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WHO International Collaborative Study of the Proposed 2nd WHO IS for Free PSA

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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 **8 October 2018** 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

The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) has recognized (2014) the need for a replacement International Standard (IS) for Free Prostate Specific Antigen (fPSA), 96/668, used for the calibration of immunoassays to measure fPSA in human serum. We report here, the evaluation of a candidate standard for fPSA by immunoassay by an international collaborative study carried out by ten laboratories in eight countries.

The geometric mean of the laboratory estimates for the fPSA content of the current standard, 96/668, agreed with the assigned content of 1 µg/vial (1.057 µg/vial (95% CI 0.988 – 1.130, n=21, GCV 15.9% with a robust geometric mean of 1.045 µg/vial). The geometric mean of the laboratory estimates for the fPSA content of 17/102 was 0.545 µg/amp (95% CI 0.508 – 0.586, n=21, GCV 17.0%) with a robust mean of 0.533 µg/amp. This was supported by the ratio of estimates of 17/102 to 96/668 of 0.516 (95% CI 0.489 – 0.544, n=21, GCV 12.5%) with a robust mean of 0.512. These data support a value assignment of 0.53 µg/amp. The results of this study also indicate that the candidate standard appears sufficiently stable, on the basis of a thermally accelerated degradation study, to serve as an IS.

The study included an assessment of the impact of the new standard on the routine measurement of fPSA in human serum samples. All laboratories contributed data during the collaborative study through the concomitant measurement of the fPSA immunoreactivity of fifteen human serum samples. The results indicate that the candidate standard is suitable for the continued calibration of immunoassay methods for the measurement of fPSA.

Therefore, it is proposed that the candidate preparation in ampoules coded, **17/102** is established as the **2nd IS for fPSA** with an assigned content of **0.53 µg** per ampoule.

Introduction

Mature Prostate specific antigen (PSA) is a 28.4 kDa kallikrein-related peptidase with a physiological role in liquefying seminal fluid (1). Clinically, total and fPSA in serum are measured by immunoassays for the purposes of diagnosing and monitoring the treatment of prostate cancers. Measurements of fPSA contribute to predictive measures such as free:total PSA ratio (2). The serum form of fPSA is a population of proteolytically inactive forms. The 1st WHO International Standard (IS) for fPSA, 96/668, was established by the WHO Expert Committee on Biological Standardization (ECBS) in 1999 and has been widely used for the calibration of immunoassays of fPSA (3,4). The IS was a batch of vials containing 1 µg fPSA purified from human seminal fluid. Stocks of the 1st IS are low and there is a requirement to replace the standard. A batch of ampoules coded 17/102 was prepared and has been evaluated

in this collaborative study to determine its immunoreactivity using current immunoassay methods and to assess its suitability to serve as an IS.

The aims for this study were therefore:

1. To value assign the candidate standard, 17/102
2. To assess the suitability of the candidate standard, 17/102, to serve as the 2nd IS for the calibration of immunoassays of fPSA.
3. To determine the stability of the candidate standard, 17/102, by comparison with ampoules stored at elevated temperatures as part of an accelerated degradation stability study.

Participants

Ten laboratories in eight countries took part in the study and are listed alphabetically, by country, in Table 1. Throughout the study, each participating laboratory is referred to by a code number. The code numbers were randomly assigned and do not reflect the order of listing.

Table 1: List of participants in order of country

BELGIUM	Dr Stefaan Marivoet Tosoh Europe N.V., Transportstraat 4, 3940 Tessenderlo.
CHINA	Dr Yu Ting National Institutes for Food and Drug Control, Institute for Medical Devices Control, No. 2 Tiantan XiLi, Dongcheng District, Beijing, 100050.
FRANCE	Dr Nathalie Ripoll, bioMerieux, Chemin de L'Orme, 69280 Marcy L'Etoile.
GERMANY	Steffen Mueller, Roche Diagnostics GmbH, Nonnenwald 2, D-82377 Penzberg.
IRELAND	Mel Costello, Abbott Ireland Diagnostics Division, Finisklin Business Park, Sligo, F91 VY44
SWEDEN	Maria Lundin, Fujirebio Diagnostics, Elof Lindälvs Gata 13, 41458 Göteborg
UK	Dr Jackie Ferguson and Katherine Partridge National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG.
UK	Dr Chris Thomas, Ortho-Clinical Diagnostics, Felindres Meadows, Pencoed, Bridgend, CF35 5PZ
USA	Ryan Masica Beckman Coulter Inc, 1000 Lake Hazeltine Drive, Chaska, MN 55318-1084.Dr
USA	Robert Pevere, Siemens Healthcare Diagnostics, 511 Benedict Avenue, Tarrytown, NY 10591

Bulk materials and processing

Purified (≥ 98%) non-complexing PSA without α-1-antichymotrypsin was supplied as a frozen, buffered solution containing 0.1% (w/v) sodium azide by Scripps Laboratories (San Diego, USA, Part no. 90024). The material was tested by the supplier and found negative for HIV1 and 2, Hepatitis B and Hepatitis C. Bovine serum albumin (BSA) from certified USA herds was obtained from EMD Millipore Corporation (Billerica, USA; Probumin, Life Science Grade, Part no.82-100). The concentration of the material was determined by the manufacturer by measuring the absorbance at 280 nm prior to the addition of sodium azide.

An 8420 ml volume of bulk formulation containing 500 ng/ml fPSA in 20 mM sodium phosphate buffer pH 7.4, 150 mM sodium chloride, 10 g/L BSA was prepared and the solution was distributed into ampoules as 1.0 ml aliquots. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen. Ampoules were stored at -20°C.

Characterization of the freeze-dried product

A total of 7209 ampoules, coded 17/102 were produced. Check-weights measured during filling demonstrated a mean fill weight of 1.0083 g (CV 0.16 %, n=302). The mean residual moisture content was 0.616% (CV 16.42%, n=12), mean headspace oxygen was 0.3% (CV 47.00 %, n=12) and the mean dry weight was 0.02074 g (CV 1.97 %, n=6).

Collaborative study for the evaluation of 17/102

The collaborative study was organised by NIBSC. All participants were provided with ampoules of the current and candidate IS, 96/668 and 17/102. Thermally-accelerated degradation samples were available in limited numbers and were distributed to participants based on assay capacity and sample availability. A study protocol, shown in Appendix 2, and instructions for use was provided with the samples.

Participants were requested to measure the fPSA content of the materials using the immunoassay(s) normally in use in their laboratory and, where possible, to perform at least two independent assays, using fresh ampoules, each assay to include all the preparations allocated, measured at no less than five dilutions in the linear part of their dose-response curve. In instances where there was not a fresh ampoule for subsequent assays, it was suggested that fresh dilutions be made from frozen stock solutions. Where dilutions of a stored stock solution were used, participants were asked to provide details of the freezing and thawing procedures used. Participants were asked to provide details of the assay method(s) used, the diluent and dilution steps, together with all raw assay data for central computation at NIBSC. Participants' own estimates of immunoreactivity as calculated by the method normally used in their laboratory were also requested**.** The ampoules provided for this study, which may be identified only by code letter, are listed in Table 2.

Table 2: Preparations provided to participants in the collaborative study.

