EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 23 to 27 October 2006

Report of a Collaborative Study to Assess the Suitability of a Replacement
for the 2nd International Standard for Anti-Measles Serum

Maureen Bentley, Peter Christian, Bernard. J. Cohen(1) and Alan Heath, National Institute for
Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Herts
EN6 3QG, U.K. and (1) Health Protection Agency Centre for Infections, 61 Colindale Avenue,
London NW9 5EQ, UK

© World Health Organization 2006

All rights reserved. Publications of the World Health Organization can be obtained from WHO Press, World Health
Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-
mail: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for
sale or for noncommercial distribution – should be addressed to WHO Press, at the above address (fax: +41 22
791 4806; e-mail: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of
any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country,
territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines
on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or
recommended by the World Health Organization in preference to others of a similar nature that are not
mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital
letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained
in this publication. However, the published material is being distributed without warranty of any kind, either
expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no
event shall the World Health Organization be liable for damages arising from its use. The named authors [editors]
alone are responsible for the views expressed in this publication.
SUMMARY

A candidate replacement standard for the 2nd International Standard (IS) for Anti-Measles Serum (NIBSC Code 66/202) was produced from a pool of defibrinated plasma. This candidate (NIBSC Code 97/648) was filled, lyophilized and sealed into ampoules in November 1997 and approximately 2700 ampoules are available to WHO. Stability studies on the candidate predicted losses of 0.78% per year and <0.001% when stored at the recommended temperature (-20°C) and activity assayed by Plaque Reduction Neutralization Test (PRNT) or Enzyme-Linked Immunosorbent Assay (ELISA) respectively. A collaborative study was performed to further characterize the candidate standard and eleven laboratories from 8 countries participated in, and completed this study. The study samples comprised the candidate (in coded duplicate) and three other lyophilized sera along with the current 2nd IS (66/202). Laboratories were invited to carry out assays by the methods that would normally be employed in that laboratory. In total five laboratories elected to perform PRNT assays, five ELISA assays and two Tissue culture Neutralizing Dose (TCND50) (microneutralization) assays. One laboratory performed both PRNT and ELISA assay. Potencies for the candidate and other samples included in the study were calculated in International Units (IU’s) by reference to the existing 2nd IS. In the PRNT assays the potency for the candidate 3rd IS was found to be 2.87 IU/ampoule while for ELISA the mean potency was estimated at 5.37 IU/ampoule. The difference in potency estimates arising from the different test methodologies has not been noted previously for anti-measles preparations. As continuity of the IU from the 1st International Reference Preparation (IRP) is documented better for PRNT we have concluded that at the present time the candidate 3rd IS should be assigned a potency only for the PRNT. Further studies will be performed to assess the suitability of the candidate for use in ELISA and to provide an appropriate unitage.

INTRODUCTION

Quantification of measles antibody in human sera is an important marker and/or diagnostic tool in assessing the immune status of individuals. Such data are invaluable in designing and monitoring immunization campaigns and in the protection of public health. Standardization of serum neutralizing activity or antibody levels is crucial in making decisions about immune status. The need for standardization of measles antibody assays was realized in the early 1960s when the 1st International Reference Preparation for anti-measles serum was established (WHO, 1964).

The dual International Standard for anti-measles and anti-polio sera (2nd International Standard Anti-Measles serum (Human)/2nd International Standard for anti-poliovirus serum types 1, 2, and 3: NIBSC Code: 66/202) was established by the Expert Committee on Biological Standardization of the World Health Organization in 1991 to replace the 1st International Reference Preparation (IRP). The original International Reference Preparation (IRP) for anti-measles serum was established in 1964 from material filled and characterized by the Statens Seruminstitut in Denmark. The IRP was established through a collaborative study (WHO, 1964) and a unitage assigned of 10 IU/ampoule. The assays used in its characterization included Plaque Reduction Neutralization Test (PRNT), Haemagglutination Inhibition (HI) and Complement-Fixation (CF).

Stocks of the 1st IRP ran very low around 1989 and a replacement for the 1st IRP was selected through a collaborative study in 1990 (Forsey et al., 1991). This was first termed the 1st International Standard (IS) (Forsey et al., 1991) but was subsequently renamed the 2nd IS to
avoid confusion with the 1st IRP (WHO, 1991). In addition, the same material was later established as the 2nd IS for Anti-Poliovirus Serum Type 1, 2 and 3 (WHO, 1992). This dual standard is the preparation 66/202.

The collaborative study for the establishment of the 2nd IS involved 4 laboratories and included HI, PRNT and ELISA. The limited data generated in this study indicated that the three methods gave similar results and a potency of 5 IU/ampoule was assigned to 66/202.

Stocks of the 2nd IS are now exhausted and there is an urgent need for a replacement both for anti-measles and anti-polio antibodies. After endorsement from WHO in November 2004 a collaborative study was launched in 2005 to find replacements for 66/202. The study to identify and characterize an anti-measles serum replacement for 66/202 involved 12 laboratories from 8 countries; a separate study has been carried out to establish an anti-polio serum replacement.

This report describes the study to identify and characterize a candidate anti-measles material and the suitability of the candidate as the 3rd International Standard. As with previous anti-measles IRPs and ISs, according to the principles laid down in ISO17511 the new 3rd IS would be an International Conventional Calibrator that has no international conventional reference measurement procedure and without traceability to SI.

**BULK MATERIALS AND PROCESSING**

**Preliminary Studies**
At the start of this study a number of filled and lyophilized human sera were available at NIBSC that had been found to have anti-measles activity. The candidate 3rd IS (97/648) was one of these samples and was still available in sufficient numbers to provide a candidate which would last for >10 years based on current usage.

To assess the suitability of the candidate for the intended use further assays were carried out in 2004 at NIBSC. The method used was a Plaque Reduction Neutralization Test (PRNT) in 24 well tissue culture plates. In this assay measles virus produces plaques in sensitive cell culture. Specific anti-measles antibodies neutralize the virus and prevent plaque formation. Dilutions of the test sera were mixed with the standard dose of challenge virus and the mixture added to the Vero cells. After incubation the plaques were counted. The dilution of the serum reducing the number of plaques by 50% was taken as the end-point. XY

**Preparation of the Candidate 3rd IS**
The candidate replacement standard, NIBSC Code 97/648, was produced from a pool of defibrinated plasma supplied by CLB, Amsterdam in September 1997. The plasma was filled, lyophilized and sealed into ampoules by The Centre for Biological Resource Materials (CBRM – NIBSC) on November 1997. The fill was originally intended for use as an anti-HAV plasma standard, however it was subsequently considered unnecessary and was not adopted for the intended use. The ampoules have been stored since production at -20°C at NIBSC. Approximately 2700 ampoules are available to WHO.

The pool, in a single container, was received by CBRM on 12/11/97 and stored overnight at +4 °C before filling. The ampoules were ink jet labelled before filling and the total number of ampoules filled on 13/11/97 was 4022. The mean weight of the fill was 1.02041g, (taken from a mean of 85 with check weights being taken at approximately two minute intervals) with a coefficient of variation of 0.31%. Freeze drying commenced 13/11/97 and was completed
18/11/97. The ampoules were stoppered and a further desiccation started 18/11/97 and completed 24/11/97 when they were sealed under dry nitrogen. Results for testing for vacuum, cracks or pin-holes showed nil failures. The mean dry weight of the fill measured by coulometric Karl Fischer was 81.17mg (taken from a mean of 6) and the residual moisture content 0.12%.

