



**World Health  
Organization**

**WHO/BS.07/2076  
ENGLISH ONLY**

**EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION  
Geneva - 8 to 12 October 2007**

**Report on a Collaborative Study to Investigate the Relationship between  
the 1<sup>st</sup> IRP and the 2<sup>nd</sup> and 3<sup>rd</sup> International Standards for Anti-Measles  
Serum/Plasma, in both ELISA and PRNT**

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## SUMMARY

The 3<sup>rd</sup> International Standard (IS) for anti-measles serum/plasma was established by ECBS in 2006. Data from the collaborative study used to calibrate the 3<sup>rd</sup> IS against the 2<sup>nd</sup> IS indicated that the standard did not behave in the same way in the two most commonly used assays for anti-measles activity (i.e. plaque reduction neutralization test (PRNT) and enzyme-linked immunosorbent assay (ELISA)). The ELISA had not been developed when the 1<sup>st</sup> International Reference Preparation (IRP) was originally characterised and established, and the very limited data from haemagglutination inhibition (HI), PRNT and ELISA were used in the calibration of the 2<sup>nd</sup> IS to the 1<sup>st</sup> IRP (Forsey et al., 1991). A unitage was therefore only assigned to the 3<sup>rd</sup> IS for virus neutralization tests (PRNT). The discovery at NIBSC of a limited number of vials of the 1<sup>st</sup> IRP prompted a collaborative study to be undertaken to examine the link for the ELISA between the 1<sup>st</sup> IRP and the 2<sup>nd</sup> and 3<sup>rd</sup> IS's, and to investigate the behaviour of the materials in a wider range of ELISA kits. The study involved 6 laboratories from 5 countries. Data was generated for ELISA from 5 laboratories using three different currently licensed kits for estimating anti-measles activity in serum samples. In addition, 3 laboratories also completed the study using PRNT. This study showed good comparability with the 2006 study for the PRNT and the ELISA when comparable reagents and kits were used. However, for the ELISA there was found to be a significant difference in the way that different kits performed. The most commonly used kit (and the only kit used in the 2006 study) produced higher estimates of the potency of the 3<sup>rd</sup> IS when calibrated against the 2<sup>nd</sup> IS (compared to the PRNT and other ELISA kits). On the basis of these data it is not appropriate to change the current recommendation that a unitage for the 3<sup>rd</sup> IS only be assigned for PRNT and not for ELISA.

## INTRODUCTION

The 3<sup>rd</sup> International Standard for Anti-Measles (Plasma), 97/648, was established by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization in October 2006. The collaborative study carried out to establish this IS (WHO, 2006) involved eleven laboratories from 8 countries, included 5 samples and involved the use of three assay techniques (i.e. Plaque Reduction Neutralization test (PRNT), Tissue Culture Neutralizing Dose (TCND<sub>50</sub>) (microneutralization) and Enzyme-linked Immunosorbent Assay (ELISA)). Data from the TCND<sub>50</sub> could not be used in the analysis of the study as one of the two laboratories that used the technique had problems with cytotoxicity of the candidate 3<sup>rd</sup> IS and the other laboratory experienced problems with the precision of the assay. Therefore, it was only possible to compare the results PRNT and ELISA in 2006 study.

The comparison of the PRNT and ELISA from the 2006 study showed that the calibration of the proposed 3<sup>rd</sup> IS was not consistent between the two methods – with the two methods effectively giving different results. As the ELISA assay had not been developed when the 1<sup>st</sup> International Reference Preparation (IRP) was established and was not in widespread use by the time the 2<sup>nd</sup> IS was established no formal link for ELISA assays was ever established for the International Unit. For these reasons it was not recommended that a unitage be assigned for the 3<sup>rd</sup> IS for the ELISA at the time that the report from the 2006 collaborative study was submitted to ECBS.

