Dr. Come Barranger, Dr. Audrey Delariviere	BioMerieux SA, Verniolle	France
Dr. Jerome Le Goff	Laboratoire de microbiologie, Hôpital Saint-Louis, APHP, Paris	France
Dr. Alke Heitmann	Altona Diagnostics GmbH, Hamburg	Germany
Dr. Christina Hellriegel	Institute of Virology, University of Cologne, Koeln	Germany
Dr. Pranav Patel	TIB Molbiol GmbH, R&D Laboratories, Berlin	Germany
PD Dr. Med. Albert Heim	Institut fur Virologie, Medizinische Hochschule Hannover, Hannover	Germany
Dr Simone Paci	Sacace Biotechnologies, Como	Italy
Dr. Ombretta Turriziani	Laboratory of Virology, UOC Microbiology and Virology, Policlinico Umberto I Sapienza University of Rome, Rome	Italy
Dr. Souichi Yamada	Department of Virology 1, National Institute of Infectious Diseases, Tokyo	Japan
Dr. Yoshinori Ito	Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya	Japan
Dr. Sandrine Medves	Fast Track Diagnostics, Esch-Sur-Alzette	Luxembourg
Dr. David Tarragó Asensio	Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda	Spain
Dr. Maria Angeles Marcos	Hospital Clinic, Barcelona	Spain
Dr Rob Schuurman	UMC Utrecht, Department of Virology	The Netherlands

Dr. Jaco J. Verweij	Laboratory for Medical Microbiology and Immunology, ETZ hospital, Tilburg	The Netherlands
Dr. Willem JG Melchers	Radboud University Medical Centre, Department of Medical Microbiology, Nijmegen	The Netherlands
Prof. Dr. H.G.M (Bert) Niesters	University Medical Center Groningen, Department of Medical Microbiology, Division of Clinical Virology, Groningen	The Netherlands
Dr. Elif Akyuz	Anatolia Tani Biyotecnoloji Uraunleri Ar-Ge San. Ve Tic. A.S., Istambul	Turkey
Dr. Elaine McCulloch	Quality Control for Molecular Diagnostics, Glasgow	United Kingdom
Dr. Mrs Sandra Gittins	The James Cook University Hospital, Middlesbrough	United Kingdom
Dr. Surendra Parmar	Addenbrookes Hospital, National Infection Services, PHE Cambridge	United Kingdom
Dr. Cynthia Wagner, Dr. Lovedeep Grewal	Siemens Healthineers, Berkeley, California	USA
Dr. Gary Fahle	National Institutes of Health, Clinical Center, DLM, Microbiology Service, Bethesda, Maryland	USA
Dr. Rola Irikat	Roche Molecular System, Inc, Pleasanton	USA
Dr. Ronald Lallar	Quidel Corporation, Ohio	USA

Appendix 2
Study protocol and IFU

Medicines & Healthcare products Regulatory Agency

NIBSC

Collaborative study to evaluate the candidate for the establishment of the 1st WHO International Standard for herpes simplex virus (HSV) for nucleic acid amplification techniques (NAT)

Study Protocol

Background and outline of the study

The World Health Organization (WHO) have endorsed a proposal to develop the 1st International Standard for Herpes simplex virus (HSV), for the standardisation of nucleic acid amplification technology (NAT)-based laboratory assays.

The candidate materials to be evaluated comprise lyophilised preparations of HSV-1 and HSV-2 in universal buffer their pre-lyophilisation liquid bulk materials and two clinical samples comprising - a HSV-1 positive swab in UTM and HSV-2 positive serum sample. The aim of this worldwide collaborative study is to determine the suitability and assign potency to the proposed candidate standard(s), using a range of NAT-based assays. Liquid frozen HSV-1 and HSV-2 patient samples are also included in order to provide a limited assessment of commutability.

In total, two lyophilised preparations and four liquid frozen samples are to be evaluated. Participants are asked to test dilutions of each lyophilised and liquid frozen sample, using their routine HSV-1 and HSV-2 NAT assays. Sufficient number of vials, of each study sample will be provided in order to evaluate each sample on four separate occasions. Where possible, we would encourage laboratories to use quantitative methods, however, data from qualitative assays will also be acceptable.