The preparation has been tested and found negative for HBsAg, HCV antibody, HIV antibody and HCV RNA by PCR. A summary of the product characteristics is shown below.

<table>
<thead>
<tr>
<th>Product Summary for the Candidate 3rd International Standard for Anti-Measles (Plasma) (97/648)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
</tr>
<tr>
<td>Number of ampoules available</td>
</tr>
<tr>
<td>Excipients/additives</td>
</tr>
<tr>
<td>Coefficient of variation of the liquid fill</td>
</tr>
<tr>
<td>Residual Moisture</td>
</tr>
</tbody>
</table>

STABILITY STUDIES

Accelerated Degradation

Methods

Samples of the candidate 3rd IS, 97/648, were removed from NIBSC storage at -20°C in January 2005 and maintained at -70°C, +4°C, +20°C and +37°C for 14 months. Samples from each temperature were tested alongside the candidate material that had been kept at -20°C by both PRNT and ELISA in March 2006. PRNT was performed by a modification of the method of Albrecht et al., (1981) while ELISA was performed using the commercial Enzygnost anti-measles IgG kit produced by Dade Behring (Marburg Germany).

In the above studies the stability of the candidate 3rd IS could not be compared directly to the 2nd IS as stocks of this material were no longer available. Data are therefore reported in terms of PRNT50 end-points rather than in IU's or as relative potencies (expressed as percentage) against the -20°C baseline samples. For the ELISA assays the potencies are given as the output from the manufacturers procedure for the kit – which are expressed in mIU/ml or as potencies (in percentage) relative to the -20°C baseline.

Results

PRNT: The samples stored at elevated temperatures were assayed concurrently by PRNT in one laboratory in three separate assays. In each assay, triplicate dilution series of each sample were used. The potencies of all samples were calculated relative to that of the -20°C sample, and are shown in Table 1.

There was no evidence of any difference between the samples stored at -20°C or -70°C. Samples stored at +4°C and +20°C retained around 80% of their neutralizing activity, although the variability of the PRNT assay means that this is not a precise estimate. At +4°C the estimates vary from 62% to 107% across the assays. At +37°C there was some loss of activity (approximately 55%).
Applying the usual Arrhenius model to the data gives a predicted % loss per year of 0.78% at -20°C. The predicted loss per month at +4°C and +20°C are 0.5% and 1.7% respectively. However these estimates are not reliable, with the standard error of the estimate greater than the estimate itself in all cases. This is the result of the poor precision of the PRNT assay when looking for small losses in potency.

There was no evidence that the candidate standard will be unstable, but studies with the PRNT assay need to continue over a longer time period to obtain more reliable predictions of stability.

**ELISA:** The samples stored at elevated temperatures were assayed concurrently by ELISA in one laboratory using the Enzygnost kit in three separate assays. In each assay, triplicates of each sample were used. The mean estimates (mIU/ml) obtained from the Enzygnost kit are shown in Table 2. The potencies of all samples were calculated relative to that of the baseline -70°C sample, and are shown in Table 3.

Applying the usual Arrhenius model to the data gives a predicted % loss per year of <0.001% at -20°C and 0.9% at +20°C. The predicted loss per month at +4°C and +20°C are 0.004% and 0.08% respectively. Even at +37°C the predicted loss per month is only 1.2%.

These results indicate that the candidate standard 97/648 is very stable when used in the ELISA assay.

**Stability on Reconstitution:**

**Methods**

Three ampoules of the candidate 3rd IS, 97/648 were reconstituted (1ml per ampoule) in sterile water and dispensed into 50 μl aliquots. One aliquot from each ampoule was tested immediately by PRNT and the remaining aliquots were stored at -20°C, +4°C or at ambient (between 15-20°C). Samples were then assayed at 4 weeks post reconstitution. The stability of the candidate 3rd IS in these studies could not be compared directly to the 2nd IS as stocks of the latter were no longer available. Data are therefore reported in terms of PRNT 50% end-point titres rather than mIU/ml.

**Results**

A summary of the results is shown in Table 4. These data indicate that storage of the material at -20°C after reconstitution does not reduce the activity of the sample. The data also indicate that after reconstitution the PRNT activity of samples stored at +4°C and ambient temperatures were observed to decrease relative to those stored at -20°C. However, these decreases may be due to assay variability but this requires further investigation.

These data indicate that short-term (up to 28 days) storage at -20°C of the reconstituted (and aliquotted) candidate 3rd IS is acceptable but that freeze-thawing and/or longer term storage and/or storage at temperatures higher than -20°C is not recommended.

**Effect of Heat Inactivation**

**Methods**

All laboratories performing the PRNT assay used a heat inactivation step, normally 56°C for 30 minutes. None of the laboratories performing an ELISA assay did so. A small study in one laboratory looked at any possible effect of the heat inactivation step on the potency estimates. Samples of the current IS 66/202 and the candidate IS 97/648 were assayed by PRNT with
and without heat inactivation concurrently in three separate assays. In each assay, triplicate dilution series of each sample were used.

**Results**
The potencies of all samples were calculated relative to that of heat inactivated 66/202, and are shown in Table 5.

There was variability between the three assays, as expected for the PRNT assay. Overall, there was no significant effect on potency of the heat inactivation.

The overall potency of 97/648 relative to 66/202 obtained from these assays for both heat inactivation and untreated samples was 2560 mIU/ml. This figure was obtained by pooling the data from the heat inactivated and non heat inactivated samples in each assay and calculating an overall geometric mean potency of 97/648 relative to 66/202.

**COLLABORATIVE STUDY**

**Study Plan**

**Aim of the study**
The aim of the study was to assess the suitability of a candidate replacement for the current International Standard (66/202) for the assay of measles antibodies and to determine a potency for anti-measles activity in International Units.

**Participants**
12 Laboratories from 8 countries agreed to participate in the collaborative study, (4 National Control Laboratories, 4 vaccine manufacturers and 4 Public Health Laboratories). Data were received only from 11 of these 12 laboratories (Appendix 1).

Laboratories are referred to by a code number throughout this report. Code numbers were allocated at random, and are not related to the order of listing in Appendix 1.

**Materials and Methods**

**Study samples**
Study samples comprised 3 samples of lyophilized human serum or defibrinated plasma and duplicates samples of the candidate replacement. These five samples were coded A – E and were distributed along with samples of the 2nd International Standard for anti-measles (66/202). The participants were provided with 3 ampoules of each coded sample and three of the International Standard for anti-measles (66/202).

All study samples had been stored at -20°C prior to the study and were distributed by courier to participants at ambient temperatures. Participants were requested to maintain samples at -20°C after receipt and prior to testing.

All of the samples were supplied as lyophilized material which required reconstitution in 1ml of distilled water. If heat inactivation of human sera was routinely carried out by participants prior to testing it was recommended that this procedure be carried out according to in-house protocols and recorded on the result sheets.

The study samples supplied are summarized below.
- Sample A - low potency sample human serum, NIBSC code 66/387
- Samples B and D - candidate standard, defibrinated human plasma, NIBSC code 97/648
- Sample C - human serum, NIBSC code 82/585
- Sample E - human serum, NIBSC code 83/571

**Study design**
Participants were requested to carry out 3 independent assays for the measles antibody content of the study samples using the assay method in routine use in their laboratory. A freshly opened and reconstituted ampoule of each preparation was used for each assay and the assay was performed on different days, generally one week apart. Each assay used a different ampoule of each sample and was tested concurrently with an ampoule of the 2nd IS.

