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**INTERNATIONAL STANDARDS FOR MINIMUM POTENCY OF  
ANTI-A AND ANTI-B BLOOD GROUPING REAGENTS**

REPORT OF THE INTERNATIONAL COLLABORATIVE STUDY TO EVALUATE  
PROPOSED GLOBAL (WHO INTERNATIONAL/CBER FDA US) MINIMUM  
POTENCY STANDARDS FOR ANTI-A AND ANTI-B BLOOD GROUPING REAGENTS

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**This document is a replacement of document WHO/BS/05.2024 - additional information is provided herewith on comparisons with previous reference materials and also on proposals to discontinue the previous WHO standards.**

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## Summary

The candidate international standards for minimum potency of anti-A and anti-B blood grouping reagents, codes 03/188 and 03/164 respectively, were evaluated against a wide range of commercial anti-A and anti-B blood grouping reagents in an international collaborative study involving 16 laboratories in 9 countries. Laboratories titrated 03/188 and 03/164 in parallel with as many commercial anti-A and anti-B blood grouping reagents, respectively, as were available to them according to specified haemagglutination methodology. Three of these laboratories and a further laboratory also titrated 03/188 and 03/164 in parallel with currently available reference preparations for anti-A and anti-B. The ratios of the mean endpoint titres of the anti-A and anti-B reagents to that of 03/188 and 03/164, respectively, within each laboratory were calculated.

The scientific advisors to the study recommend that a 1 in 8 dilution of the candidate IS for anti-A, 03/188, and a 1 in 4 dilution of the candidate IS for anti-B, 03/164, should define the recommended minimum potencies of anti-A and anti-B blood grouping reagents, respectively, in tube tests. The majority of the study participants endorsed these recommendations. Manufacturers of grouping reagents can exceed these recommended specifications which are the *minimum* titres considered necessary to ensure safety.

## Introduction

The quality of blood grouping reagents is clearly an important factor for safe blood transfusion, yet there is currently no appropriate international standardization of monoclonal anti-A or anti-B blood grouping reagents. Although WHO has International Standards for anti-A and anti-B, they were prepared many years ago and represent grouping reagents available at that time i.e. sera from immunized donors, whereas current anti-A and anti-B grouping reagents consist of monoclonal antibody preparations. There is little or no demand for the existing WHO standards. CBER/FDA distributes reference preparations for minimum potency of anti-A and anti-B grouping reagents, but again, they consist of anti-sera rather than monoclonal antibodies.

Suitable international reference reagents are needed to ensure minimum standards of potency of such reagents [1,2], particularly as CE marking for *in vitro* diagnostic products is now mandatory under Directive 98/79/EC. Reagents used for typing the major blood groups are listed in Annex II of the Directive, which defines those products that are considered to present a serious health risk in the event of failing to perform as intended, although potency requirements are not specifically stated. A new International Standard for minimum potency of anti-D blood grouping reagents (99/836), a monoclonal IgM anti-D preparation, was established by the WHO in 2004 [3]. The aim of the present collaborative study was to evaluate lyophilized monoclonal IgM anti-A and anti-B preparations using haemagglutination titrations in tubes, to determine, by international consensus, an appropriate dilution of the reconstituted contents to specify the recommended minimum acceptable potency of anti-A and anti-B blood grouping reagents in tube tests to differentiate 'safe' from 'unsafe' reagents.

The candidate minimum potency standards are not intended to determine specificity.

## Materials and methods

### *Candidate International Standards for Minimum Potency of anti-A and anti-B grouping reagents*

Culture supernatant containing IgM murine monoclonal anti-A BRIC 131 was kindly donated by the International Blood Group Reference Laboratory, Bristol, UK. Culture supernatant containing IgM murine monoclonal anti-B ES4 was kindly donated by Diagnostics Scotland, Edinburgh, UK. At NIBSC, the supernates were dispensed into glass ampoules (~1ml/ampoule), lyophilized, and coded 03/188 and 03/164, respectively. Fill details are summarized below:

	<i>Anti-A 03/188</i>	<i>Anti-B 03/164</i>
<i>Mean weight of the dispensed solution (number of fill weights measured)</i>	1.0044g (127)	1.0051g (106)
<i>Imprecision of the filling (coefficient of variation)</i>	0.08%	0.08%
<i>Residual moisture</i>	0.8%	1.2%
<i>Number of ampoules for distribution as WHO reference reagent</i>	4500	4500

### *Collaborative study participants*

A total of sixteen laboratories in nine countries worldwide participated in the study (Appendix 1). Each was assigned a code number, which does not reflect the order of listing. The participants included manufacturers of blood grouping reagents and expert immunohaematology laboratories.