The 3<sup>rd</sup> IS has therefore been assigned a unitage of 3 IU /ampoule - but this unitage can only be applied in estimation of anti-measles titre by virus neutralization assays, and more specifically PRNT.

In late 2006, a limited number of samples of the 1<sup>st</sup> IRP were located at NIBSC and it was decided that these samples should be used in a limited collaborative study to examine the relationship between the 1<sup>st</sup> IRP and the 2<sup>nd</sup> and 3<sup>rd</sup> IS's for ELISA and PRNT. While the inclusion of the 1<sup>st</sup> IRP did allow for a more complete study to be carried out in terms of tracing the International Unit, it was accepted that 2<sup>nd</sup> IS has replaced the 1<sup>st</sup> IRP and has been in use for so long that the current study could not provide any re-calibration of the 2<sup>nd</sup> or 3<sup>rd</sup> IS's against the 1<sup>st</sup> IRP.

Nevertheless, the inclusion of all three standards along with a wider range of ELISA kits than used in the original study did allow for a comparison of the PRNT and ELISA to be compared over a range of samples and also allowed further assessment to be made of the suitability of assigning an ELISA unitage for the 3<sup>rd</sup> IS.

## **COLLABORATIVE STUDY**

### **Study Plan**

#### ***Aim of the study***

The aim of this small study was to investigate the link between the 3<sup>rd</sup> IS (97/648), the 1<sup>st</sup> IRP and the 2<sup>nd</sup> IS by both ELISA and PRNT and to ascertain if it may be possible to assign an ELISA unitage to the 3<sup>rd</sup> IS.

#### ***Participants***

Six laboratories from 5 countries agreed to participate in the collaborative study including: 3 National Control Laboratories, 1 vaccine manufacturer and 2 Public Health 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***

The study comprised three different samples of lyophilized human serum/plasma and one of which was included as a duplicate. The four samples were coded A – D. As only limited amounts of the 1<sup>st</sup> IRP and the 2<sup>nd</sup> IS were available it was decided that samples would need to be reconstituted and aliquotted prior to use. For each coded sample three separate ampoules represented by the numbers 1, 2 and 3 were taken and reconstituted in 1ml of distilled water. The reconstituted samples were then dispensed into 100µl aliquots in vials and frozen at -70°C before dispatch to participants.

All study samples were distributed by courier on dry ice to participants, who were then requested to maintain samples at -20°C after receipt and prior to testing.

The study samples supplied are summarized below:

Sample A – 1<sup>st</sup> International Reference Preparation for Anti-Measles Serum

Sample B - 2<sup>nd</sup> International Standard for Anti-Measles Serum, 66/202

Samples C and D – 3<sup>rd</sup> International Standard for Anti-Measles Serum, 97/648

Each participant therefore received 12 tubes labeled: A1, A2, A3; B1, B2, B3; C1, C2, C3, D1, D2 and D3.

#### ***Study design***

Participants were requested to carry out 3 independent assays for the measles antibody content of the study samples using either the PRNT and/or ELISA method in routine use in their

laboratory. Three laboratories carried out the ELISA assay only, 1 laboratory carried out the PRNT only and two laboratories carried out both assays. Therefore, a total of 5 laboratories carried out the ELISA and 3 the PRNT.

Participants were requested to ensure that a freshly thawed vial of each preparation was used for each assay and the assay was performed on different days, generally one week apart and that each assay used a different vial of each sample.

The study was designed to allow for the following to be investigated and assessed:

- The relationship between the PRNT and ELISA (Enzygnost kit) for each sample
- The comparability of the current (smaller) study with the previous study
- The relationship of the 1<sup>st</sup> IRP to the 2<sup>nd</sup> and 3<sup>rd</sup> IS's in both the ELISA and PRNT
- The possible effect that ELISA-kit may have on the results seen in the two studies.