Due to the shortage of some materials included in the study it was suggested that at least 3 aliquots of each reconstituted ampoule be made and that one aliquot be used for the assay and the remainder frozen at -20°C to be used for further assays or re-tests. Laboratories that performed more than one test were provided with only one set of samples and had to follow these instructions if assays could not be completed on the same day.

**Statistical Methods**
The PRNT assays were analyzed using a modified Spearman-Kärber method to determine a 50% neutralizing end-point (NEP50) for each sample in each assay. A potency estimate for each sample, in mIU/ml, was obtained by taking the ratio of the estimated NEP50 for the sample and that of the current IS 66/202, and multiplying by the assigned unitage of 5000 mIU/ml for 66/202.

The assays for Tissue Culture Neutralization Dilution (TCND50) were analyzed using Spearman-Kärber, and potencies relative to 66/202 calculated in a similar fashion.

For the ELISA assays, the estimates returned by the participants were used. These were based on calculations defined in the manufacturers instruction from the kit. Potencies were calculated relative to 66/202.

For each sample, laboratory mean estimates of potency were calculated as geometric means of individual assay estimates. Overall method means were calculated as geometric means of individual laboratory means. Variation between laboratories was assessed by calculating percent geometric coefficients of variation (%GCV).

The variation between assays within laboratories was assessed by calculating geometric coefficients of variation (%GCV) between assays of potency estimates for each sample. An overall figure for each laboratory and method was obtained by taking a pooled estimate of the %GCV across the five study samples A – E.

The variation within assays between the coded duplicate samples B and D was assessed by calculating the difference in log potency estimates of B and D within each assay, and calculating the root mean square average of this difference across assays (square root of the average squared difference). This was then expressed in percentage terms as the average fold difference (lowest to highest). For example, a two-fold difference between potency estimates of B and D in an assay would represent a 100% difference.
The difference in potency estimates for the candidate standard relative to 66/202 between PRNT and ELISA methods was assessed using a non-parametric Wilcoxon Two-sample rank test. The differences between laboratories using an individual method were assessed using an analysis of variance, with allowance for multiple comparisons.

Assay Methods and Data Received
Details of methods used by the 11 laboratories that submitted data are shown in Table 6. Three basic assays were performed. Plaque reduction neutralization (PRNT), microneutralization in a format similar to TCID_{50} assays – termed for this study Tissue Culture Neutralizing Dilution 50% (TCND_{50}) - and Enzyme-Linked Immunosorbent Assay (ELISA). The PRNT assay was performed by 5 laboratories, TCND_{50} by two laboratories, and ELISA by 5 laboratories – with 1 laboratory completing both PRNT and ELISA. The TCND_{50} assays are coded T or TCND_{50} in the figures and tables.

PRNT Assays:
Assay methods amongst the laboratories that performed this assay generally followed the methodology of Albrecht et al, 1981. However, there were differences between laboratories with respect to the neutralization conditions and strain of virus utilized and in how each laboratory completed the assay.

Laboratory 1 provided data from three assays each from two operators. In each assay, samples A-E were tested in duplicate dilution series, with 66/202 as a single dilution series.

Laboratories 4, 9 and 12 provided data from three assays. In each assay, samples A-E were tested in a single dilution series. Laboratories 4 and 12 tested 66/202 in triplicate dilution series while Laboratory 9 tested it as a single dilution series.

Laboratory 3 provided data from three assays. They returned raw plaque counts for samples A-E (in duplicate) and for 66/202. They included an additional sample for 66/202 that had been reconstituted at an earlier date. They returned calculated potencies relative to both samples of 66/202. The geometric means of the two calculated potencies were used for further analysis.

TCND_{50} Assays:
As for the PRNT assay the specific conditions used by the two laboratories performing this assay were slightly different (see Table 6). In terms of the assay format, Laboratory 6 provided data from three assays. For all three assays they were unable to obtain a 50% endpoint for samples B or D, the candidate standard, and reported problems with cytotoxicity of the reconstituted sample. Eight replicate wells were used per dilution and sample.

Laboratory 8 provided data from three assays. For each assay, the dilution series of each sample was tested only in single wells. The resultant estimates of neutralizing dilution (ND_{50}) will therefore be less precise than assays using 8 replicates per dilution and sample.

ELISA Assays
For the ELISA test 5 of the 6 laboratories used the Enzygnost kit (Dade Behring) which provides a potency for a sample expressed in IU/ml. The calculation method specified that estimates of IU/ml with this kit are based on a calibration against the 1st IRP. Assays were conducted and data returned from laboratories in the following formats. Unless otherwise stated the Enzygnost kit was used.
Laboratories 2 and 5 provided data from three assays, using the Enzygnost kit, with results expressed as mIU/ml based on the kit calculation method. These results were converted to potencies (mIU/ml) relative to 66/202 which was included in each assay.

Laboratories 4 and 10 provided data from three assays, using the Enzygnost kit. Results were expressed as mIU/ml based on the kit calculation method and calculated potencies relative to 66/202 were also provided.

Laboratory 11 provided data from three assays, using an in-house method. Results were expressed as mIU/ml relative to 66/202, as requested.

Laboratory 12 provided data from two assays, using the Enzygnost kit. The first assay did not include 66/202, and so no potencies relative to 66/202 could be calculated. There were large discrepancies between the results for samples A–E between the two assays in terms of the calculated IU from the kit for the other samples. For the second assay that included 66/202 the reported optical densities for the study samples were below that reported for a negative control. The ELISA results from this assay were not included in any subsequent analysis.

RESULTS

Potency relative to 66/202:
The laboratory geometric mean estimates of potency relative to 66/202 are given in Table 7. They are also shown in histogram form in Figure 1.

From the table and figures it is clear that the results from the ELISA assays gave higher potency estimates relative to 66/202 for samples A – E than the other assay methods. The TCND50 and PRNT assays appear to be in broad agreement with each other – albeit that there were limited data from the TCND50 method.

The difference in potency estimates for B & D (pooled) relative to 66/202 between the PRNT and ELISA methods was highly significant (p<0.0001). However, the difference between laboratories using the PRNT assay was not significant. Note, that although there was considerable variability between laboratories, relative to the within laboratory between assay variability, the between laboratory variability was not significant.

The overall mean (geometric mean of individual laboratory means) estimates of potency relative to 66/202 are shown in Table 8, for the ELISA and PRNT methods. The estimates for the duplicate samples B and D are in good agreement and so the overall mean for samples B & D are also shown in Table 8.

The difference between ELISA and PRNT methods was reasonably consistent across all the study samples A – E and was close to two-fold greater for the ELISA than for the PRNT.

The overall potency of 97/648 (B & D) relative to 66/202 obtained from the study of heat inactivation was 2560 mIU/ml. This figure is in good agreement with the overall estimate of 2869 mIU/ml obtained in the collaborative study with the PRNT assay (table 8).

The within assay variability was assessed by comparing the results of the coded duplicate samples B and D within individual assays. The average “% difference” for each laboratory and method is shown in Table 9 A two-fold difference between potency estimates of B and D in an assay would represent a 100% difference. There was considerable variation in performance between different laboratories, although it must be remembered that the figures
are based on a limited number of assays (three in most cases). Generally the ELISA method had much better agreement between duplicate samples than PRNT, as expected. Exceptions were the PRNT from laboratory 4 which had very good agreement (11%), and the ELISA assays from laboratory 11, which have poorer agreement (39%) than for other laboratories using ELISA. The pooled estimates (overall average) for the two methods are 22% and 50% for ELISA and PRNT respectively. These figures represent the “typical” precision that can be expected within an assay.