Study samples

Study samples are as follows:

Sample Name	Formulation	Volume	Storage Temperature
Sample A	Liquid frozen HSV-1	1.0 mL	-70 °C
Sample B	Liquid frozen HSV-2	1.0 mL	-70 °C
Sample C	Lyophilised HSV-1	1.0 mL	-20 °C
Sample D	Lyophilised HSV-2	1.0 mL	-20 °C
Sample E	Liquid frozen HSV-1	1.0 mL	-70 °C
Sample F	Liquid frozen HSV-2	1.0 mL	-70 °C

Study samples comprise two lyophilised preparations in 3 mL screw top glass vials (coded; sample C and sample D), four liquid frozen samples (coded; sample A, sample B, sample E, and sample F). Sufficient vials of each study sample are provided for

World Health Organization

evaluation on four separate occasions. Upon receipt, lyophilised samples should be stored at -20 °C or below. Liquid frozen samples should be stored at -70 °C or below.

* **CAUTION:** Study samples A-F contain infectious HSV-1 and HSV-2 and should be handled only in appropriate containment facilities by fully trained and competent staff in accordance with national safety guidelines. These preparations contain material of human origin. Care should be taken when opening vials to avoid cuts. See instructions for use for further details. The study samples MUST NOT be used for any purpose other than for the performance of this study.

Study protocol

Participants are requested to test each study sample, using their routine HSV-1 and HSV-2 NAT assay, on four separate occasions. It is critical that the first assay results are reviewed to assess whether all dilutions tested are within the linear range of the assay and to determine the correct dilutions for the method prior to subsequent assays.

Prior to each assay run, samples C and D must be reconstituted with 1.0 mL of deionised, nuclease-free molecular-grade water and left for a minimum of 20 minutes with occasional agitation before use.

Dilutions of samples A-D should be prepared in the sample matrix appropriate for the extraction procedure (e.g. Universal transport medium). Samples E and F should be tested undiluted in quantitative assays and for qualitative assays diluted in UTM or serum respectively.

Each sample must be extracted prior to amplification.

For each independent assay, study samples A-F should be tested within the same assay run. Independent assays should be performed on separate days, using a fresh vial of each sample.

Below, are specific instructions for the dilution and testing of study samples, using either quantitative or qualitative assays.

NB: Sample A and C contain approximately $8x10^7$ copies/mL HSV-1 and sample B and D contain approximately $2x10^8$ copies/mL HSV-2 DNA when reconstituted in 1.0 mL nuclease-free water. Sample E contains approximately $1x10^5$ copies/mL HSV-1 and sample F contain approximately $5x10^4$ copies/mL HSV-2 DNA.

For quantitative assays:

For the first assay participants are requested to test each sample at suggested dilutions below to ensure the results are within the linear range of the assay. For each of three subsequent assays, participants are requested to test samples A and B, at a single dilution within the linear range of the assay (Samples A-B; 10^{-3}), samples C-D at three serial dilutions within the linear range of the assay (Samples C-D; 10^{-2}, 10^{-3} and 10^{-4}). Samples E and F should be tested undiluted. If practicable, please test replicates of each dilution of each sample within the same assay run.

For qualitative assays:

For the first assay, participants are requested to test ten-fold serial dilutions of each sample, in order to determine the end-point (e.g. for samples A to D 10^{-4} to 10^{-9}. The results from the first

assay must be reviewed to determine the correct dilutions for the method prior to subsequent assays.

For the remaining three assays, participants are requested to test the dilution at the assay end-point (limit of detection) determined in assay 1, and a minimum of two half-log10 serial dilutions either side of the pre-determined end-point i.e., at least five dilutions in total (e.g. if for samples A to D, the LOD determined in assay one is $10^{-7.5}$, then test $10^{-6.5}$, 10^{-7}, $10^{-7.5}$, 10^{-8}, $10^{-8.5}$). If practicable, please test replicates of each dilution of each sample within the same assay run. It is important that the dilution series spans the limit of detection for the assay and the data includes positive and negative results.

If further clarification on the study protocol is required and also if the number of tests is too onerous for your laboratory please do get in contact us so that the protocol can be amended for your laboratory.

Reporting of results

The results of each assay (HSV-1 and HSV-2 copies/mL or qualitative result; positive / negative) and methodology used, should be recorded on the Result Reporting Form accompanying the samples. Where applicable, please also include the crossing point / threshold cycle for each result. Results should be returned to NIBSC as soon as possible and **before the end of December 2017**.