Code	Preparation	Content
Not coded	1st IS for fPSA (96/668)	1 µg per ampoule
H	Candidate 2nd IS for fPSA (17/102) stored at -20°C	Nominally, 0.5 µg per ampoule
F, G, I, J	Accelerated thermal degradation (ATD) samples of 17/102 stored at +4°C, +37°C, +45°C and +20°C (respectively) for 6 m 27 d	Content assumed to be identical to 17/102 stored at -20ºC

In addition to the ampouled preparations, participants were provided with a panel of fifteen human serum samples coded PSASerum1 to PSASerum15 with estimated fPSA concentration so 0.1-7.0 µg/L. The assays contributed by each laboratory are shown in Table 3.

Table 3: Immunoassay methods contributed

Lab	Method	Details of calibration of kit/method	Total Number of assays	Number of assays with ATDs	Number of assays with serum samples	Number of serum samples measured
1(1)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	2	15
1(2)	Automated chemiluminescent immunoassay (in development)	WHO 96/668	2	0	2	15
2	Automated chemiluminescent immunoassay	WHO 96/670	2	0	2	15
3(1)	Automated chemiluminescent immunoassay	WHO 96/668	2	2	2	15
3(2)	Automated chemiluminescent immunoassay	WHO 96/668	2	2	2	15
4	Enzyme-linked fluorescent immunoassay	WHO 96/668	2	2	2	15
5	Automated chemiluminescent immunoassay	WHO 96/668	2	2	2	15
6(1)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	0*	0
6(2)	Automated chemiluminescent immunoassay	National Standard	3	0	3	15
6(3)	Automated chemiluminescent immunoassay	National Standard	4	0	2	15
6(4)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	2	15
6(5)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	0*	0
6(6)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	2	15
6(7)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	0*	0
6(8)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	0*	0
6(9)	Automated chemiluminescent immunoassay	National Standard	2	0	2	15
6(10)	Enzyme-linked fluorescent immunoassay	WHO 96/668	2	0	1	15
6(11)	Flowcytometry Fluorescence luminance method	National Standard	2	0	2	15
6(12)	Time-resolved immunofluorometric assay	WHO 96/668	2	0	2	15
7(1)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	2	15
7(2)	Automated chemiluminescent immunoassay	WHO 96/668	2	0	2	15
8	Automated chemiluminescent immunoassay	WHO 96/668	2	1	1	15
9	Enzyme-linked immunosorbent assay	WHO 96/668	2	0	2	15
10	Automated chemiluminescent immunoassay	WHO 96/668	2	0	2	15

* insufficient serum available, method replicated by other participants

Statistical analysis

Assessment of immunoreactivity of 17/102 and the current WHO IS, 96/668
Analysis was performed with PSA concentrations as reported by the participants, using results from the nominal concentration range of 0.5 – 8.0 µg/L only. Results from an assay run were considered valid for a sample if the slope of the fitted regression line for $\log_{10}$ estimated concentration against $\log_{10}$ nominal concentration was in the range [0.90, 1.11] and the 90% confidence interval for the slope was within [0.80, 1.25].

Results from all valid assay runs were corrected for dilution factor and combined to generate unweighted geometric mean (GM) estimates for each laboratory and these laboratory means were used to calculate overall unweighted geometric mean estimates. Variability between laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^s$-1$\}\times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates). Due to possible outliers and anomalous results, Huber's robust mean was also calculated using the R package 'WRS2' (5).

Assessment of commutability
Data used for analysis. All reported results were $\log_{10}$ transformed for analysis to achieve approximately constant scatter over the range of concentrations used. A consensus value for each sample, shown in Table 4, was calculated as Huber's robust mean of laboratory means using the R package 'WRS2' (5). Bias values were then calculated for all reported results as the difference between the reported value and the study consensus value for that sample. For each individual laboratory and method, bias values were then analysed using a linear mixed model with assay run included as a random effect and sample (96/668, 17/102 or serum) included as a fixed effect. Calculations for this part were performed using Minitab 17 (6), with Dunnett's method used to estimate the difference in bias for the reference standards with serum samples.

Determination of commutability criteria. The standard deviation of the bias values for serum samples only was calculated within each laboratory and a pooled value, s_P, was calculated across all laboratories. Possible commutability criteria representing the maximum acceptable difference in bias were then set as $\pm 2s_P$. Reference standards were to be concluded as commutable if the 90% confidence interval on the difference in bias was fully contained within the commutability criteria. For the purpose of this commutability assessment, the bias for serum samples has been assumed to be constant over the concentration range used.

Assessment of stability
The relative immunoreactivities of the accelerated thermal degradation samples were used to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay (7), and hence predict the degradation rates when stored at a range of temperatures.

Results

Data returned for analysis
Assay validity

Data were contributed by 10 laboratories who performed in total 51 individual assays from 24 methods (18 different methods). The following exclusions were made as the slope of the fitted regression line exceeded 1.11 and therefore did not meet the validity criteria described above.

- laboratory 6, method 6, both assay runs and both samples
- laboratory 6, method 11, assay run 1
- laboratory 10, 96/668 in assay run 1, 17/102 in both assay runs.

As this gave no valid results for 17/102 from laboratory 10, this laboratory was excluded from further analysis. All remaining results were included in further analysis. Differences in estimates between assay runs did not exceed 29% (laboratory 8, sample 96/668).

Estimates of free PSA content

A summary of estimates obtained for both standards is shown in Tables 5-6 and Figure 1. The estimate obtained for 96/668 by laboratory 6, method 8 was identified as an outlier using Grubbs' test (8) and overall estimates are also presented excluding this lab/method. As shown in Table 6, the geometric mean of the laboratory estimates for the fPSA content of 96/668 agreed with the assigned content of 1 µg/vial (1.057 µg/vial (95% CI 0.998 – 1.130, n=21, GCV 15.9% with a robust geometric mean of 1.045 µg/vial). The geometric mean of the laboratory estimates for the fPSA content of 17/102 was 0.545 µg/amp (95% CI 0.508 – 0.586, n=21, GCV 17.0%) with a robust mean of 0.533 µg/amp. This was supported by the geometric mean of the estimates of ratio of 17/102 to 96/668 of 0.516 (95% CI 0.489 – 0.544, n=21, GCV% 12.5%) with a robust mean of 0.512.

Assessment of the free PSA concentration of human serum samples and reference standard commutability

Fifteen human serum samples with estimated fPSA concentrations ranging from 0.1 – 7 µg/L were supplied to all laboratories. Data from 19 methods contributed to an assessment of commutability. Commutability criteria calculated using $2s_P$ were ±0.100, or 0.795 to 1.258 on the untransformed scale, i.e. the bias for a reference standard must be demonstrated to be not less than 79.5% and not more than 125.8% of the bias observed for serum samples. Estimates of the difference in bias together with 90% confidence intervals are shown in Table 7, including data from all nominal concentrations, and Appendix Table 1 for each nominal concentration separately. Comparisons of the estimated differences in bias with the possible commutability criteria are shown in Figure 2. A comparison of inter-laboratory variability for reported mean values for serum samples with mean values expressed relative to 96/668 or 17/102 within each laboratory is shown in Table 8.