The within laboratory, between assay variability was assessed by calculating the %GCV of potency estimates between individual assays within a laboratory. The pooled estimate across the 5 study samples A – E for each laboratory and assay method are shown in Table 10. For the ELISA method, the between-assay variability was very similar for all laboratories, with %GCV between 13% and 23%. The overall between-assay %GCV for the ELISA method was 19%. For the PRNT assays, the variability was greater, as expected. There were considerable differences in performance between laboratories however, with the between assay %GCV ranging from 32% (laboratory 3) to 108% (laboratory 1). The overall between assay %GCV for the PRNT method was 48%, but quoting an overall figure masks the difference in performance between individual laboratories. These figures highlight the lack of precision of a single PRNT assay. Depending on the purpose of the assay, it may be necessary to consider replicate assays.

The variability between laboratories was also expressed as a geometric coefficient of variation (%GCV), and these data are shown in Table 11. The overall variability between laboratories was high, particularly for the low potency sample A i.e. the %GCV between laboratories was 85% for A, and around 50% for samples B-E. Looking at the results from PRNT and ELISA methods separately, it is clear that the ELISA method was much more consistent between laboratories. Although results for the low potency sample A were still highly variable with a %GCV of 50%, for samples B-E the results were more consistent with a %GCV of around 10%.

**Potency relative to Candidate Standard Sample B**
The potencies of the study samples A, C and E were calculated relative to the candidate standard sample B, taking B to have a hypothetical unitage of 3000 mIU/ml. The overall mean potencies for samples A, C, D and E are shown in Table 12, with the between laboratory %GCVs shown in Table 13. The individual laboratory potency estimates are also shown in histogram form in Figure 2.

When the samples were analyzed using an assigned unitage for the candidate 3rd IS, the differences between the PRNT and ELISA methods were much reduced, and no longer significant. As a result, the overall variability (between laboratory %GCV – Table 13) was less than for the potencies calculated relative to 66/202. The variability for the low potency sample A was higher than for the other samples for both methods, as before but for samples C, D and E, the variability between laboratories using the PRNT method was lower. This is to be expected for sample D, a coded duplicate of B, as assaying a sample against itself should be consistent independent of method. The ELISA method again showed good consistency between laboratories.

**Estimates of mIU/ml from the Enzygnost kit:**
The Enzygnost kit used by the majority of laboratories performing ELISA provides estimates of mIU/ml for samples, based on a calculation method defined in the manufacturers instructions. These estimates are based on a calibration against the 1st IRP for anti-measles serum. Three of the laboratories that used the Enzygnost kit in the collaborative study
returned the kit estimates of mIU/ml for the study samples, as well as the requested estimates relative to the current IS 66/202.

The geometric mean estimates obtained for 66/202 by laboratories 2, 4 and 5 were 7877, 6985 and 8341 mIU/ml respectively. The overall mean of these three laboratories was 7713 mIU/ml. The assigned unitage for 66/202 is 5000 mIU/ml. Clearly the calibration of the Enzygnost kit against the 1st IRP was inconsistent with the unitage of the 2nd IS 66/202, with results being around 50% higher.

This effect was also seen in the ELISA data from the accelerated degradation study. The three assays of the sample of 97/648 stored at -20°C gave an estimate of 7855 mIU/ml based on the kit calculations (Table 2). The overall mean estimate of 97/648 relative to 66/202 from all ELISA assays in the collaborative study was 5366 mIU/ml. Again this represents a discrepancy with the kit estimates being around 50% higher.

**DISCUSSION**

The current study was initiated to find a replacement for the current (2nd) International Standard for anti-measles serum. Eleven laboratories contributed to the study and applied the full range of methods that are commonly in current use for the estimation of anti-measles titre in human serum or plasma i.e. PRNT, microneutralization (TCND50) and ELISA.

Data for the TCND50 was relatively limited but seems to be in agreement with that generated by the PRNT assay – which was not unexpected as both rely on the neutralization of virus activity in an *in vitro* assay system. However, when potencies were calculated against the current, 2nd IS (assigned a unitage of 5 IU/ampoule), using the either the PRNT or ELISA method there was a significant 2 fold difference in the potency obtained. This may not be surprising as the ELISA assay measures the amount of anti-measles IgG and not the amount of neutralizing antibody. Moreover, the reactivity of test sera in measles IgG ELISA or PRNT reflects differences in the specificity of antibodies measured by the two methods. ELISA based on cell culture grown measles antigen, such as the Dade Behring ELISA, detects predominantly antibody to nucleocapsid antigen, which is the most abundant viral protein in measles-infected cell culture (Griffin, 2001). Neutralization assays, however, detect antibody directed predominantly against the haemagglutinin protein, though there is also a smaller contribution by neutralizing antibodies against the fusion protein (Griffin, 2001).

The findings of the collaborative study thus indicated that there was a problem with the definition of the IU – and that a single unitage could not be assigned for the candidate IS that was applicable to both ELISA and PRNT. Following on from this we then considered the continuity of the IU.

**Continuity of the IU**

The 1st International Reference Preparation (IRP) for anti-measles serum was established in 1964 as the result of a collaborative study performed by the by the Statens Seruminstitut in Denmark. The IRP was assigned an arbitrary unitage of 10 IU/ampoule from this study in which the participating laboratories had used Plaque Reduction Neutralization (PRNT) (5 laboratories), Haemagglutination Inhibition (HI) (4 laboratories) and Complement-Fixation (CF) (4 laboratories).

Stocks of the 1st IRP ran very low around 1989 and a replacement for the 1st IRP was selected through a collaborative study in 1990 (Forsey et al., 1991). This was first termed the 1st International Standard (Forsey *et al.*, 1991) but was subsequently renamed the 2nd IS to avoid
confusion with the 1st IRP (WHO, 1991). However this study involved only 4 laboratories with 2 laboratories using HI, 1 laboratory PRNT and 1 laboratory using ELISA. The limited data generated in this study indicated that the three methods gave similar results and a potency of 5 IU/ampoule was assigned to 66/202, based on the calibration against the 1st IRP.

The study for the 1st IRP showed that there was good agreement between HI and PRNT assays and so the 3 assays (2 HI and 1 PRNT) used to calibrate the 2nd IS against the 1st IRP provided a limited, but consistent link to the IU established with the 1st IRP. The studies reported here would, likewise, indicate that there is good continuity for the IU through to the candidate 3rd IS as estimated by PRNT – but this is not true for ELISA.

ELISA was not included in the study to establish the 1st IRP and there was limited use of this assay (1 laboratory) in the study to establish the 2nd IS, albeit that ELISA gave consistent results with the other assays used in this study. The ELISA assay was used extensively in the current study; however, the quantitative results using ELISA were not consistent with those from the neutralization assays. There is therefore no established link between the 1st IRP, the 2nd IS and the candidate 3rd IS using ELISA. The situation is complicated further by the fact that the commercial ELISA kit used by 4 out of 5 laboratories in this study was calibrated by the kit manufacturer directly against the 1st IRP. This calibration gives results in IU for 66/202 that were inconsistent with the assigned unitage of 5IU.