### *Methods*

Participants were requested to perform direct haemagglutination tests in tubes according to a detailed methodology protocol provided (Appendix 2).

### *Study design*

Each participant was provided with 9 ampoules of each of the candidate international minimum potency standards 03/188 and 03/164. Participants were requested to perform haemagglutination titrations of reconstituted 03/188 and 03/164 in parallel with as many anti-A and anti-B blood grouping reagents as were available to them. They were asked to test the anti-A reagents against the following red cell ABO phenotypes: A<sub>1</sub> (one donor), A<sub>2</sub> (one donor), A<sub>2</sub>B (two donors, not pooled), B (one donor) and O (one donor). They were asked to test the anti-B reagents against the following ABO phenotypes: B (two donors, not pooled), A<sub>1</sub>B (two donors, not pooled), A<sub>1</sub> (one donor) and O (one donor). Participants were requested to test two independent doubling dilution series from neat of reconstituted 03/188 and 01/164 and each anti-A and anti-B reagent, respectively, in three assays on each of three days using the pooled contents of 3 ampoules of each of 03/188 and 03/164 each day. Participants were requested to record the grade of the haemagglutination reaction at each dilution on the results sheets provided according to specified criteria, and return the results

together with their estimates of the endpoint titres (defined as the last dilution giving 1+ grade agglutination) of 03/188, 03/164 and the anti-A and anti-B reagents.

#### *Statistical analysis*

The endpoint titres were used as the basis for analysis. For each reagent tested by each laboratory, a geometric mean titre was calculated for each red cell phenotype, by calculating the geometric mean across replicates on each day, and calculating a geometric mean across the three days, giving a single mean titre for each laboratory, reagent and cell phenotype. Geometric means were used as the endpoint titration method is based on a doubling dilution scale, e.g. the geometric mean of 1:8 and 1:32 is 1:16, the mid-dilution point between them; a simple arithmetic mean would give  $(8 + 32)/2 = 20$ . The ratio of the mean titre of each anti-A and anti-B reagent to those of 03/188 and 03/164, respectively, was then calculated for each laboratory.

#### *Comparison with existing IS and other reference preparations for anti-A and anti-B*

The WHO collaborating centres (laboratories 06, 12, 14, 17) performed additional titrations, in a separate exercise, of the candidate international minimum potency standards, 03/188 and 03/164, in parallel with existing WHO IS for anti-A and anti-B blood typing sera and/or the current CBER/FDA reference anti-A and anti-B blood grouping reagents and/or the British Minimum Potency Reference Preparations for anti-A and anti-B (the latter consist of lyophilizates of the same monoclonal anti-A and anti-B used to prepare the candidate international minimum potency standards). The reference preparations are listed in Appendix 3. The study design was the same as that used for the main collaborative study, with the existing reference materials being treated as reagents.

### **Results**

#### *Data received*

Data were received from sixteen laboratories. These laboratories tested 23 different anti-A reagents, along with the candidate international minimum potency standard 03/188, and 25 different anti-B reagents along with the candidate international minimum potency standard 03/164. The reagents are listed in Appendices 4 and 5, and include CBER's reference reagents as these were tested by laboratory 03.

The last dilution that gave 1+ grade haemagglutination (or in some cases 2+ grade where the next dilution gave +/- grade agglutination) was taken as the endpoint titre for analysis.

#### *Mean titres of 03/188 and anti-A reagents*

The mean titres obtained by the participants using 03/188 and the anti-A reagents are shown in Tables 1a-d, for each laboratory and reagent for each blood group A phenotype (all anti-A reagents were negative with the group B and O cells). The mean titres are also plotted in histogram form, where each box represents the titre obtained by a particular laboratory with a particular reagent, for each phenotype and combined phenotypes, in Figures 1a-d. The boxes are labelled with the laboratory code number, and the reagent code. The results for 03/188 are coloured coded differently from the other reagents in Figures 1a-c. The results for the different phenotypes are coloured coded differently in Figure 1d, which shows the combined results for all of the blood group A phenotypes. From Figure 1d it can be seen that the titres tended to be highest for the A<sub>1</sub> cells, which have the strongest expression of A antigen, and lowest for the A<sub>2</sub>B cells, which have the weakest expression of A antigen (amongst the phenotypes tested). The titres for the A<sub>2</sub> cells were intermediate.