Stability of 17/102

Estimates of the immunoreactivity of ampoules stored at elevated temperatures for a period of 6 months, 27 d are summarized in Table 9. Analysis showed a predicted loss of fPSA immunoreactivity per year of 0.096% when stored at -20°C.

Summary

The current WHO International Standard for fPSA, 96/668, has been available since 1999 for the calibration of immunoassays of fPSA. Stocks are low and there is need to replace this standard to ensure continued harmonization of measurements of fPSA. The free PSA standard, 96/668, was prepared from a stock solution of purified fPSA which had been assigned a concentration by measuring the absorbance at 280 nm. The molar extinction

coefficient used to define the concentration had been previously determined by measuring the absorbance of multiple batches of fPSA preparations which had been quantified by amino acid analysis (4, 9, 10). Immunoassay assessment of 96/668 by methods available at the time (which included assays of total PSA) provided estimates of the PSA immunoreactivity of 96/668 of 1.10 µg/vial (95% confidence interval, 0.99-1.21 µg/vial) (3).

A candidate batch of ampoules, coded 17/102 was prepared using non-complexing PSA purified from human seminal fluid. The candidate standard was filled with a fPSA content of 0.5 µg/amp based on the stated fPSA concentration determined by absorbance at 280 nm by the supplier prior to the addition of sodium azide. The aim of the collaborative study was to evaluate the fPSA immunoreactivity of the candidate standard by comparison to the current WHO IS for fPSA, 96/668. As described above, the robust geometric mean of the fPSA content estimates of the current standard was 1.045 µg/vial whereas the robust geometric mean of the fPSA content estimates of the candidate standard was 0.533 µg/amp (Tables 6 and Figure 1). The robust geometric mean of the ratio of the content estimates of 17/102 to 96/668 determined by each laboratory method was 0.512. An assignment of 0.53 µg/amp to 17/102 is proposed.

Diagnostic standards require an assessment of commutability to evaluate if there is equivalence in the relationship between the assay response to dilutions of the reference material and the response to representative clinical samples. A difference in bias approach allows, for each laboratory method, comparison of the bias from the mean consensus value for each dilution of the reference material to be compared to that observed for the serum samples. For this study, the limits of commutability were statistically defined.

As shown in Table 7, for the majority of the methods (12 of 19), both the current and candidate standards were considered fully commutable within the defined limits and for four methods, commutability was inconclusive. For two methods, the reference materials were considered non-commutable, in one of these, only the current standard was considered non-commutable. No trend in bias was observed in the inconclusive or non-commutable results. Furthermore, the estimates and confidence intervals obtained for the difference in bias were comparable between the current and candidate standards for each method (Figure 2) indicating that the commutability of 17/102 is likely to be equivalent to that of the current standard. Comparable inter-laboratory variability (GCV (%)) determined for the fPSA concentrations of the serum samples when expressed in terms of the kit, current and candidate standards (Table 8) further demonstrates that the introduction of 17/102 is unlikely to have a negative impact on the between-laboratory variability of fPSA measurement. It is noted that there is higher variability in measurements of fPSA (GCV >22-25%) compared to measurements of total PSA (GCV 14-16%) (11). The multiple forms of fPSA may contribute to this variability. These include proenzyme forms which retain up to 7 amino acids as a leader sequence, internally cleaved forms and minor variants that appear intact but are enzymatically inactive due to structure and conformational changes (12). These changes do not result in detectable changes in the separation characteristics of non-complexing fPSA and active fPSA. The seminal-fluid derived fPSA used to prepare 17/102 was characterized by the supplier as having no or minimal chymotrypsin-like activity.

Finally, the stability of the 17/102 was assessed by the measurement of accelerated thermal degradation samples by four laboratories using five methods (Table 9). The candidate IS, 17/102, was predicted to exhibit a loss of free PSA immunoreactivity per year of 0.096% when stored at -20°C indicating that it is sufficiently stable to be a WHO IS.

Proposal

It is recommended that the preparation in ampoules coded 17/102 is established as the WHO 2nd IS for free PSA with an assigned fPSA content of 0.53 µg/amp.

Acknowledgements

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Table 4. Consensus values obtained for all samples as used for the assessment of the commutability of 96/668 and 17/102 by difference in bias.

Sample:	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6	Serum 7	Serum 8	Serum 9
Robust Mean $\log_{10}$ µg/L	0.36	0.29	0.82	0.12	0.22	0.11	-0.61	-0.02	-0.40
Robust Geometric Mean µg /L	2.28	1.95	6.57	1.32	1.66	1.28	0.24	0.96	0.40
Sample:	Serum 10	Serum 11	Serum 12	Serum 13	Serum 14	Serum 15	96/668 (0.5)	96/668 (1)	96/668 (2)
Robust Mean $\log_{10}$ µg/L	-0.46	0.85	0.77	0.82	-0.62	-0.81	-0.27	0.02	0.32
Robust Geometric Mean µg /L	0.35	7.15	5.87	6.68	0.24	0.16	0.54	1.04	2.10
Sample:	96/668 (4)	96/668 (8)	17/102 (0.5)	17/102 (1)	17/102 (2)	17/102 (4)	17/102 (8)		
Robust Mean $\log_{10}$ µg/L	0.62	0.92	-0.26	0.02	0.32	0.62	0.91		
Robust Geometric Mean µg /L	4.18	8.23	0.55	1.04	2.08	4.17	8.17		

Values in brackets after reference standard samples indicate nominal free PSA concentrations (µg/L)

Table 5. Individual estimates of the PSA content of 96/668 (µg/vial) and 17/102 (µg/ampoule)

Lab	Method	Assay	Assay estimates 96/668	Assay estimates 17/102	Geometric Mean (GM) 96/668	Geometric Mean (GM) 17/102	17/102 rel. to 96/668
1	1	1	1.106	0.594	1.052	0.599	0.569
1	1	2	1.001	0.605			
1	2	1	1.083	0.586	1.034	0.591	0.572
1	2	2	0.988	0.595			
2	1	1	1.038	0.529	1.004	0.505	0.503
2	1	2	0.971	0.483			
3	1	1	1.079	0.537	1.057	0.529	0.500
3	1	2	1.035	0.522			
3	2	1	1.083	0.551	1.048	0.543	0.518
3	2	2	1.014	0.535			
4	1	1	1.122	0.740	1.170	0.769	0.657
4	1	2	1.221	0.799			
5	1	1	0.949	0.469	0.950	0.463	0.487
5	1	2	0.951	0.457			
6	1	1	1.003	0.537	0.965	0.529	0.548
6	1	2	0.928	0.522			
6	2	1	0.977	0.440	1.041	0.475	0.456
6	2	2	1.085	0.492			
6	2	3	1.065	0.494			
6	3	1	1.049	0.571	1.124	0.565	0.503
6	3	2	1.175	0.581			