Therefore, at this time we do not consider that it is possible to assign a single unitage for the 3rd IS and so would propose that a unitage be assigned for PRNT only until the issues concerning ELISA are resolved.

**Use of the candidate 3rd IS**

In all of the assays performed in the collaborative study there was only one report of any problems associated with the candidate IS. This was from laboratory 6 who found that the candidate 3rd IS was cytotoxic in their TCND₅₀ assay and could not obtain an end-point for the IS in the assay. There was no further investigation by the laboratory as to the possible causes of the observed cytotoxicity. As relatively few laboratories completed the TCND₅₀ assay we carried out a small trial at NIBSC where all of the study samples were assayed by TCND₅₀ assay. While we observed some cytotoxicity at low dilutions, the end-point dilution for virus neutralization was far below these dilutions and we considered it would therefore not be a problem in routine use of the candidate IS.

When the potencies of study samples A, C and E against the candidate 3rd IS (97/648) were calculated having assigned the latter a theoretical unitage of 3000 mIU/ampoule, there were improved estimates for the between laboratory agreement compared to those derived using 66/202 (see Tables 11 and 13). This indicates that calibration against the candidate 3rd IS provides the level of standardization that was achievable with the previous (2nd) IS and that the candidate 3rd IS is therefore suitable for use as an IS.

Interestingly it can also be seen in Table 12 that when calibrations were made against the candidate 3rd IS that the differences between the estimates obtained for the PRNT and ELISA are no longer apparent. This suggests that 97/648 may be more similar to A, C & E than to 66/202 in relative contents of neutralizing antibody and ELISA measurable antibody – but this clearly needs further investigation.

**Stability of the candidate 3rd IS**

Samples of the candidate 3rd IS, 97/648, were removed from NIBSC storage at -20°C in January 2005 and maintained at -70°C, +4°C, +20°C and +37°C for 14 months. These stability
studies conducted using PRNT (1 laboratory) and ELISA (1 laboratory) showed that the candidate 3\textsuperscript{rd} IS had good stability at the proposed storage temperature of -20°C. Estimated potency losses when stored at -20°C were 0.78% and <0.001% per year when assayed by PRNT or ELISA respectively. The accelerated degradation data at the higher temperatures of 20°C and 37°C indicated that there may be differences in stability of the factors that these two test methods actually measure. In the case of ELISA (Enzygnost kit) this is anti-measles IgG. In the case of PRNT, however, neutralizing activity is due primarily to IgG and other immunoglobulins but may also involve other components of the preparation. Further studies are on-going to investigate this and samples of the candidate 3\textsuperscript{rd} IS from routine storage (-20°C) will be assayed at regular intervals against a -70°C baseline to garner additional data on the real-time stability of the preparation.

Additional stability studies on the candidate 3\textsuperscript{rd} IS involved as assessment of the effect of heat inactivation (commonly used in PRNT at 56°C for 30 minutes) on the reconstituted preparation and the stability of the reconstituted preparation at different temperatures. These studies showed that there was no significant loss of activity after heat inactivation and that storage at -20°C of the reconstituted sample did not lead to a loss of activity (Table 4). Data at the higher temperatures (ambient at 15-20°C and 37°C) for the reconstituted candidate indicated that storage at these temperatures after reconstitution could not be recommended. Further studies are being undertaken to assess the effect of storage after reconstitution and will be used to update the Instructions for use on the Candidate 3\textsuperscript{rd} IS.

Further studies
Data generated from ELISA in the current study was mainly from a single commercial kit, which was expected as the kit is dominant in the market. The findings suggest that it would be useful to test other available ELISA kits based on different antigens and assay formats to assess whether the discrepancy observed between PRNT and ELISA holds true for those too. This study is currently underway in collaboration with another laboratory.

Furthermore, the discrepancy in results from PRNT and ELISA when estimates were calibrated against 66/202 raises a number of questions about the establishment of a unitage for the candidate 3\textsuperscript{rd} IS for ELISA. We plan further investigations of the unitage link between 1\textsuperscript{st} IRP, 2\textsuperscript{nd} IS and candidate 3\textsuperscript{rd} IS using stored materials available at NIBSC. It is expected that this study will be completed for submission to ECBS in 2007.

CURRENT STOCKS & USAGE OF THE IS

The current IS for anti-measles serum (66/202) is a dual standard with anti-poliovirus serum and as such the exact use to which dispatched material have been put is difficult to ascertain. However, for both uses the standard has been used at the rate of around 100 ampoules per year for the last 5 years. On this basis and given that the 3\textsuperscript{rd} anti-measles IS would have a single use we would anticipate that current stock levels (2700) would last in excess of 20 years.

RECOMMENDATIONS

It is recommended that:

1) 97/648 be established as the 3\textsuperscript{rd} International Standard for Anti-Measles. As it is derived from defibrinated plasma rather than serum it is also recommended that it be termed:

3\textsuperscript{rd} International Standard for Anti-Measles (Plasma) (NIBSC Code: 97/648)
and that it be assigned a unitage of 3 IU per ampoule. This unitage, however, applies only to the use of the standard in estimation of anti-measles titre by virus neutralization assays, and more specifically in plaque reduction neutralization assays.

The unit assigned in the 3rd IS is traceable to the current (2nd) IS. The previous standard will be discontinued and there is no formal metrological traceability between the two.

No formal determination of commutability for the new (3rd) IS has been made but the data presented in this report demonstrate that it is fit for the intended purpose i.e. the determination of anti-measles activity by virus neutralization assay.

2) A small study be initiated with the limited samples available to establish the relationship between the 1st RP, the 2nd IS and the candidate 3rd IS for both ELISA and PRNT. This study would initially involve only 2 laboratories but may be extended to include more laboratories at a later stage depending on the availability of material.
REFERENCES

WHO (1992) Expert Committee on Biological Standardization; forty-second report Technical Report Series, 822, 7-8
SUMMARY OF COMMENTS FROM PARTICIPANTS

Laboratory 1
No comments received.

Laboratory 2
A number of editorial changes/clarifications requested. No major comments on recommendations. Final draft revised in response to comments.

Laboratory 3
A number of editorial changes/clarifications requested. No major comments on recommendations. Final draft revised in response to comments.

Laboratory 4
A number of editorial changes/clarifications requested. No major comments on recommendations. Final draft revised in response to comments.

Laboratory 5
Replied but had no comments.

Laboratory 6
No comments received.

Laboratory 7
Did not participate.

Laboratory 8
Agreed with the proposals for the further work.

Laboratory 9
Minor editorial/clarifications requested. No major comments on recommendations. Final draft revised in response to comments.

Laboratory 10
No comments received.

Laboratory 11
Replied but had no comments.