### ***Statistical Methods***

The PRNT assays were analyzed using a modified Spearman-Kärber method to determine a 50% neutralizing end-point (NEP<sub>50</sub>) for each sample in each assay. A potency estimate for each sample, in mIU/ml, was obtained by taking the ratio of the estimated NEP<sub>50</sub> for the sample and that of sample A (1<sup>st</sup> IRP) or sample B (2<sup>nd</sup> IS) and multiplying by the assigned unitage of 10,000 mIU/ml for 1<sup>st</sup> IRP or 5,000 mIU/ml for 2<sup>nd</sup> IS.

The ELISAs were analyzed as parallel line assays, using the log transformed optical density and after selecting regions of the dose-response curve for linearity. Potencies of samples B-D were calculated relative to sample A and potencies of samples A, C and D were calculated relative to sample B. In addition, the estimates of each replicate and dilution were calculated in kit units, according to the method defined in the instructions (using a kit standard curve for lab 3A). After correcting for dilution factor, an overall mean estimate for each sample and assay was calculated. Only ODs above 0.1 were used.

### ***Assay Methods and Data Received***

Data were received from the 3 laboratories that completed the PRNT and from the 5 laboratories that completed ELISA.

#### ***ELISA:***

For the ELISA, 4 of the 5 laboratories used the Enzygnost kit (Behring, Marburg, Germany) while one laboratory used both the IBL (IBL-America, Minneapolis, USA) and Serion (Virion/Serion, Würzburg, Germany) kits. All three kits are designed for the detection of anti-measles IgG. Further details of the IgG used in the different kits were not available

#### ***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.

The following virus strains were used; laboratory 3 PH26, laboratory 4 Ed wt, laboratory 6 low passage Edmonston

## Results

The laboratory geometric mean potencies of samples B-D relative to sample A (the 1<sup>st</sup> IRP, with assigned unitage 10,000 mIU/ml) are shown in Table 1. The results, converted to potencies relative to sample B (the 2<sup>nd</sup> IS, with assigned unitage 5,000 mIU/ml), are shown in Table 2. Tables 3 and 4 give the overall geometric means by assay method – in which each of the ELISA kits used in the study are treated separately. Mean values for the duplicated samples C and D are also shown in Tables 3 and 4.

Tables 5 and 6 give the mean estimates for the ELISA assays as deduced from the method outlined in the documentation accompanying the kit. The Enzygnost kit is stated to be calibrated in mIU/ml, against the 1<sup>st</sup> IRP. The Serion kit is stated to be calibrated in mIU/ml, against the 2<sup>nd</sup> IS. The IBL kit gives quantitative units - but there is no statement about traceability to the IS and the unitage is not expressed in IU/ml.

To compare the three ELISA methodologies (kits) used in the current study the data for the calibration of the 3<sup>rd</sup> IS against the 2<sup>nd</sup> IS was used (Table 4). The mean estimate from the Enzygnost assay is 5189 with 95% confidence interval (based on the variability between the laboratory estimates) of 4870 – 5529. Likewise, the 99% CI is 4618 – 5831. With values from the IBL and Serion kits being 3074 and 3248, respectively, we can see that both are well outside the 95% and 99% CI's and shows difference between the ELISA methods used in the current study.

## DISCUSSION

### *Consistency with 2006 study (WHO/BS/06.2031)*

The amount of material available for the current study meant that only a limited number of assays could be conducted and hence, a limited number of laboratories could be used in the study. To ensure consistency with the previous study, laboratories were selected to perform the PRNT and ELISA (with the Enzygnost kit) on the basis of the first study (i.e. with the intention that overall means for the two tests should be the same across the two studies). The best way of assessing this between study consistency is to look at the calibration of the 3<sup>rd</sup> IS to the 2<sup>nd</sup> IS in the two studies. In the current study these data are shown in Table 4 (C&D) where the values obtained were 2996 and 5189 mIU/ml for the PRNT and Enzygnost ELISA respectively. In the 2006 study the values obtained were 2869 and 5366, respectively – indicating very close agreement between the two studies.