6	3	3	1.149	0.571			
6	3	4	1.128	0.539			
6	4	1	0.949	0.436	0.954	0.476	0.499
6	4	2	0.959	0.520			
6	5	1	1.532	0.696	1.529	0.707	0.462
6	5	2	1.526	0.717			
6	6	1	*Excluded (see text)*				
6	6	2	*Excluded (see text)*				
6	7	1	0.986	0.523	1.016	0.522	0.514
6	7	2	1.047	0.521			
6	8	1	0.416	0.442	0.439	0.441	1.005
6	8	2	0.462	0.440			
6	9	1	1.067	0.452	1.041	0.445	0.427
6	9	2	1.017	0.438			
6	10	1	1.473	0.738	1.421	0.772	0.543
6	10	2	1.370	0.808			
6	11	1	*Excluded (see text)*		0.711	0.483	0.679
6	11	2	0.711	0.483			
6	12	1	1.015	0.549	1.001	0.520	0.519
6	12	2	0.987	0.493			
7	1	1	1.064	0.526	1.066	0.527	0.494
7	1	2	1.067	0.529			
7	2	1	1.079	0.515	1.103	0.529	0.480
7	2	2	1.127	0.544			
8	1	1	0.941	0.443	1.070	0.457	0.427
8	1	2	1.218	0.473			
9	1	1	1.071	0.585	1.064	0.583	0.548
9	1	2	1.057	0.581			
10	1	1	*Excluded (see text)*				
10	1	2	*Excluded (see text)*				

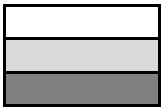
Table 6. Overall estimated PSA content of 96/668 (µg/vial), 17/102 (µg/ampoule) and 17/102 relative to 96/668

Sample	GM	95% LCL	95% UCL	GCV	n	Robust GM
96/668	1.015	0.914	1.127	26.7%	22	1.040
	1.057*	0.988	1.130	15.9%	21	1.045*
17/102	0.540	0.503	0.580	17.3%	22	0.529
	0.545*	0.508	0.586	17.0%	21	0.533*
17/102:96/668	0.532	0.491	0.577	20.1%	22	0.516
	0.516*	0.489	0.544	12.5%	21	0.512*

GM: Geometric Mean, LCL: Lower Confidence Limit, UCL: Upper Confidence Limit
GCV: Geometric Coefficient of Variation (%), n: number of estimates used in calculation
* excludes laboratory 6, method 8

Table 7. Overall estimated difference in bias between the serum samples and the current and candidate standards

Lab	Method	96/668 Difference in bias			17/102 Difference in bias		
		Estimate	90% Confidence Interval		Estimate	90% Confidence Interval	
1	1	0.079	0.059	0.100	0.022	0.002	0.043
1	2	0.074	0.050	0.098	0.016	-0.008	0.040
2	1	0.055	0.046	0.065	0.052	0.043	0.062
3	1	-0.097	-0.117	-0.078	-0.098	-0.118	-0.079
3	2	-0.117	-0.131	-0.104	-0.133	-0.147	-0.119
4	1	0.058	0.034	0.083	-0.061	-0.085	-0.036
5	1	0.074	0.060	0.087	0.084	0.070	0.098
6	2	0.052	-0.012	0.116	0.092	0.028	0.156
6	3	-0.059	-0.080	-0.038	-0.061	-0.082	-0.040
6	4	-0.009	-0.048	0.029	-0.009	-0.048	0.030
6	6	0.040	-0.025	0.104	0.014	-0.051	0.079
6	9	-0.078	-0.110	-0.046	-0.010	-0.042	0.022
6	10	-0.013	-0.044	0.017	-0.049	-0.079	-0.019
6	11	0.139	0.108	0.170	0.008	-0.023	0.039
6	12	-0.020	-0.056	0.016	-0.037	-0.073	-0.001
7	1	0.021	0.007	0.034	0.024	0.010	0.037
7	2	0.023	0.008	0.038	0.040	0.025	0.055
8	1	-0.113	-0.175	-0.050	-0.096	-0.157	-0.034
9	1	0.020	0.006	0.035	-0.020	-0.034	-0.006



Commutable
Inconclusive
Not Commutable

Table 8. Inter-laboratory variability (GCV (%)) of the free PSA concentration of serum samples, PSASerum1–15, ([1]) as reported by the participant, ([2]) expressed in terms of the current standard, 96/668 and ([3]) in terms of the candidate standard, 17/102

Serum Sample	N	GCV[1]	GCV[2]	GCV[3]
1	18	14.6%	19.0%	18.4%
2	18	16.5%	16.4%	18.2%
3	18	18.3%	17.2%	19.9%
4	18	19.4%	15.1%	18.6%
5	18	20.1%	21.1%	21.1%
6	18	19.8%	20.8%	21.4%
7	18	39.6%	30.6%	35.3%
8	18	18.6%	21.3%	21.6%
9	18	25.9%	24.4%	26.4%
10	18	25.9%	24.0%	25.3%
11	18	14.4%	20.2%	18.6%
12	18	19.0%	18.2%	18.1%
13	18	18.7%	14.8%	18.9%
14	18	38.7%	35.3%	37.1%
15	18	32.8%	32.1%	33.3%
Pooled GCV across serum samples		**23.7%**	**22.6%**	**24.1%**

Table 9. Estimated potencies of accelerated thermal degradation samples relative to samples stored at -20°C for 17/102

Lab	Method	+4°C	+20°C	+37°C	+45°C
3	1	1.02	0.96	0.97	0.94
3	2	0.98	0.95	0.92	0.92
4	1	0.98	0.94	0.96	0.90
5	1	1.04	1.04	0.94	0.90
8	1	1.01	1.02	0.97	0.97
GM		1.00	0.98	0.95	0.92
95% LCL		0.97	0.93	0.93	0.89
95% UCL		1.04	1.04	0.98	0.96

GM: Geometric Mean, LCL: Lower Confidence Limit, UCL: Upper Confidence Limit
Predicted loss in potency per year when stored at -20°C is 0.096%

Figure 1a. Laboratory estimates of the free PSA content of 96/668 (µg/vial) and 17/102 (µg/ampoule)

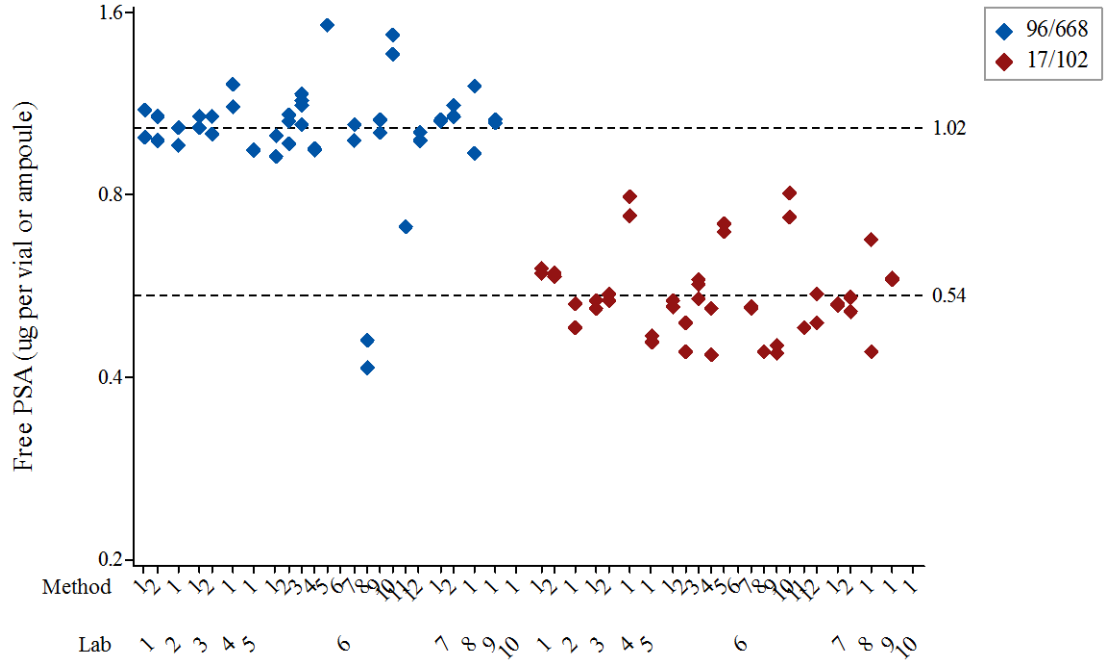


Figure 1b. Laboratory geometric mean estimates of the free PSA content of 17/102 (µg/ampoule, unshaded boxes) and 96/668 (µg/vial, shaded boxes)