Laboratory 12
No comments received.
Table 1
Accelerated Degradation Study – all samples exposed at the indicated temperature for 14 months.
PRNT Assays.
Potencies of 97/648 relative to -20°C sample (expressed as percentage)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Assay 1</th>
<th>Assay 2</th>
<th>Assay 3</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>-70°C</td>
<td>84.5</td>
<td>126.6</td>
<td>91.3</td>
<td>99.2</td>
</tr>
<tr>
<td>-20°C</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>+4°C</td>
<td>61.5</td>
<td>87.2</td>
<td>107.3</td>
<td>83.2</td>
</tr>
<tr>
<td>+20°C</td>
<td>90.0</td>
<td>72.9</td>
<td>89.2</td>
<td>83.6</td>
</tr>
<tr>
<td>+37°C</td>
<td>37.5</td>
<td>39.2</td>
<td>62.0</td>
<td>45.0</td>
</tr>
</tbody>
</table>

Table 2
Accelerated Degradation Study
ELISA Assays
Potencies of 97/648 (mIU/ml) by Enzygnost kit

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Assay 1</th>
<th>Assay 2</th>
<th>Assay 3</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>-70°C</td>
<td>7028</td>
<td>7252</td>
<td>7690</td>
<td>7316</td>
</tr>
<tr>
<td>-20°C</td>
<td>8053</td>
<td>7905</td>
<td>7612</td>
<td>7855</td>
</tr>
<tr>
<td>+4°C</td>
<td>6982</td>
<td>7721</td>
<td>8106</td>
<td>7588</td>
</tr>
<tr>
<td>+20°C</td>
<td>6747</td>
<td>7216</td>
<td>7750</td>
<td>7226</td>
</tr>
<tr>
<td>+37°C</td>
<td>6139</td>
<td>6199</td>
<td>6123</td>
<td>6154</td>
</tr>
</tbody>
</table>

Table 3
Accelerated Degradation Study
ELISA Assays
Potencies of 97/648 relative to -70°C sample (expressed as percentage)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Assay 1</th>
<th>Assay 2</th>
<th>Assay 3</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>-70°C</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>-20°C</td>
<td>114.7</td>
<td>109.0</td>
<td>99.0</td>
<td>107.4</td>
</tr>
<tr>
<td>+4°C</td>
<td>99.4</td>
<td>106.5</td>
<td>105.4</td>
<td>103.7</td>
</tr>
<tr>
<td>+20°C</td>
<td>98.1</td>
<td>99.5</td>
<td>100.8</td>
<td>98.8</td>
</tr>
<tr>
<td>+37°C</td>
<td>87.4</td>
<td>85.5</td>
<td>79.6</td>
<td>84.1</td>
</tr>
</tbody>
</table>
Table 4
Stability of Reconstituted Candidate 3rd IS
PRNT Assays
Reciprocal end-point dilutions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (Days)</th>
<th>Assay 1</th>
<th>Assay 2</th>
<th>Assay 3</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly Reconstituted</td>
<td>0</td>
<td>99</td>
<td>133</td>
<td>109</td>
<td>114</td>
</tr>
<tr>
<td>Freshly Reconstituted&lt;sup&gt;(1)&lt;/sup&gt;</td>
<td>28</td>
<td>240</td>
<td>-</td>
<td>-</td>
<td>240</td>
</tr>
<tr>
<td>Reconstituted and -20°C</td>
<td>28</td>
<td>420</td>
<td>485</td>
<td>369</td>
<td>425</td>
</tr>
<tr>
<td>Reconstituted and RT</td>
<td>28</td>
<td>243</td>
<td>304</td>
<td>271</td>
<td>273</td>
</tr>
<tr>
<td>Reconstituted and +4°C</td>
<td>28</td>
<td>298</td>
<td>315</td>
<td>283</td>
<td>299</td>
</tr>
</tbody>
</table>

<sup>(1)</sup> Only a single ampoule of the candidate assayed at this time.

Table 5
Effect of heat inactivation on potency of candidate 3rd IS
Potencies relative to heat inactivated 66/202 (mIU/ml)

<table>
<thead>
<tr>
<th></th>
<th>Assay 1</th>
<th>Assay 2</th>
<th>Assay 3</th>
<th>Geometric mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>66/202 not heat inactivated</td>
<td>5333</td>
<td>3649</td>
<td>3868</td>
<td>4222</td>
</tr>
<tr>
<td>97/648 heat inactivated</td>
<td>2895</td>
<td>2962</td>
<td>1500</td>
<td>2343</td>
</tr>
<tr>
<td>97/648 not heat inactivated</td>
<td>2628</td>
<td>3107</td>
<td>1613</td>
<td>2362</td>
</tr>
</tbody>
</table>
### Table 6
Details of Methods used by Participants in the Collaborative Study

<table>
<thead>
<tr>
<th>PRNT</th>
<th>Lab. No</th>
<th>Neutralization time and temp</th>
<th>Vol/well (Virus + antibody)</th>
<th>Virus strain</th>
<th>Heat inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2hrs at 37°C</td>
<td>50μl</td>
<td>Edmonston Zagreb (E-Z)</td>
<td>30min at 56°C</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2hrs at 36°C</td>
<td>100μl</td>
<td>Low passage Wild-type Edmonston</td>
<td>30min at 56°C</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2hrs at 37°C</td>
<td>50μl</td>
<td>Ed wt</td>
<td>30min at 56°C</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1.5hrs at 4°C</td>
<td>50μl</td>
<td>PH 26</td>
<td>30min at 56°C</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2hrs at 37°C</td>
<td>50μl</td>
<td>Edmonston wt US-FDA (Ed HeK7V4PP3 V4)</td>
<td>30min at 56°C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TCND&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Lab. No</th>
<th>Neutralization time and temp</th>
<th>Vol/well (Virus + antibody)</th>
<th>Target titre of virus dil</th>
<th>Heat inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>1.5 hrs at RT</td>
<td>100μl</td>
<td>100 CCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>30min at 56°C</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2hrs at 37°C</td>
<td>100μl</td>
<td>100 CCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>none</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ELISA</th>
<th>Lab. No</th>
<th>Kit Manufacturer</th>
<th>Name of Kit</th>
<th>Diluent</th>
<th>Heat inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>Dade Behring</td>
<td>Enzygnost Anti-MV /IgG</td>
<td>Kit sample buffer diluent</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Dade Behring</td>
<td>Enzygnost Anti-MV/ IgG</td>
<td>Kit sample buffer diluent</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Dade Behring</td>
<td>Enzygnost Anti-MV /IgG</td>
<td>Kit sample buffer diluent</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Dade Behring</td>
<td>Enzygnost Anti-MV /IgG</td>
<td>Kit sample buffer diluent</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>In-house method</td>
<td></td>
<td>Tween20 +1% BSA in PBS</td>
<td>none</td>
</tr>
</tbody>
</table>
Table 7
Individual Laboratory Geometric Mean
Potency relative to 66/202 in mIU/ml

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>Method(^{(2)})</th>
<th>N</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P</td>
<td>6</td>
<td>424</td>
<td>2109</td>
<td>2475</td>
<td>1784</td>
<td>1789</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>3</td>
<td>1170</td>
<td>5068</td>
<td>5737</td>
<td>4802</td>
<td>6624</td>
</tr>
<tr>
<td>3</td>
<td>P</td>
<td>3</td>
<td>193</td>
<td>2841</td>
<td>3872</td>
<td>1950</td>
<td>4183</td>
</tr>
<tr>
<td>4</td>
<td>E</td>
<td>3</td>
<td>1137</td>
<td>6281</td>
<td>7164</td>
<td>6318</td>
<td>7759</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>3</td>
<td>295</td>
<td>4426</td>
<td>4846</td>
<td>4583</td>
<td>3509</td>
</tr>
<tr>
<td>6</td>
<td>T</td>
<td>3</td>
<td>815</td>
<td>5109</td>
<td>6006</td>
<td>5675</td>
<td>7138</td>
</tr>
<tr>
<td>8</td>
<td>T</td>
<td>3</td>
<td>248</td>
<td>ND(^{(4)})</td>
<td>4862</td>
<td>ND(^{(4)})</td>
<td>3968</td>
</tr>
<tr>
<td>9</td>
<td>P</td>
<td>3</td>
<td>313</td>
<td>2500</td>
<td>5000</td>
<td>2500</td>
<td>3150</td>
</tr>
<tr>
<td>10</td>
<td>E</td>
<td>3</td>
<td>592</td>
<td>2336</td>
<td>3923</td>
<td>2014</td>
<td>3988</td>
</tr>
<tr>
<td>11</td>
<td>E</td>
<td>3</td>
<td>969</td>
<td>5528</td>
<td>6439</td>
<td>5325</td>
<td>7502</td>
</tr>
<tr>
<td>12</td>
<td>P</td>
<td>2</td>
<td>368</td>
<td>4384</td>
<td>5835</td>
<td>4339</td>
<td>6149</td>
</tr>
</tbody>
</table>

\(^{(1)}\) Samples B and D are coded duplicates.