The mean titres for each reagent tested were compared to the concurrently tested candidate international minimum potency standard 03/188 by calculating the ratio of the mean titre of the reagent to that of 03/188. For example, if a reagent had a mean titre of 256, and 03/188 had a mean titre of 128, the ratio is 2. The resulting ratios are shown in Tables 2a-b in ascending order of size of ratio, for each phenotype, and plotted in histogram form in Figures 2a-d. The range of ratios was similar for each phenotype (Figure 2d).

#### *Mean titres of 03/164 and anti-B reagents*

The mean titres obtained by the participants using 03/164 and the anti-B reagents are shown in Tables 3a-d, for each laboratory and reagent for each blood group B phenotype (all anti-B reagents were negative with the group A and O cells). The mean titres are also plotted in histogram form, where each box represents the titre obtained by a particular laboratory with a particular reagent, for each phenotype and combined phenotypes, in Figures 3a-c. The boxes are labelled with the laboratory code number, and the reagent code. The results for 03/164 are coloured coded differently from the other reagents in Figures 3a-b. The results for the different phenotypes are coloured coded differently in Figure 3c, which shows the combined results for all the blood group B phenotypes. The titres with B cells tended to be slightly higher than those with A<sub>1</sub>B cells.

The ratios of the mean titres of the anti-B reagents to the mean titres of 03/164 are shown in Tables 4a-b in ascending order of size of ratio, for each phenotype, and plotted in histogram form in Figures 4a-c. The range of ratios was similar for each phenotype (Figure 4c).

#### *Stability of 03/188 and 03/164*

Ampoules of 03/188 and 03/164 were stored at elevated temperatures following the fills in 2004. Titration results following just over 10 months storage for 03/188 and nearly 23 months for 03/164 are shown in Tables 5 and 6. Ampoules of 03/188 stored at +45°C would not reconstitute; ampoules of 03/164 stored at +45°C and +37°C would not reconstitute. There was no loss of potency for 03/188 in ampoules stored at +37°C (or any other temperature) compared to those stored at -70°C. Although overall, there appeared to be a slight loss of potency in ampoules stored at elevated temperatures for 03/164, ampoule 1 from -70°C had the same geometric mean titre as ampoule 2 from +20°C. Also, the end-point titres of tests from ampoules from all temperatures were a mix of 256 and 512 and a one-tube difference in haemagglutination titre is not considered significant. The slight differences in overall mean titres may therefore not be significant. More time at the elevated temperatures is needed.

As the length of time at the elevated temperatures was relatively short for 03/188 and 03/164, an indication of the intrinsic stability of the monoclonal anti-A and anti-B used in the fills was also provided by examining ampoules of the British Minimum Potency Preparations for anti-A and anti-B (88/722 and 88/724, respectively), which consist of the same monoclonal antibodies (BRIC 131 and ES4, respectively) and which were ampouled at the beginning of 1989.

*Anti-A, 88/722:* Ampoules stored at -20°C, +4°C and +20°C for nearly 16 years were assayed concurrently using haemagglutination methodology. Samples stored at +37°C could not be reconstituted. The result of each assay is a titre (eg 128, 256, 512 etc) that is a semi-quantitative measurement. The raw assay data are therefore not suitable for analysis using the usual Arrhenius model of accelerated degradation. However, from the overall geometric mean titres (GMT's) shown in Table 7, it can be seen that the potency (titre) of the samples stored at +4°C have dropped only slightly compared to those stored at -20°C. This suggests that the anti-A monoclonal antibody BRIC 131 is not intrinsically unstable.