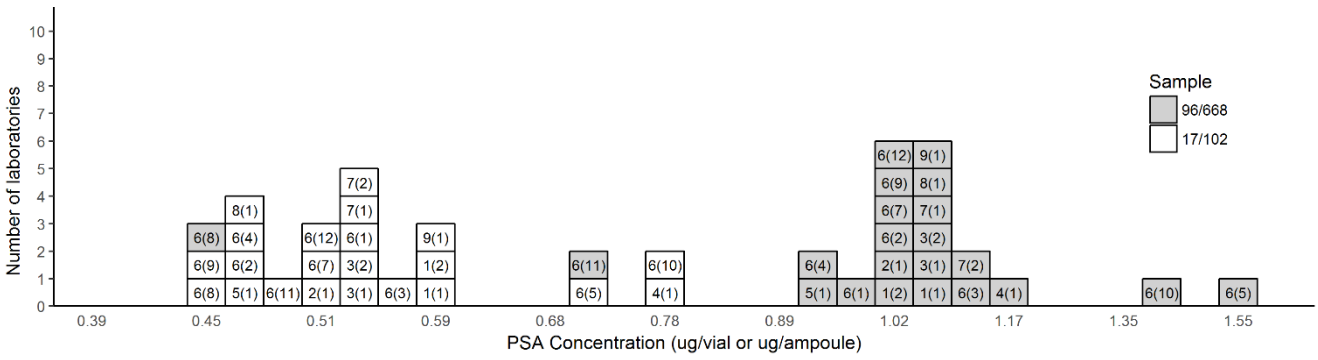
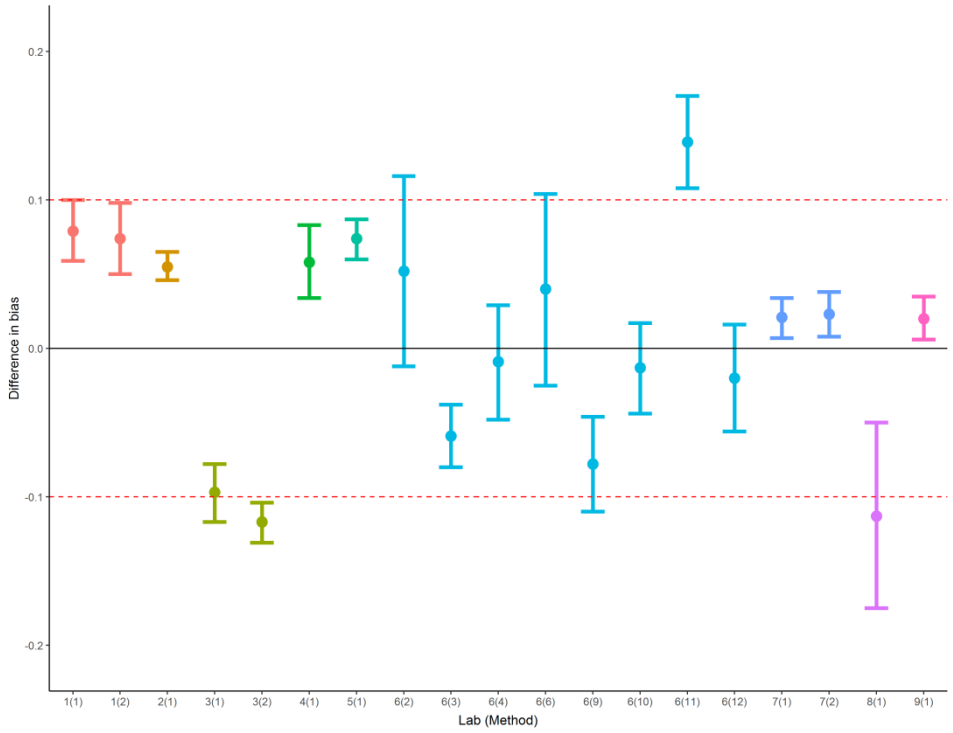
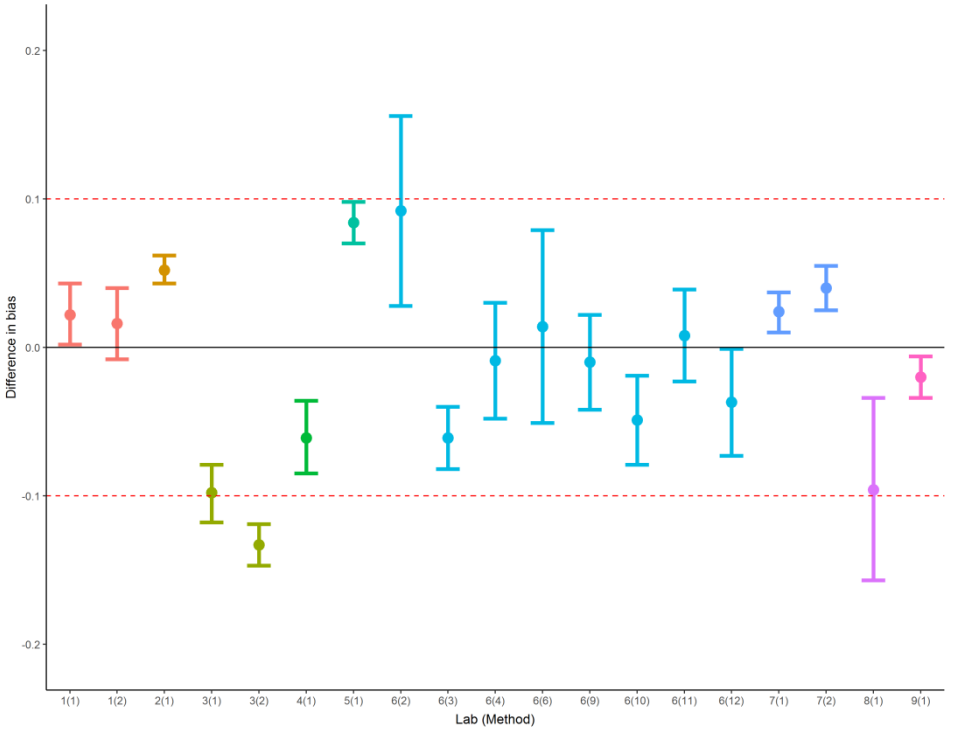


Figure 2. The overall estimated difference in bias (with 90% confidence limits) between the serum samples and (a) the current IS, 96/668, and (b) the candidate IS, 17/102 for each method.