### *Differences in ELISA Methodologies*

The calibration of the 3<sup>rd</sup> IS to the 2<sup>nd</sup> IS showed a significant difference when the results obtained with different kits in the current study were compared (see Table 4) the Enzygnost kits gave a high estimate for the 3<sup>rd</sup> IS, (5189 mIU/ml) which was similar to that observed in the 2006 study (5366 mIU/ml), and the IBL and Serion kits gave lower estimates (3074 and 3248 mIU/ml, respectively) in line with the overall geometric mean PRNT, and in contrast to the Enzygnost kit.

The number of assays that it was possible to perform with the IBL and Serion kits was relatively limited and it is possible that the single laboratory that completed these studies may well be an outlier. Without further study it is not possible to determine the magnitude of the effect that ELISA kit has on the assay of anti-measles activity. However, what the results do indicate is that there are no reasons to reverse the recommendation previously made that a unitage should not be assigned for ELISA at the present time. Suggestions for further work are outlined below.

*Traceability of the IU*

The 1<sup>st</sup> IRP was assigned an arbitrary unitage of 10 IU. The 2<sup>nd</sup> IS was calibrated against the 1<sup>st</sup> IRP in a small scale study ((Forsey et al., 1991) in an attempt to provide continuity of the IU.

Using the 2<sup>nd</sup> IS as the “baseline” against which calibrations were made (Table 4) we can trace a link for the IU between the 1<sup>st</sup> IRP and 2<sup>nd</sup> IS from the current study. The data for the PRNT gave a lower estimate than the assigned 10 IU/ml for the 1<sup>st</sup> IRP (i.e. around 7 IU/ml) while for the ELISA the results were between 8 and 9 IU/ml. Considering the severely limited data that were available when the 2<sup>nd</sup> IS was established, the agreement between the 2<sup>nd</sup> IS and the 1<sup>st</sup> IRP is good given that the stability of the 1<sup>st</sup> IS was never fully characterized and that some of the above losses may actually be due to the long-term stability of the 1<sup>st</sup> IS samples.

Alternately, taking the 1<sup>st</sup> IRP as the “baseline” (Table 3), the 2<sup>nd</sup> IS is found to have a higher unitage than the assigned 5 IU/ml, which is 7.4 IU/ml for PRNT and between 5 and 6 IU/ml for the ELISA. In effect this is comparable to what was undertaken for the 1991 collaborative study to establish the 2<sup>nd</sup> IS. Because of time constraints, the 1991 study was a limited study and only included 3 laboratories and 4 assays – 1 PRNT, 1 ELISA and 2 haemagglutination-inhibition assays (HI). A summary of the results from this collaborative study are reproduced in Table 7. Even though the original study to establish the 2<sup>nd</sup> IS had only single assays for the PRNT and ELISA there is good agreement for these assays with the current study. In the case of the PRNT, the values obtained were 7404 and 6900 mIU/ml for the current and original studies, respectively, while for the ELISA the values were 6016 and 5900 mIU/ml for the current and original studies, respectively. As can be seen in Table 7, while the overall potency estimate for the 2<sup>nd</sup> IS is 5,000 mIU/ampoule, this is actually more of a reflection of the low value obtained in the two HI assays; if only the results of the PRNT and ELISA testing had been considered a figure of 6400 mIU/ampoule would have been more appropriate and actually would be in better agreement with the estimate from the current study i.e. overall mean from the ELISA and PRNT is 6515 mIU/ampoule. However as the 2<sup>nd</sup> IS has been the definition of the IU for 17 years the relationship of the 1<sup>st</sup> IRP to the 2<sup>nd</sup> IS is of academic interest only and there is no suggestion that the assigned unitage of the 2<sup>nd</sup> IS should be adjusted retrospectively.