\(^{(2)}\) Method; E = Elisa, P=PRNT, T = TCND\(_{50}\)

\(^{(3)}\) ND – not determined due to cytotoxicity of the reconstituted sample.

\(^{(4)}\) TCID\(_{50}\) with single replicate wells.

Table 8
Overall Means by Assay Method
Potency relative to 66/202 in mIU/ml
Geometric Mean of Individual Laboratory Means

<table>
<thead>
<tr>
<th>Assay</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>B &amp; D Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRNT</td>
<td>350</td>
<td>3067</td>
<td>4030</td>
<td>2684</td>
<td>3646</td>
<td>2869</td>
</tr>
<tr>
<td>ELISA</td>
<td>855</td>
<td>5390</td>
<td>6108</td>
<td>5343</td>
<td>7230</td>
<td>5366</td>
</tr>
</tbody>
</table>
**Table 9**

Within Assay – Between Duplicate Variability
Average fold difference in potency estimates of B & D relative to 66/202

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>Method(1)</th>
<th>N</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P</td>
<td>6</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>P</td>
<td>3</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>E</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>P</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>T</td>
<td>3</td>
<td>ND(2)</td>
</tr>
<tr>
<td>8</td>
<td>T</td>
<td>3</td>
<td>ND(3)</td>
</tr>
<tr>
<td>9</td>
<td>P</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>E</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>11</td>
<td>E</td>
<td>3</td>
<td>39</td>
</tr>
<tr>
<td>12</td>
<td>P</td>
<td>2</td>
<td>42</td>
</tr>
<tr>
<td>Overall</td>
<td>E</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Overall</td>
<td>P</td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

(1) Method: E = ELISA, P=PRNT, T = TCND<sub>50</sub>
(2) ND – not determined due to cytotoxicity of the reconstituted sample.
(3) TCND<sub>50</sub> with single replicate wells. Insufficient precision to assess.
Table 10

Within Laboratory – Between Assay %GCV
Pooled estimate from potency estimates relative to 66/202 for samples A - E

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>Method(1)</th>
<th>N</th>
<th>%GCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P</td>
<td>6</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>P</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>E</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>P</td>
<td>3</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>T</td>
<td>3</td>
<td>ND(2)</td>
</tr>
<tr>
<td>8</td>
<td>T</td>
<td>3</td>
<td>ND(3)</td>
</tr>
<tr>
<td>9</td>
<td>P</td>
<td>3</td>
<td>66</td>
</tr>
<tr>
<td>10</td>
<td>E</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>E</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>12</td>
<td>P</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td>Overall</td>
<td>E</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Overall</td>
<td>P</td>
<td></td>
<td>66</td>
</tr>
</tbody>
</table>

(1) Method: E = ELISA, P = PRNT, T = TCND50
(2) ND – not determined due to cytotoxicity of the reconstituted sample.
(3) TCND50 with single replicate wells. Insufficient precision to estimate %GCV.

Table 11

Between laboratory %GCV of potency relative to 66/202

<table>
<thead>
<tr>
<th>Assay</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>Pooled*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>85</td>
<td>48</td>
<td>33</td>
<td>61</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>PRNT</td>
<td>52</td>
<td>42</td>
<td>38</td>
<td>59</td>
<td>57</td>
<td>49</td>
</tr>
<tr>
<td>ELISA</td>
<td>50</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

* Pooled estimates for samples B-E
Table 12
Potency relative to Candidate 3rd IS (Sample B)
Overall Means by Assay Method
(B = 3000 mIU/ml set as an arbitrary unitage)
Geometric Mean of Individual Laboratory Means (mIU/ml)

<table>
<thead>
<tr>
<th>Assay</th>
<th>A</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRNT</td>
<td>342</td>
<td>3942</td>
<td>2625</td>
<td>3565</td>
</tr>
<tr>
<td>ELISA</td>
<td>476</td>
<td>3400</td>
<td>2974</td>
<td>4024</td>
</tr>
</tbody>
</table>

Table 13
Between laboratory %GCV of potency relative to Candidate 3rd IS (SampleB)

<table>
<thead>
<tr>
<th>Assay</th>
<th>A</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>64</td>
<td>22</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>PRNT</td>
<td>89</td>
<td>18</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>ELISA</td>
<td>45</td>
<td>4</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

FIGURE LEGENDS (Figures 1 and 2)

Each box represents the mean estimate from one laboratory, and is labeled with the laboratory code number. The boxes are also shaded to indicate the assay method, and labeled with a code letter to denote the assay method (P, T or E for PRNT, TCND30 or ELISA respectively).
FIGURE 1
Laboratory geometric mean estimates of potency relative to 66/202

Sample A

Sample B
FIGURE 1 (continued)
Laboratory geometric mean estimates of potency relative to 66/202

Sample C

Sample D
FIGURE 1 (continued)
Laboratory geometric mean estimates of potency relative to 66/202

Sample E

<table>
<thead>
<tr>
<th>Potency mlU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>375</td>
</tr>
<tr>
<td>1 P</td>
</tr>
<tr>
<td>6 T</td>
</tr>
<tr>
<td>4 E</td>
</tr>
</tbody>
</table>

*Note:* The diagram shows the distribution of potency estimates across different samples.
FIGURE 2
Individual laboratory potency estimates relative to Proposed 3rd IS (Sample B)

Sample A

Sample C
FIGURE 2 (Continued)
Individual laboratory potency estimates relative to sample B

Sample D

Sample E
APPENDIX 1
List of Participants

Dr Isabelle Parent  
GSK Biologicals  
CLINLABS –P44  
Rue de l’Institut, 89  
B-1330 Rixensart  
Belgium  
isabelle.parent@gskbio.com

Dr Noemi Caro  
Chiron Vaccines  
Via Fiorentina 1  
53100 Siena  
ITALY  
noemi.car@chiron.com

Veronik Hutse  
Scientific Institute of Public Health  
Section Virology - Department Microbiology  
Juliette Wytsmanstreet 14  
1050 Brussels  
Belgium  
veronik.hutse@iph.fgov.be

Dr Judy Beeler  
LPRVD, DVP, OVRR, CBER  
Building 29A, Room 3B05, HFM 463  
29 Lincoln Drive  
Bethesda  
Maryland 20892  
USA  
beeler@cber.fda.gov

Dr Niteen Wairagkar and Dr Naseem Shaik  
National Institute of Virology  
20- A, Dr Ambedkar Road  
PO Box-11  
Pune - 411001  
India  
niteenw@hotmail.com;  
or niteen@yahoo.com  
njsaikh2000@yahoo.com