(a) WHO 1st IS 96/668



(b) Candidate IS 17/102



Appendix 1

Table 1. Estimated difference in bias with serum samples (individual dilutions)

Lab	Method	Nominal conc. (µg/L)	96/668 Difference in bias			17/102 Difference in bias		
			Estimate	90% Confidence Interval		Estimate	90% Confidence Interval	
1	1	0.5	0.069	0.041	0.097	0.043	0.014	0.071
		1	0.125	0.097	0.154	0.005	-0.023	0.034
		2	0.066	0.038	0.095	0.004	-0.025	0.032
		4	0.067	0.039	0.096	0.025	-0.003	0.054
		8	0.070	0.041	0.098	0.035	0.007	0.064
1	2	0.5	0.047	0.012	0.081	0.016	-0.018	0.051
		1	0.114	0.080	0.149	-0.004	-0.038	0.031
		2	0.065	0.031	0.100	0.010	-0.025	0.045
		4	0.070	0.036	0.105	0.027	-0.007	0.062
		8	0.074	0.039	0.108	0.030	-0.005	0.064
2	1	0.5	0.069	0.053	0.084	0.071	0.055	0.087
		1	0.049	0.033	0.065	0.048	0.033	0.064
		2	0.054	0.038	0.070	0.049	0.033	0.065
		4	0.054	0.038	0.070	0.056	0.040	0.072
		8	0.051	0.035	0.067	0.037	0.021	0.053
3	1	0.5	-0.117	-0.146	-0.089	-0.112	-0.141	-0.084
		1	-0.111	-0.140	-0.083	-0.115	-0.143	-0.086
		2	-0.089	-0.118	-0.061	-0.102	-0.131	-0.074
		4	-0.086	-0.114	-0.057	-0.089	-0.118	-0.061
		8	-0.083	-0.112	-0.055	-0.072	-0.101	-0.044
3	2	0.5	-0.129	-0.152	-0.106	-0.139	-0.162	-0.117
		1	-0.132	-0.155	-0.110	-0.142	-0.165	-0.120
		2	-0.111	-0.134	-0.088	-0.144	-0.167	-0.122
		4	-0.112	-0.135	-0.090	-0.125	-0.148	-0.103
		8	-0.102	-0.124	-0.079	-0.113	-0.136	-0.090
4	1	0.5	0.056	0.016	0.096	-0.051	-0.091	-0.010
		1	0.058	0.017	0.098	-0.058	-0.098	-0.018
		2	0.076	0.036	0.117	-0.054	-0.094	-0.014
		4	0.064	0.024	0.105	-0.052	-0.092	-0.011
		8	0.038	-0.003	0.078	-0.089	-0.129	-0.048
5	1	0.5	0.099	0.085	0.114	0.139	0.124	0.153
		1	0.068	0.054	0.083	0.083	0.068	0.097
		2	0.074	0.059	0.089	0.069	0.054	0.084
		4	0.066	0.051	0.081	0.073	0.059	0.088
		8	0.060	0.045	0.075	0.057	0.042	0.071
6	2	0.5	0.113	0.017	0.209	0.157	0.061	0.253
		1	-0.015	-0.111	0.081	0.054	-0.042	0.150
		2	0.042	-0.054	0.138	0.090	-0.007	0.186
		4	0.063	-0.033	0.159	0.088	-0.008	0.184
		8	0.057	-0.039	0.153	0.071	-0.025	0.167
6	3	0.5	-0.029	-0.057	-0.001	-0.024	-0.052	0.005
		1	-0.076	-0.104	-0.048	-0.082	-0.110	-0.054
		2	-0.061	-0.089	-0.033	-0.071	-0.099	-0.043
		4	-0.063	-0.091	-0.035	-0.063	-0.091	-0.035
		8	-0.063	-0.091	-0.035	-0.067	-0.095	-0.039

6	4	0.5	-0.019	-0.077	0.039	-0.004	-0.062	0.054
		1	-0.011	-0.069	0.047	-0.003	-0.061	0.055
		2	0.012	-0.045	0.070	0.016	-0.042	0.074
		4	-0.009	-0.067	0.049	0.009	-0.049	0.067
		8	-0.020	-0.078	0.038	-0.062	-0.120	-0.004
6	6	1	0.151	0.079	0.224	0.130	0.058	0.202
		2	0.054	-0.019	0.126	0.018	-0.054	0.090
		4	-0.026	-0.098	0.047	-0.046	-0.119	0.026
		8	-0.020	-0.093	0.052	-0.046	-0.118	0.027
6	9	0.5	-0.049	-0.096	-0.002	0.025	-0.022	0.072
		1	-0.067	-0.114	-0.020	-0.006	-0.053	0.041
		2	-0.085	-0.132	-0.038	-0.004	-0.051	0.043
		4	-0.085	-0.132	-0.038	-0.021	-0.068	0.026
		8	-0.102	-0.149	-0.055	-0.043	-0.090	0.004
6	10	0.5	-0.031	-0.067	0.005	-0.064	-0.100	-0.028
		1	-0.024	-0.060	0.012	-0.052	-0.087	-0.016
		2	-0.002	-0.037	0.034	-0.039	-0.075	-0.004
		4	0.003	-0.033	0.039	-0.041	-0.077	-0.005
6	11	1	0.184	0.146	0.222	0.052	0.014	0.090
		2	0.185	0.147	0.223	0.020	-0.018	0.057
		4	0.122	0.085	0.160	-0.007	-0.045	0.031
		8	0.066	0.029	0.104	-0.033	-0.071	0.005
6	12	0.5	-0.020	-0.073	0.034	-0.040	-0.094	0.014
		1	-0.028	-0.081	0.026	-0.049	-0.102	0.005
		2	-0.041	-0.095	0.013	-0.051	-0.104	0.003
		4	-0.003	-0.057	0.051	-0.024	-0.078	0.030
		8	-0.007	-0.061	0.047	-0.020	-0.074	0.034
7	1	0.5	0.007	-0.013	0.027	0.022	0.002	0.042
		1	0.015	-0.005	0.036	0.021	0.001	0.041
		2	0.033	0.013	0.053	0.031	0.011	0.051
		4	0.029	0.009	0.049	0.019	-0.001	0.039
		8	0.019	-0.001	0.039	0.027	0.007	0.047
7	2	0.5	0.009	-0.013	0.032	0.040	0.018	0.063
		1	0.024	0.002	0.047	0.038	0.015	0.060
		2	0.038	0.015	0.060	0.040	0.017	0.062
		4	0.021	-0.002	0.043	0.034	0.012	0.056
		8	0.025	0.003	0.047	0.047	0.025	0.070
8	1	0.5	-0.095	-0.173	-0.017	-0.077	-0.154	0.001
		1	-0.141	-0.219	-0.064	-0.130	-0.208	-0.053
		2	-0.121	-0.199	-0.043	-0.107	-0.185	-0.029
		4	-0.107	-0.185	-0.029	-0.086	-0.163	-0.008
		8	-0.101	-0.179	-0.023	-0.079	-0.157	-0.002
9	1	0.5	0.033	0.012	0.054	-0.001	-0.022	0.020
		1	0.016	-0.004	0.037	-0.020	-0.041	0.000
		2	0.026	0.006	0.047	-0.019	-0.040	0.002
		4	0.021	0.001	0.042	-0.022	-0.043	-0.002
		8	0.004	-0.017	0.025	-0.037	-0.058	-0.016

Commutable
Inconclusive
Not Commutable

Appendix 2

Study Protocol
International Collaborative Study to establish a replacement WHO International Standard for Prostate Specific Antigen (free)

Introduction

The 1st International Standard (IS) for Prostate Specific Antigen (free), NIBSC code 96/668, was established by the Expert Committee on Biological Standardization (ECBS) in 1999 and has been widely used to calibrate immunoassays for free PSA (WHO, 2002). Determinations of the concentration of free PSA in serum contribute to the diagnosis and management of prostate cancer. Stocks of the 1st IS are low and there is a requirement to replace this standard.