*Kit estimates in mIU:*

Of the three kits used in the current study, two of them - Enzygnost and Serion - can produce estimates of mIU/ml that are derived from calibrations of the kit against the 1<sup>st</sup> IRP and 2<sup>nd</sup> IS respectively. While the Enzygnost kit is stated to be calibrated against the 1<sup>st</sup> IRP it actually gives results above the assigned 10 IU/ml, with a mean across laboratories of 12.8 IU/ml (Table 5) - a 28% overestimate. Likewise, it also gives results that are higher for the 2<sup>nd</sup> IS, with a mean of 7.6 IU/ml - a 52% overestimate. In a similar vein, the Serion kit also produced overestimates of the potency of the 1<sup>st</sup> IRP and 2<sup>nd</sup> IS but in this case were almost exactly 2-fold higher than the assigned unitage for both the standards.

*Further Work*

The current study has indicated that there some aspects of the standardization of anti-measles sera/plasma that need to be followed-up in further studies. The previous collaborative study (WHO, 2006) and the current study both indicated that the calibration of the 3<sup>rd</sup> IS against the 2<sup>nd</sup> IS is not consistent between PRNT and ELISA – particularly for the Enzygnost kit. At the

moment this kit is used by about 90% of the laboratories that use an anti-measles IgG kit and so the results for this kit are very important and any assignment of unitage would need to take this kit into account.

The current study also indicated that there were significant differences in the behaviour of the 3<sup>rd</sup> IS between the Enzygnost ELISA kit and other ELISA kits. As only a single laboratory was able to complete the assays for other kits one further piece of work indicated is to expand the number of labs and the number of kits to be tested. In addition, as a total of only 5 samples have been examined across the two collaborative studies it is our intention to increase the range of samples examined using the methods and kits utilized in the current study to provide a better insight into what the full range of behaviours for anti-measles serum/plasma may be when tested by different assay methods.

Finally, the data from the current study indicate that the result for the HI relative to the PRNT and ELISA in the study for establishing the 2<sup>nd</sup> IS (Forsey et al., 1991) may have led to a lower potency for the 2<sup>nd</sup> IS being established than what was appropriate – certainly for PRNT and ELISA. While this is of mostly academic concern as the HI test is used very little today, it would certainly be of interest to gather some data that may further elucidate the differences and consequences of such differences in establishing anti-measles standards and reference materials.

#### *Summary*

The current study was designed to allow for the following to be investigated and assessed:

- The relationship between the PRNT and ELISA (Enzygnost kit) for each sample
- The comparability of the current (smaller) study with the previous study
- The relationship of the 1<sup>st</sup> IRP to the 2<sup>nd</sup> and 3<sup>rd</sup> IS's in both the ELISA and PRNT
- The possible effect that ELISA-kit may have on the results seen in the two studies

Against these objectives the following was achieved:

- A relationship between the PRNT and ELISA with the Enzygnost kit for each of the three samples (the three IS's) was established and found to be in-line with that established previously (WHO, 2006).
- There was good comparability with the previous study (WHO, 2006) for the PRNT and Enzygnost ELISA
- The relationship between the 1<sup>st</sup> IRP, 2<sup>nd</sup> and 3<sup>rd</sup> IS cannot be summarized by the relative assigned potencies of 10 IU, 5IU and 3 IU respectively. Calibration between these reference materials may have been confounded through time by the relative use with which certain techniques (in particular HI) were applied and the method by which a single unitage for a given standard was calculated.
- The effect of ELISA kit may be significant and the range of kits and samples that should be examined and the number of laboratories using such kits needs to be increased to provide a more complete picture.

On the basis of the above findings it would not seem to be appropriate at this point in time that a unitage for ELISA be assigned to the 3<sup>rd</sup> IS.

## RECOMMENDATIONS

It is recommended that:

- 1) the 3<sup>rd</sup> International Standard for Anti-Measles (Plasma) (NIBSC Code: 97/648) **should not** at the current time be assigned a unitage for use in ELISA.
- 2) the assigned unitage of 3 IU per ampoule for 97/648 be retained and that it be recognized that this unitage applies to the use of the standard in estimation of anti-measles titre by virus neutralization assays, and more specifically by plaque reduction neutralization test (PRNT).