*Anti-B, 88/724:* Ampoules stored at -20°C, +4°C, +20°C and +37°C for nearly 16 years were assayed concurrently using haemagglutination methodology. From the overall geometric mean titres (GMT's) shown in Table 8, it can be seen that the potency (titre) of the samples stored at +20°C and +37°C has dropped only slightly compared to the potency of those stored at -20°C. The titre of samples stored at +4°C has not dropped below that of samples stored at -20°C. This suggests that the anti-B monoclonal antibody ES4 is not intrinsically unstable. There is therefore no haemagglutination data to suggest that 03/188 and 03/164 will not be adequately stable at -20°C. However, ongoing real-time haemagglutination studies will be essential to monitor the stability of these preparations. It is proposed to carry out haemagglutination titrations on ampoules stored at -20°C and on ampoules stored at -70°C and the elevated temperatures annually to look for developing trends in absolute and relative haemagglutination titres.

*Comparison with existing IS and other reference preparations for anti-A and anti-B*

The four WHO collaborating centres (laboratories 06, 12, 14, 17 (two operators designated 17a and 17b)) performed additional titrations of the candidate international minimum potency standards, 03/188 and 03/164, in parallel with existing IS for anti-A and anti-B blood typing sera and/or the current CBER/FDA reference anti-A and anti-B blood grouping reagents and/or the British Minimum Potency Reference Preparations for anti-A and anti-B (the latter consist of lyophilizates of the same monoclonal anti-A and anti-B used to prepare the candidate international minimum potency standards). The ratios of the mean titres of the anti-A and anti-B reference materials to the mean titres of the candidate international minimum potency standards, 03/188 and 03/164, are shown in Figures 5 and 6 respectively. Also included are the titration results for the CBER/FDA preparations, which were tested in the main collaborative study above for laboratory 03.

The overall geometric mean ratios (across all phenotypes) for the three different preparations are:

Anti-A

BMPRP	1.02	n=5
CBER	0.10	n=18
WHO	0.25	n=12

Anti-B

BMPRP	0.85	n=5
CBER	0.31	n=18
WHO	0.83	n=12

For the anti-A therefore, the existing WHO IS and the CBER anti-A are about ¼ and 1/10 as potent, respectively, as the new candidate IS 03/188; for the anti-B, the existing WHO IS and the CBER anti-B are about 4/5 and 1/3 as potent, respectively, as the new candidate IS 03/164. The BMPRP for anti-A (88/722), when reconstituted and titrated from neat, has a similar potency to the candidate IS 03/188; whereas the BMPRP for anti-B (88/724) is about 4/5 as potent as the candidate IS 03/164. The recommended minimum potency specifications of the BMPRP are a 1 in 14 dilution for the anti-A, and a 1 in 4 dilution of the anti-B, based on an earlier comparative study with the WHO IS and CBER preparations [4].

## Discussion

The plots in Figures 1 and 3 show considerable variation in haemagglutination endpoint titres for the anti-A and anti-B reagents between reagents and laboratories (up to 1024-fold within a phenotype). Although the titres for each of the candidate international minimum potency standards 03/188 and 03/164 showed less variation within a phenotype (8-32-fold differences), the results show that even when using the same method, haemagglutination tests vary widely in their sensitivity and reproducibility between laboratories.

The plots of the titration ratios in Figures 2 and 4 do not show differences within the group A or the group B phenotypes, respectively. All ratios fell between 0.062 and 4 for both the combined group A phenotypes and the combined group B phenotypes (Figures 2d and 4c respectively, excluding one outlier in Figure 4c).

For the anti-A reagents, the most frequent ratios were 0.5 and 1, i.e. the most common mean endpoint titre amongst the anti-A reagents tested was a half or the same as that of 03/188 (Figure 2d). For the anti-B reagents, the most frequent ratio was 2, i.e. the most common mean endpoint titre amongst the anti-B reagents was double that of 03/164.

Most anti-A reagents would meet a minimum potency specification of an eight-fold dilution of 03/188; most anti-B reagents would meet a minimum potency specification of a four-fold dilution of 03/164, although minimum potency specifications corresponding to a four or five-fold dilution of reconstituted 03/188 and a two or three-fold dilution of 03/164 would be more in line with the current quality of most anti-A and anti-B reagents, respectively. Following the collaborative study in 2004, when the participants and scientific advisors considered whether a 1 in 4, a 1 in 5 or a 1 in 8 dilution (or any other dilution) of the reconstituted contents of 03/188 should define the minimum potency of anti-A blood grouping reagents, there was not a clear majority in favour of one dilution (Table 9). When the participants and scientific advisors considered whether a 1 in 2, a 1 in 3 or a 1 in 4 dilution (or any other dilution) of the reconstituted contents of 03/164 should define the minimum potency of anti-B blood grouping reagents, again, there was not a clear majority in favour of one dilution (Table 9).