The 1st International Standard consisted of a batch of vials coded 96/668 which contained free PSA purified from seminal fluid (Stamey et al., 1998). The candidate standard, coded 17/102, contains non-complexing (free) PSA, which is formulated in a similar matrix to that of the current IS and has a nominal PSA content derived from the absorptivity of the purified material at 280 nm (Scripps Laboratories, San Diego). A trial formulation was evaluated through the PSA quality assurance scheme organised by UK NEQAS (D. Patel, UK NEQAS, Sheffield) prior to preparation of the candidate standard.

We now intend to organize a collaborative study with expert laboratories to aid in the value assignment of the candidate standard, coded 17/102.

The aims of the study would be:

1. to value assign the candidate standard, 17/102
2. to assess the suitability of 17/102 to calibrate immunoassays of free PSA
3. to assess the stability of 17/102 after accelerated thermal degradation

Materials

The materials to be provided to collaborators are listed in Table 1. Each participant will be allocated a set of preparations based on assay capacity and sample availability.

Preparation	**Contents**
1st IS for PSA (free), coded 96/668	1 µg free PSA per vial
Candidate standard, coded 17/102	Nominally, 0.5 µg free PSA per ampoule
ATD samples of 17/102	Nominally, 0.5 µg free PSA per ampoule
Human serum samples (n=15)	Volumes of 0.5 – 1.2 ml provided according to assay requirements. The serum samples contain 0.1 to 7 µg/L free PSA

Table 1: Preparations to be provided to participants

[Note: Not all participants will receive accelerated thermal degradation (ATD) samples. Participants receiving ATD samples will receive ampoules coded 17/102 Sample F to 17/102 Sample J]

1st IS for PSA (free), coded 96/668
The 1st IS contains the residue, after freeze-drying, of 2 ml of a solution of 20 mM Phosphate buffered saline, pH 7.4 which contained 10 g/L bovine serum albumin, 500 µg/L PSA (free). The 1st IS has been tested and found to be negative for HBsAg, anti-HIV and HCV RNA.

Candidate replacement IS, coded 17/102
The candidate IS contains the residue, after freeze-drying, of 1 ml of a solution of 20 mM sodium phosphate buffer pH 7.4 which contained 150 mM sodium chloride, 10 g/L bovine serum albumin and 500 µg/L non-complexing (free) PSA. The stock solution of non-complexing (free) PSA, purified from seminal fluid, was certified negative for HIV 1 and 2, Hepatitis B and Hepatitis C by the Supplier.

Accelerated thermal degradation (ATD) samples
Ampoules of the candidate IS which have been incubated at +4°C, +20°C, +37°C and +45°C for 6 months will be included in the study to assess the stability of the candidate standard. Ampoules will be identified by a code letter. Not all participants will receive accelerated thermal degradation (ATD) samples. Participants receiving ATD samples will receive ampoules coded 17/102 Sample F to 17/102 Sample J.

Human serum samples
Samples coded PSASerum1 to PSASerum15 contain human serum with a free PSA content of between 0.1 and 7 µg/L. The serum samples coded PSASerum1 to PSA Serum13 were prepared by dilution of clinical remnant samples containing elevated PSA (Cerba Specimen Services, Saint-Ouen l'Aumône, France) in a normal male serum pool (First Link UK, Wolverhampton, UK). The clinical remnant and serum diluent identity numbers are indicated on the label. The serum samples coded PSASerum14 and PSASerum15 contain individual normal male serum obtained from First Link UK and the batch numbers for these are indicated on the label. Samples coded PSASerum1 to PSASerum15 were tested at NIBSC and were found to be non-reactive for HBsAg, HIV antibody and HCV RNA by PCR.

These materials are only to be used for this study and in accordance with the UK Human Tissue Act or equivalent national legislation and are to be destroyed at the end of the collaborative study.

All material of human origin should be considered as potentially hazardous and handled with appropriate care. It should be used and discarded according to your own laboratory's safety procedures.

Handling of the Preparations
On receipt, vials/ampoules should be stored at -20°C or below until use. Before opening, vials and ampoules should be brought to room temperature to minimise moisture uptake. Serum samples should be stored at -20°C or below until use. Serum samples should be thawed, brought to room temperature and mixed gently before measuring.

Tests requested
Participants are requested to carry out the **free PSA** immunoassay method(s) in use in their laboratory and which are used clinically for the measurement of patient samples. Participants are requested to prepare dilutions of the ampouled/vialled preparations and to measure, in triplicate*, the free PSA content of these and the free PSA content of the serum samples. The test concentrations are described below.

Participants are asked to perform **two independent runs**. An independent run consists of the measurement of one set of test samples ('dilutions') prepared from previously unopened vials/ampoules and one set of serum samples (n=15) which have been thawed specifically for that run. An independent run will use a single calibrated kit, integral or plate as required for your method.
Each independent run will also include the measurement of the kit calibrators or in-house standards for that method.
*If using a 96 well plate format, please measure the dilutions of the current and candidate standards in triplicate and the serum samples in duplicate.

Common tests sample concentrations
In order to compare different assay methods, participants are asked to prepare and measure the free PSA content of eight dilutions of 96/668 and 17/102 which are common to all participants. These are **16**, **8**, **4**, **2**, **1, 0.5, 0.25** and **0.125 µg/L** free PSA. Participants are asked to include all possible common concentrations and to prepare additional test samples as required, to ensure that a minimum of **five points in the linear part of the dose response curve are measured**.

Stock solutions
The contents of the vials or ampoules should be reconstituted in water to prepare a stock solution of 500 ng/ml. Where a fresh vial will not be available for subsequent assays, the stock solution may be aliquotted and snap frozen for use in subsequent assays. Where this is the case, participants are requested to provide details of freeze-thaw steps.

Assay diluent
Participants are asked to prepare further dilutions in a suitable assay diluent. The diluent should include protein to prevent surface adsorption (typically 0.1 % (w/v) bovine serum albumin or 0.1 % (w/v) human serum albumin). The free PSA in 96/668 has some enzymatic activity and can react with protease inhibitors. It should not be mixed with any serum-based matrix containing such activity (Stamey et al.,1998).

Participants are requested to provide details of the reconstitution of the ampoules, all pre-dilutions and the dilutions used to prepare the test samples.

Submission of data
Participants are asked to submit data as an Excel file.