A copy of the revised Instructions for use are provided (Appendix 2)

## REFERENCES

- Albrecht, P., Herrman, K. and Burns, G.R. (1981) Role of virus strain in conventional and enhanced plaque neutralization test. *J Virol Methods* 1981, **3**, 251-260.
- Forsey, T., Heath, A. and Minor, P. (1991) The 1<sup>st</sup> International Standard for anti-measles serum. *Biologicals*, **19**, 237-241.
- WHO (2006) Report of a Collaborative Study to Assess the Suitability of a Replacement for the 2<sup>nd</sup> International Standard for Anti-Measles Serum. WHO/BS/06.2031.

## SUMMARY OF COMMENTS FROM PARTICIPANTS

### Laboratory 1

No comments received.

### Laboratory 2

No comments received.

### Laboratory 3

Minor editorial/clarifications requested.  
Final draft revised in response to comments.

### Laboratory 4

Minor editorial/clarifications requested.  
Agreed with the recommendations

### Laboratory 5

No comments received

### Laboratory 6

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

**Table 1**

**Potency in mIU/ml vs 1<sup>st</sup> IRP (=10,000 mIU/ml)  
Geometric Mean of 3 Assays**

Lab	Method	Sample		
		B	C	D
1	Enzygnost	6753	6918	7279
2	Enzygnost	6608	6688	6442
4	Enzygnost	6306	7161	6596
5	Enzygnost	5395	5441	5560
3A	IBL	5332	3179	3127
3B	Serion	5700	3657	3746
3P	PRNT	9244	5036	3926
4P	PRNT	6117	5316	4711
6	PRNT	7178	4228	3644

**Table 2**

**Potency in mIU/ml vs 2<sup>nd</sup> IS (=5,000 mIU/ml)  
Geometric Mean of 3 Assays**

Lab	Method	Sample		
		A	C	D
1	Enzygnost	7404	5122	5389
2	Enzygnost	7567	5061	4874
4	Enzygnost	7929	5678	5230
5	Enzygnost	9268	5043	5153
3A	IBL	9377	2981	3169
3B	Serion	8773	3209	3287
3P	PRNT	5406	2724	2123
4P	PRNT	8175	4346	3851
6	PRNT	6966	2945	2539

**Table 3**

**Potency in mIU/ml vs 1<sup>st</sup> IRP (=10,000 mIU/ml)  
Geometric Mean of Lab Means**

Method	N Labs	Sample			
		B	C	D	C & D
Enzygnost	4	6242	6516	6439	6479
IBL	1	5332	3179	3127	3153
Serion	1	5700	3657	3746	3702
PRNT	3	7404	4837	4069	4436

**Table 4**

**Potency in mIU/ml vs 2<sup>nd</sup> IS (=5,000 mIU/ml)  
Geometric Mean of Lab Means**

Method	N Labs	Sample			
		A	C	D	C & D
Enzygnost	4	8010	5220	5158	5189
IBL	1	9377	2981	3169	3074
Serion	1	8773	3209	3287	3248
PRNT	3	6753	3267	2748	2996

**Table 5**

**Estimates of potency in mIU/ml from Enzygnost or Serion Kit**

Lab	Method	Sample				
		A	B	C	D	C & D
1	Enzygnost	13178	8183	8504	9054	8775
2	Enzygnost	10672	6409	6289	5904	6093
4	Enzygnost	14008	8791	9455	8672	9055
5	Enzygnost	13467	7267	6924	7436	7175
<i>Overall</i>	<i>Enzygnost</i>	<i>12763</i>	<i>7608</i>	<i>7693</i>	<i>7663</i>	<i>7678</i>
3B	Serion	19445	10302	6606	6910	6756