In general, manufacturers were in favour of the greater dilutions, i.e. a less potent 'minimum potency', whereas users tended to favour lower dilutions, i.e. a more potent 'minimum potency'. The lack of consensus may also reflect the strength of the particular reagents a participant is used to working with.

In view of this lack of consensus, it was therefore decided to carry out further parallel titrations, in 2005, with existing anti-A and anti-B reference materials (WHO IS for anti-A and anti-B, CBER reference reagents for anti-A and anti-B, British minimum potency reference preparations for anti-A and anti-B) in the WHO collaborating centres to help choose an appropriate and acceptable minimum potency specification for each of the new candidate IS. The results of these additional titrations are included in this report.

The existing WHO IS for anti-A and the CBER anti-A were overall about  $\frac{1}{4}$  and  $\frac{1}{10}$  as potent, respectively, as the new candidate IS for anti-A, 03/188, when titrated from the neat, reconstituted contents. However, the WHO recommended minimum potency specification of 64 IU/ml (titre of 64, based on the 1<sup>st</sup> IS for anti-A blood typing serum [5]) is equivalent to just over a 1 in 7 dilution of the reconstituted contents of the 2<sup>nd</sup> IS for anti-A blood typing

serum, and therefore roughly equivalent to a 1 in 28 dilution of the candidate IS 03/188. The BMPRP for anti-A (88/722), when reconstituted and titrated from neat, had a similar potency to the candidate IS 03/188, but the recommended minimum potency specification for the BMPRP for anti-A is actually a 1 in 14 dilution of the reconstituted contents, which is therefore equivalent to a 1/14 dilution of the new candidate IS for anti-A.

The existing WHO IS for anti-B and the CBER anti-B were overall about 4/5 and 1/3 as potent, respectively, as the new candidate IS for anti-B 03/164, when titrated from the neat, reconstituted contents. However, the WHO recommended minimum potency specification of 64 IU/ml (titre of 64, based on the 1<sup>st</sup> IS for anti-B blood typing serum [5]) is just over a 1 in 13 dilution of the reconstituted contents of the 3<sup>rd</sup> IS for anti-B blood typing serum, and therefore roughly equivalent to a 1 in 16 dilution of the candidate IS. The potency of the British minimum potency reference preparation for anti-B, 88/724 (when reconstituted and titrated from neat) was approximately equivalent to a 4 in 5 dilution of 03/164. However, the recommended minimum potency specification for 88/724 is actually a 1 in 4 dilution of the neat, reconstituted contents, which is therefore roughly equivalent to a 1 in 5 dilution of the new candidate IS for anti-B.

The purpose of the IS for minimum potency is to differentiate 'safe' from 'unsafe' grouping reagents, not to define an optimum potency. In the US, blood grouping reagents are licensed, and the current US CBER/FDA reference reagents are intended to be the minimum potency needed to ensure that grouping reagents give the correct assignment of blood type as opposed to what manufacturers are capable of making. Therefore, based largely on CBER/FDA's experience from inspections and medical device reports, the scientific advisors to the study proposed to the participants in that a 1 in 8 dilution of the candidate IS for anti-A, 03/188, and a 1 in 4 dilution of the candidate IS for anti-B, 03/164, should define the recommended minimum potencies of anti-A and anti-B blood grouping reagents, respectively. These specifications are comparable to those of the current CBER/FDA and British preparations (which were based on those of CBER/FDA [4]), but more stringent than those of the existing but outdated WHO IS. Manufacturers of grouping reagents can exceed these recommended specifications which are the *minimum* titres considered necessary to ensure safety.