Dr Juan Ruiz-Gómez  
Instituto Nacional de Salud Pública  
Laboratorio de Evaluacion de Vacunas Virales  
Av. Universidad No. 655  
Col. Sta. Ma. Ahuacatitlan Co. 62508  
Cuernavaca  
Mexico  
juiruiz@correo.insp.mx

Dr Bernard Cohen  
Enteric, Respiratory and Neurological Virus Laboratory  
Centre for Infections  
Health Protection Agency  
61 Colindale Avenue  
London NW9 5HT  
UK  
bernard.cohen@hpa.org.uk

Anne Anderson  
NIBSC  
Blanche Lane  
South Mimms  
Potters Bar  
Herts  
EN5 3QG  
aanderson@nibsc.ac.uk

Dr. Novilia Sjafri Bachtiar  
Bagian Evaluasi Produk  
Bio Farma  
Jl. Pasteur no. 28 Bandung  
Indonesia 40161  
novilia@biofarma.co.id

Kei Numazaki, M.D., Ph.D.  
Chief of Measles Laboratory  
Virology III  
National Institute of Infectious Diseases  
Gakuen 4-7-1, Musashi-muryayama  
Tokyo, 208-0011  
Japan  
numazaki@nih.go.jp

Dr Rocio D Marchese  
Merck & Co. Inc  
WYN – 1  
466 Devon Park Drive  
Wayne  
PA 19087-1916  
rocio_marchese@merck.com
PROPOSED INSTRUCTIONS FOR USE

3rd INTERNATIONAL STANDARD FOR ANTI-MEASLES
NIBSC CODE: 97/648

1. CAUTION

This preparation contains material of human origin which has been tested and found negative for HBsAg, HCV antibody and HIV antibody.

As with all materials of biological origin, the preparation should be regarded as potentially hazardous to health. The container and its contents should be used and discarded according to your own laboratory procedures. Such procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening the containers to avoid cuts.

THIS MATERIAL IS NOT FOR ADMINISTRATION TO HUMANS

2. BACKGROUND


Stocks of the above standard are now exhausted and collaborative study was run in 2005/06 to establish a replacement. The 3rd International Standard was established by ECBS in 2006 (WHO, 2007) and is available from NIBSC.

3. UNITAGE

For use in Plaque Reduction Neutralization Test (PRNT) assays the reconstituted material will contain 3.0 IU anti-measles activity (3,000 milli-IUs).

This preparation has not been calibrated for use in ELISA assays and/or a unitage assigned for this use.

Please also note that this material may not be suitable for use in TCND50 neutralization assays due to cytotoxicity of the preparation at low dilutions. It is recommended that if intended for this purpose it be tested prior to use in the appropriate cell/assay system.

If you have any further questions concerning the unitage or use of this material then please contact: Maureen Bentley (mbentley@nibsc.ac.uk) at NIBSC. A full copy of the collaborative study (WHO, 2006) report is also available upon request.

4. CONTENTS AND USE
4.1 Contents
Each ampoule contains a freeze-dried residue comprising (under an atmosphere of nitrogen) human serum containing antibodies against measles virus. Each ampoule should be reconstituted in 1ml of distilled water.

4.2 Preparation of Standard
The candidate replacement standard, NIBSC Code 97/648 was produced from a pool of defibrinated plasma supplied by CLB, Amsterdam. The plasma was filled, lyophilized and sealed into ampoules at NIBSC in November 1997.

The mean weight of the fill was 1.02041g, (taken from a mean of 85 samples) with a coefficient of variation of 0.31%. The mean dry weight of the fill measured by coulometric Karl Fischer was 81.17mg (taken from a mean of 6) and the residual moisture content 0.12%

The preparation has been tested and found negative for HBsAg, HCV antibody, HIV antibody and HCV RNA by PCR.

The ampoules have been stored since production at -20°C at NIBSC. A summary of the product characteristics is shown below.

<table>
<thead>
<tr>
<th>Product Summary for the 3rd International Standard for Anti-Measles Serum (97/648)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
</tr>
<tr>
<td>Excipients/additives</td>
</tr>
<tr>
<td>Coefficient of variation of the liquid fill</td>
</tr>
<tr>
<td>Residual Moisture</td>
</tr>
</tbody>
</table>

4.3 Storage and Use
Unopened ampoules should be stored at -20°C or below until use. It is recommended that samples be used as soon after receipt as possible.

After re-constitution samples may be aliquotted and stored frozen (ideally at -70°C) for further use. Studies have shown that reconstituted samples are stable for up to 28 days at this temperature. For longer periods of storage recipients should use their own in-house criteria to determine the length of time for which reconstituted samples can be retained.

Please note that the 3rd IS is provided as a reagent for calibrating your own in-house reference material(s). With this in mind recipients should remember that the supply of this reagent will be limited to 3 ampoules per organization per year.

**IT IS NOT INTENDED THAT THIS PRODUCT BE USED AS A WORKING REFERENCE AND SHOULD ONLY BE USED TO CALIBRATE YOUR OWN REFERENCE.**

5. DIRECTIONS FOR OPENING THE DIN AMPOULE

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.

6. CITATION

In all publications in which this preparation is used as an assay calibrant, it is important that name and address of NIBSC are cited correctly.

7. STABILITY

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 with assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. Once reconstituted, diluted or aliquoted users should determine the stability of the material according to their own method of preparation, storage and use.

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

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

8. PRODUCT LIABILITY

8.1 Information emanating from NIBSC is given after the exercise of all reasonable care and skill in it compilation, preparation and issue, but is provided without liability in its application and use.

8.2 This product is intended for use as a standard or reference material in laboratory work in relation to biological research, manufacturing or quality control testing of biological products or in the field of *in vitro* diagnostics. It is the responsibility of the user to ensure that he/she has the necessary technical skills to determine the appropriateness of this product for the proposed application. Results obtained from this product are likely to be dependent on conditions of use and the variability of materials beyond the control of NIBSC.

NIBSC accepts no liability whatsoever for any loss or damage arising from the use of this product, whether loss of profits, or indirect or consequential loss or other wise,
including, but not limited to, personal injury other than as caused by the negligence of NIBSC. In particular, NIBSC accepts no liability whatsoever for:

a) results obtained from this product; and or
b) non delivery of goods or for damages in transit.

8.3 In the event of any replacement of goods following loss or damage, a customer accepts as a condition of receipt of a replacement product, acceptance of the fact that the replacement is not to be construed as an admission of liability on NIBSC’s behalf.

REFERENCES

### Physical properties (at room temperature)

<table>
<thead>
<tr>
<th>Physical appearance</th>
<th>White/yellowish freeze-dried cake.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fire hazard</td>
<td>None</td>
</tr>
</tbody>
</table>

### Chemical properties

<table>
<thead>
<tr>
<th>Stable</th>
<th>Yes</th>
<th>Corrosive:</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hygroscopic</td>
<td>No</td>
<td>Oxidising:</td>
<td>No</td>
</tr>
<tr>
<td>Flammable</td>
<td>No</td>
<td>Irritant:</td>
<td>No</td>
</tr>
<tr>
<td>Other (specify)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Handling: *For in vitro use only, not for administration to humans.*

### Toxicological properties

- Effects of inhalation: *Not established*
- Effects of ingestion: *Not established*
- Effects of skin absorption: *Not established*

### 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.*

---

* = *