**Table 6**

**Estimates of potency in U/ml from IBL Kit**

Lab	Method	Sample				
		A	B	C	D	C & D
3A	IBL	253	137	82	100	91

**Table 7**

**Estimates of the Potency of the 2<sup>nd</sup> IS in mIU/ml vs 1<sup>st</sup> IRP (=10,000 mIU/ml) – from the Original Collaborative Study (Forsey *et al.*, 1991)**

PRNT	ELISA	HI (A)	HI(B)	MEAN
6,900	5,900	3,200	4,500	5,100

**APPENDIX 1**  
**List of Participants**

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## APPENDIX 2

## PROPOSED INSTRUCTIONS FOR USE

3<sup>rd</sup> 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**

The dual International Standard for anti-measles and anti-polio sera (2<sup>nd</sup> International Standard Anti-Measles serum (Human)/2<sup>nd</sup> 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 (WHO, 1992)

Stocks of the above standard are now exhausted and collaborative study was run in 2005/06 to establish a replacement. The 3<sup>rd</sup> 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 IU anti-measles activity (3,000 milli-IU's).

**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 TCND<sub>50</sub> 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](mailto: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.

<b>Product Summary for the 3<sup>rd</sup> International Standard for Anti-Measles Serum (97/648)</b>	
Presentation	Ampoule
Excipients/additives	None
Coefficient of variation of the liquid fill	0.31%
Residual Moisture	0.12%

### 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 3<sup>rd</sup> 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. LIABILITY AND LOSS**

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

It is the responsibility of the Recipient to determine the appropriateness of the materials supplied by the Institute to the Recipient ("the Goods") for the proposed application and ensure that it has the necessary technical skills to determine that they are appropriate. Results obtained from the Goods are likely to be dependant on conditions of use by the Recipient and the variability of materials beyond the control of the Institute.

All warranties are excluded to the fullest extent permitted by law, including without limitation that the Goods are free from infectious agents or that the supply of Goods will not infringe any rights of any third party.

The Institute shall not be liable to the Recipient for any economic loss whether direct or indirect, which arise in connection with this agreement.

The total liability of the Institute in connection with this agreement, whether for negligence or breach of agreement or otherwise, shall in no event exceed 120% of any price paid or payable by the Recipient for the supply of the Goods.

If any of the Goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.

## 9. REFERENCES

WHO (1964) International Standard for anti-measles virus serum. WHO/BS/680.

WHO (1992) Expert Committee on Biological Standardization; Forty-second report. Technical Report Series, **822**, 7-8.

WHO (2006) Report of a Collaborative Study to Assess the Suitability of a Replacement for the 2<sup>nd</sup> International Standard for Anti-Measles Serum. WHO/BS/06.2031.

WHO (2007) Expert Committee on Biological Standardization; Fifty-sixth report. Technical Report Series.

3<sup>rd</sup> INTERNATIONAL STANDARD FOR ANTI-MEASLES (PLASMA)  
97/648

**MATERIAL SAFETY SHEET**

<b>Physical properties (at room temperature)</b>	
Physical appearance	White/yellowish freeze-dried cake.
Fire hazard	None

<b>Chemical properties</b>			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	None		
Handling:	<i>For in vitro use only, not for administration to humans.</i>		

<b>Toxicological properties</b>	
Effects of inhalation:	<i>Not established</i>
Effects of ingestion:	<i>Not established</i>
Effects of skin absorption:	<i>Not established</i>

<b>Suggested First Aid</b>	
Inhalation	<i>Seek medical advice</i>
Ingestion	<i>Seek medical advice</i>
Contact with eyes	<i>Wash with copious amounts of water. Seek medical advice.</i>
Contact with skin	<i>Wash thoroughly with water.</i>

<b>Action on Spillage and Method of Disposal</b>	
<i>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.</i>	
<i>Absorbent materials used to treat spillage should be treated as biologically hazardous waste.</i>	

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