For each run, participants are required to provide details of:
- the diluent used to prepare the test samples
- the volumes used to prepare the dilution series of each vial/ampoule
- the signal (absorbance, RLU, counts) for each replicate of the kit or in house calibrators
- the signal (absorbance, RLU, counts) for each replicate of the test samples
- the signal (absorbance, RLU, counts) for each replicate of the serum samples
- the reported concentration (µg/L) for each replicate of the test samples
- the reported concentration (µg/L) for each replicate of the serum samples

A suggested reporting table is shown in **Appendix 1.**

Participants' estimates of the free PSA content of the ampouled preparations are requested, as calculated by the method normally used in their laboratory. However, it must be noted that data

from all participating laboratories will be analysed at NIBSC and this may result in small differences between participants' estimates and the values reported after central computation.

Use of Samples
The use of the current and candidate standard and serum samples is restricted to this study.

Publication
The publication of data arising from the use or analysis of the candidate standard or serum samples is not permitted.

Report
A preliminary report will be prepared and circulated to all participants for comment before submission to the Expert Committee on Biological Standardization of the WHO. Participating laboratories will be identified by a laboratory number only and any requests to treat information in confidence will be respected.

For further information, please contact Dr Jackie Ferguson (e-mail: jackie.ferguson@nibsc.org)
National Institute for Biological Standards and Control (http://www.nibsc.org)
Tel: 44 (0) 1707 641000; Fax: 44 (0) 1707 641057

References
Stamey TA., Chen Z., Prestigiacomo AF. 1998, Clin. Biochem. **31**, 475-481
WHO 2002, ECBS 50th Report, Technical Report Series, **904**, pg 18

Appendix:
a. Example data reporting table for recording the free PSA content of the test samples
Please submit as an Excel file.

International Collaborative Study to establish a replacement WHO International Standard for Prostate Specific Antigen (free)							
PSA FREE		**Platform:**			**Method:**		
Run No.:		**RLU/Absorbance Units/Counts**			**Reported fPSA concentration (µg/L)**		
***Sample**	**Nominal fPSA (µg/L)**	**1**	**2**	**3**	**1**	**2**	**3**
Baselines	0						
Kit standard 1							
Kit standard 2							
Kit standard 3							
Kit standard 4							
Kit standard 5							
96/668 dil^{n} 1	16						
96/668 dil^{n} 2	8						
96/668 dil^{n} 3	4						
96/668 dil^{n} 4	2						
96/668 dil^{n} 5	1						
96/668 dil^{n} 6	0.5						
96/668 dil^{n} 7	0.25						
96/668 dil^{n} 8	0.125						
17/102 dil^{n} 1	16						
17/102 dil^{n} 2	8						
17/102 dil^{n} 3	4						
17/102 dil^{n} 4	2						
17/102 dil^{n} 5	1						
17/102 dil^{n} 6	0.5						
17/102 dil^{n} 7	0.25						
17/102 dil^{n} 8	0.125						
PSASerum1							
PSASerum2							
PSASerum3							
PSASerum4							
PSASerum5							
PSASerum6							
PSASerum7							
PSASerum8							
PSASerum9							
PSASerum10							
PSASerum11							
PSASerum12							
PSASerum13							
PSASerum14							
PSASerum15							

*expand table for coded ampoules and/or additional dilutions

Appendix 3

WHO International Standard
2nd International Standard for Prostate Specific Antigen (free)
NIBSC Code: 17/102
DRAFT instructions for use
(Version1, Dated XX/11/2019)

1. INTENDED USE

The 1st International Standard (IS) for Prostate Specific Antigen (free) in ampoules coded 96/668, has been widely used for the calibration of immunoassays for free PSA. Stocks of the 1st IS are exhausted and the WHO Expert Committee on Biological Standardization (ECBS) has recognized (2014) the need for a replacement IS. The 1st IS for PSA contained seminal plasma-derived free PSA and had an assigned content of 1 µg PSA per vial (1). Prepared using non-complexing, seminal-fluid-derived free PSA, the 2nd WHO IS for PSA (free) was established at the 69th Meeting of WHO ECBS (2018). This material replaces the 1st IS for PSA (free), 96/668, which is discontinued.

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 regards 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

Each ampoule of the International Standard contains 0.53 µg free PSA

4. CONTENTS

Country of origin of biological material: USA
Each ampoule contains the residue, after freeze-drying, of a 1.0 ml volume of a solution of 20 mM sodium phosphate buffer pH 7.4, 150 mM sodium chloride, 10 g/L bovine serum albumin (BSA) and 0.53 µg/ml non-complexing prostate specific antigen (free).

5. STORAGE

Unopened ampoules 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

DIN ampoules have an "easy-open" coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar. Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by

the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution. For all practical purposes each ampoule contains the same quantity of the substances listed above. Depending on the intended use, dissolve the total contents of the ampoule in a known volume of a suitable diluent. Users should make their own investigations into the type of diluent suitable for their use. If extensive dilutions are prepared, a carrier protein should be added. The ampoules do not contain bacteriostat and solutions of the material should not be assumed to be sterile.

8. PREPARATION OF AMPOULES

A bulk solution of purified non-complexing (free) PSA without α-1-antichymotrypsin (Scripps Laboratories, San Diego, USA) was prepared in 20 mM sodium phosphate buffer pH 7.4, 150 mM sodium chloride, 10 g/L BSA at a concentration of 500 ng/ml. The solution was distributed into ampoules as 1.0 ml aliquots. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen. Ampoules were stored at -20°C.

The batch of ampoules, coded 17/102, was evaluated in a collaborative study in which ten laboratories in eight countries participated providing

1. To value assign the candidate standard, 17/102
2. To assess the suitability of the candidate standard, 17/102, to serve as the 2nd IS for the calibration of immunoassays of free PSA.
3. To determine the stability of the candidate standard, 17/102, by comparison with ampoules stored at elevated temperatures as part of an accelerated degradation stability

Reported estimates of content of 17/102 from each method gave a geometric mean estimate of 0.545 µg/amp (95% CI 0.508 – 0.586, n=21) with a robust mean of 0.533 µg/amp. The geometric mean of the ratio of content estimates for 17/102 to 96/668 for each method was 0.516 (95% CI 0.489 – 0.544, n=21) with a robust geometric mean of 0.512.

9. STABILITY

Analysis of accelerated thermal degradation samples of 17/102, measured by participants in the collaborative study showed a predicted loss of 0.096 % of free PSA immunoreactivity per year when stored at -20ºC. NIBSC follows the policy of WHO with respect to its reference materials. It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended. Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference materials should be stored on receipt as indicated on the label. In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

10. REFERENCES

(1) Rafferty, B., Rigsby, P., Rose, M., Stamey, T. and Gaines Das, R (2000) Reference Reagents for Prostate-specific Antigen (PSA): Establishment of the First International Standards for Free PSA and PSA (90:10). *Clin Chem* **46**, 1310-1317

11. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of all the participants in the collaborative study and Dina Patel, UK NEQAS Immunology, Immunochemistry & Allergy, Sheffield, UK.

12. FURTHER INFORMATION

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

13. CUSTOMER FEEDBACK

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

14. CITATION

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

15. MATERIAL SAFETY SHEET

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

16. 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 11of the Conditions.

17. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.02 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. Attached: No

18. 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_biol efstandardsrev2004.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.