#### *Feedback from participants*

Of the 14 laboratories that expressed an opinion in 2004, including those of the scientific advisors, 4 considered a 1/8 dilution of 03/188 appropriate and 6 considered a 1/4 dilution of 03/164 appropriate to define the minimum potency specifications of anti-A and anti-B blood grouping reagents, respectively. Following the recommendation of the scientific advisors in 2005 to adopt these specifications, a number of participants revised their opinions or responded for the first time: a further 4 laboratories agreed to these specifications for both IS. Therefore, of a total of 17 laboratories, representing the scientific advisors and the participants, 13 agreed that a 1/8 dilution of 03/188 and a 1/4 dilution of 03/164 should define the minimum potency of anti-A and anti-B blood grouping reagents. Of the 4 laboratories that did not agree, 2 laboratories maintained their opinions from 2004 that a 1/4 dilution of 03/188 and a 1/2 dilution of 03/164 should define the minimum potency specifications, one laboratory did not respond at all either in 2004 or 2005, and one laboratory that considered 1/16 dilutions of each candidate IS to be appropriate in 2004 did not respond in 2005.

## Recommendations

It is recommended that a 1 in 8 dilution of the candidate IS for anti-A, 03/188, and a 1 in 4 dilution of the candidate IS for anti-B, 03/164, should define the recommended minimum potencies of anti-A and anti-B blood grouping reagents in tube tests, respectively.

### Notes.

1. Stocks of 03/188 and 03/164 will be shared with CBER/FDA for distribution as US Minimum Potency Reference Reagents.
2. The British Minimum Potency Reference Reagents for anti-A and anti-B blood grouping reagents, 88/722 and 88/724, respectively, will be discontinued upon establishment of the candidate International Minimum Potency Reference Reagents, 03/188 and 03/164.
3. Based on the advice of the members of the scientific advisory group, it is proposed that the 2nd WHO International Standard for anti-A (code number W1001) and the 3<sup>rd</sup> IS for anti-B (code W1002) be discontinued.

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## References

1. WHO consultation on review and replacement of the current WHO International Standards for blood grouping reagents: CBER/FDA, USA, September 1998.
2. Review and update of the WHO International Standards for blood grouping reagents: Amsterdam, the Netherlands, October 1999.
3. Thorpe SJ, Fox B, Heath AB, Scott M, de Haas M, Kochman S, Padilla A: An International Standard for specifying the minimum potency of anti-D blood-grouping reagents: evaluation of a candidate preparation in an international collaborative study. *Vox Sang* 2006; 90: 131-139.
4. Report on an interlaboratory trial and workshop to produce specifications for BCR reference materials for major blood grouping reagents (anti-A, anti-B and anti-D monoclonals). Provided by Professor ML Scott, IBGRL, Bristol, UK.
5. Holburn AM, Moore BPL, David-West AS, Lema RA, Kasili EG, Cazal P, Lothe F, von Steffens E, on behalf of the World Health Organization, League of Red Cross Societies and International Society of Blood Transfusion: The production of ABO and D (Rh<sub>0</sub>) grouping reagents. LAB/81.1. Reagents for the 1990s. Blood Bank Reagents Standards Workshop, Bethesda MD, USA, National Institutes of Health, November 1990.

Table 1a Laboratory mean titres using anti-A reagents with A<sub>1</sub> red cells

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
03/188	1149	645	256	1149	645	1024	1149	724	3649	2048	1626	2580	3649	1149	1626	683
110						108										
131						102										
26A												1085				
94A			128													
BCA		512												574		242
CBERA			32													
CSLA							1825									
DCLA																228
DEVA		575														
DGA													8192			
DMA													3251			
DSA							1149							575	813	
GCA											2299					
ICA					228						575					
LOA									228				2048	456		
MTA					323											
NIIDA								256								
NOA	1024															
P/CA												256				
PKA												1825				
SIA				362						724						
SCA									4598			2048				
TLA	1024				575	1448	1448		1448				4096	645		

**Table 1b Laboratory mean titres using anti-A reagents with A<sub>2</sub> red cells**

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
03/188	406	406	128	456	323	724	912	NT	645	1024	912	912	1825	813	512	271
110						108										
131						91										
26A												304				
94A			64													
BCA		512												456		171
CBERA			16													
CSLA							1149									
DCLA																108
DEVA		512														
DGA													4096			
DMA													3649			
DSA							912							512	256	
GCA											1024					
ICA					128						287					
LOA									64				1024	287		
MTA					128											
NIIDA								NT								
NOA	512															
P/CA												128				
PKA												575				
SIA				128						406						
SCA									1290			912				
TLA	512				512	1024	1149		362				1825	512		

NT = not tested

Table 1c Laboratory mean titres using anti-A reagents with A<sub>2</sub>B red cells (first donor)