The data should not be published or cited before the formal establishment of the standard by the WHO ECBS, without the expressed permission of the NIBSC study organiser.

All requests for further information and completed Result Reporting Forms should be returned electronically to R Minhas: **Rehan.Minhas@nibsc.org**

Alternatively, results may be faxed or mailed to: Fax: +44 (0)1707 641366

Address: Mr R Minhas, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG.

Data analysis

Data from the study will be analysed at NIBSC. The analysis will assess the concentration of each sample, relative to each other, and the sensitivities of the different assay methods. Individual participants' 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 including proposed recommendations, for comment, before it is further distributed. It is normal practice to acknowledge participants as contributors of data rather than co-authors in publications describing the establishment of the standard.

^

Herpes simplex virus (HSV-1 and HSV-2), for nucleic acid amplification technology (NAT)
WHO HSV CS608 Samples A-F
Version 1.0, 19th September 2017

1. CONTENTS

Upon receipt, samples A-B and E-F should be stored at -60°C or below. Samples C and D should be stored at -20°C or below.

Samples C and D contain 1.0 mL of lyophilized Herpes simplex virus (HSV-1 and HSV-2) in a 3 mL crimp top glass vial. Prior to use, these samples must be reconstituted with 1.0 mL of deionised, nuclease-free, molecular-grade water and left for a minimum of 20 minutes with occasional agitation before use.

Sample A-B and E-F contain 1.0 mL of liquid frozen Herpes simplex virus (HSV-1 and HSV-2) in 2 mL Sarstedt tubes. Prior to use these samples must be thawed and mixed gently.

Further instructions for the dilution and testing of these study samples are provided in the study protocol accompanying the samples.

2. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS OR ANIMALS IN THE HUMAN FOOD CHAIN.

These samples contain Herpes simplex virus (HSV-1 and HSV-2) and material of human origin.

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 probably will 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. DIRECTIONS FOR OPENING THE SCREW CAP VIALS.

Vials have a screw cap and stopper. The cap should be removed by turning anti-clockwise, please note on removal of the cap, the stopper may remain in the vial or be removed with the cap. Care should be taken on removal of cap to prevent the contents escaping.

4. CITATION

In any circumstance where the Recipient publishes a reference to NIBSC materials, it is important that the title of the preparation and any NIBSC code number, and the name and address of NIBSC are cited correctly.

5. LIABILITY AND LOSS

Medicines and Healthcare Products Regulatory Agency

World Health Organization

National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG
WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory

T +44 (0)1707 641000
nibsc.org

5.1. Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (http://www.nibsc.org/terms_and_conditions.aspx) apply to the exclusion of all other terms and are hereby incorporated into this document by reference.

5.2. Unless the context otherwise requires, the definitions in the Conditions shall apply.

5.3. Nothing in this document or the Conditions shall limit or exclude NIBSC's liability for fraud or fraudulent misrepresentation, death or personal injury caused by its negligence, or the negligence of its employees.

5.4. Subject to clause 5.3:

5.4.1. NIBSC shall under no circumstances whatsoever be liable to the Recipient, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, for any loss of data, loss of profit, loss of business or goodwill, or any indirect or consequential loss or damage suffered or incurred by the Recipient arising in relation to the supply of the Materials or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or by any other person; and

5.4.2. NIBSC's total liability to the Recipient in respect of all other losses arising under or in connection with the Contract, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, shall in no circumstances exceed 100% of the fees paid to NIBSC for the Materials.

5.5. The Recipient shall defend, indemnify and hold NIBSC, its officers, employees and agents harmless against any loss, claim, damage or liability including reasonable legal costs and fees (of whatsoever kind or nature) made against NIBSC which may arise as a result of the wilful act, omission or negligence of the Recipient or its employees, the breach of any of the terms of the Contract, or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or on its behalf.

6. MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	Liquid or lyophilized		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other	Contains human herpes simplex virus and material of human origin		
Handling:	See contents and caution, section 1 and 2		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation, contains infectious HSV		
Effects of ingestion:	Not established, avoid ingestion, contains infectious HSV		
Effects of skin absorption:	Not established, avoid skin contact, contains infectious HSV		
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 a virucidal agent. Rinse area with a virucidal agent followed by water. Absorbent materials used to treat spillage should be treated as biologically hazardous waste.			

Appendix 3

Proposed IFU for HSV-1 (Nibsc code: 16/368) established Material

Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

WHO International Standard
1st WHO IS for Herpes Simplex Virus type-1 (HSV-1) DNA Nucleic Acid Amplification Techniques
NIBSC code: 16/368
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

The 1st WHO International Standard for Herpes Simplex Virus type-1 (HSV-1) DNA, NIBSC code 16/368, is intended to be used in the standardization of nucleic acid amplification technology (NAT)-based assays for HSV-1. The reference comprises a whole virus preparation of HSV type 1, formulated in a universal buffer comprising 10mM Tris buffer, 0.5 % human serum albumin, 0.1% trehalose. The material has been lyophilized in 1 mL aliquots and stored at -20 °C. The material was evaluated in a worldwide collaborative study involving 30 laboratories performing a range of NAT-based assays for HSV.

2. CAUTION

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

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

3. UNITAGE

This material has been assigned a concentration 7.21 log10 International Units (IU)/vial when reconstituted in 1 mL of nuclease-free water, based on the results of a worldwide collaborative study. Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the vial content and was determined to be +/-0.24%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each vial contains the lyophilized equivalent of 1mL of HSV-1 in 10mM Tris buffer, 0.5 % human serum albumin, 0.1% trehalose.

5. STORAGE

Vials of lyophilized standard should be stored at -20 °C
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: if a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
The materials should be reconstituted with 1 mL of deionized, nuclease-free molecular-grade water and left for a minimum of 20 minutes with occasional agitation before use. The product should be reconstitued just prior to use, freeze thawing of the product once reconstituted is not recommended.

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.

Accelerated thermal degradation tests have been carried out at NIBSC are are avaible in the ECBS report

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

9. REFERENCES

WHO/ECBS 10.2020

10. ACKNOWLEDGEMENTS

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11. FURTHER INFORMATION

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

12. CUSTOMER FEEDBACK

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13. CITATION

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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: Lyophilized powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	Contains infectious Herpes Simplex Virus type-1 and human serum albumin		

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

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UK Official Medicines Control Laboratory

World Health Organization

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.

Action on Spillage and Method of Disposal

Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water.
Absorbent materials used to treat spillage should be treated as biological waste.

15. LIABILITY AND LOSS

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

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 1 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached:** No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_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.

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Proposed IFU for HSV-2 (Nibsc code: 17/122) established Material

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WHO International Standard
1st WHO IS for Herpes Simplex Virus type-2 (HSV-2) DNA Nucleic acid Amplification Techniques
NIBSC code: 17/122
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

The 1st WHO International Standard for Herpes Simplex Virus type-2 (HSV-2) DNA, NIBSC code 17/122, is intended to be used in the standardization of nucleic acid amplification technology (NAT)-based assays for HSV-2. The reference comprises a whole virus preparation of HSV type 2, formulated in a universal buffer comprising 10mM Tris buffer, 0.5 % human serum albumin, 0.1% trehalose. The material has been lyophilized in 1 mL aliquots and stored at -20 °C. The material was evaluated in a worldwide collaborative study involving 30 laboratories performing a range of NAT-based assays for HSV.

2. CAUTION

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

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

3. UNITAGE

This material has been assigned a concentration 7.32 log10 International Units (IU)/vial when reconstituted in 1 mL of nuclease-free water, based on the results of a worldwide collaborative study. Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the vial content and was determined to be +/-0.27%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each vial contains the lyophilized equivalent of 1mL of HSV-1 in 10mM Tris buffer, 0.5 % human serum albumin, 0.1% trehalose.

5. STORAGE

Vials of lyophilized standard should be stored at -20 °C
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: if a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

7. USE OF MATERIAL

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

The materials should be reconstituted with 1 mL of deionized, nucleasefree molecular-grade water and left for a minimum of 20 minutes with occasional agitation before use. The product should be reconstitued just prior to use, freeze thawing of the product once reconstituted is not recommended.

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.

Accelerated thermal degradation tests have been carried out at NIBSC and are avaible in the ECBS report

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

9. REFERENCES

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