Reagent (NIBSC code)	Laboratory															
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16
03/188	203	144	81	64	144	287	256	362	203	256	512	406	2048	406	256	136
110							54									
131							40									
26A												171				
94A			64													
BCA		91												91		68
CBERA			6													
CSLA							575									
DCLA																72
DEVA		102														
DGA													3649			
DMA													1290			
DSA							406							203	256	
GCA											512					
ICA					57						203					
LOA									36				1024	228		
MTA					36											
NIIDA								256								
NOA	203															
P/CA												64				
PKA												362				
SIA				23						72						
SCA									362			512				
TLA	181				228	323	456		161				2299	256		

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
03/188	203	144	81	64	144	287	256	362	203	256	512	406	2048	406	256	136
110							54									
131							40									
26A												171				
94A			64													
BCA		91												91		68
CBERA			6													
CSLA							575									
DCLA																72
DEVA		102														
DGA													3649			
DMA													1290			
DSA							406							203	256	
GCA											512					
ICA					57						203					
LOA									36				1024	228		
MTA					36											
NIIDA								256								
NOA	203															
P/CA												64				
PKA												362				
SIA				23						72						
SCA									362			512				
TLA	181				228	323	456		161				2299	256		

**Table 1d Laboratory mean titres using anti-A reagents with A<sub>2</sub>B red cells (second donor)**

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
03/188	181	144	45	144	128	256	NT	NT	287	114	512	483	256	NT	256	144
110						45										
131						32										
26A												256				
94A			32													
BCA		81														96
CBERA			4													
CSLA							NT									
DCLA																76
DEVA		114														
DGA													456			
DMA													362			
DSA														256		
GCA											406					
ICA					36						144					
LOA							36						128			
MTA					16											
NIIDA								NT								
NOA	228															
P/CA												64				
PKA												512				
SIA				36						32						
SCA									1024			575				
TLA	228				128	228			256				256			

NT = not tested

**Table 2a Ratios of the mean titres of anti-A reagents to the mean titres of 03/188 (ascending order) for A<sub>1</sub> and A<sub>2</sub> cells**

<i>A<sub>1</sub> cells (1 donor)</i>			<i>A<sub>2</sub> cells (1 donor)</i>		
<i>Reagent</i>	<i>Lab</i>	<i>Ratio</i>	<i>Reagent</i>	<i>Lab</i>	<i>Ratio</i>
LOA	09	0.06250	LOA	09	0.09921
131	06	0.09921	131	06	0.12500
P/CA	12	0.09921	CBERA	03	0.12500
110	06	0.10511	P/CA	12	0.14031
CBERA	03	0.12500	110	06	0.14865
S1A	04	0.31498	S1A	04	0.28062
DCLA	16	0.33371	ICA	11	0.31498
ICA	05	0.35355	26A	12	0.33371
ICA	11	0.35355	LOA	14	0.35355
NIIDA	08	0.35355	S1A	10	0.39685
S1A	10	0.35355	DCLA	16	0.39685
BCA	16	0.35355	ICA	05	0.39685
LOA	14	0.39685	MTA	05	0.39685
TLA	09	0.39685	94A	03	0.50000
26A	12	0.42045	DSA	15	0.50000
BCA	14	0.50000	LOA	13	0.56123
DSA	14	0.50000	BCA	14	0.56123
MTA	05	0.50000	TLA	09	0.56123
94A	03	0.50000	PKA	12	0.62996
DSA	15	0.50000	BCA	16	0.62996
TLA	14	0.56123	DSA	14	0.62996
LOA	13	0.56123	TLA	14	0.62996
PKA	12	0.70711	DSA	07	1.00000
BCA	02	0.79370	SCA	12	1.00000
SCA	12	0.79370	TLA	13	1.00000
TLA	05	0.89090	GCA	11	1.12246
NOA	01	0.89090	TLA	01	1.25992
TLA	01	0.89090	CSLA	07	1.25992
DEVA	02	0.89090	TLA	07	1.25992
DMA	13	0.89090	NOA	01	1.25992
DSA	07	1.00000	BCA	02	1.25992
TLA	13	1.12246	DEVA	02	1.25992
TLA	07	1.25992	TLA	06	1.41421
SCA	09	1.25992	TLA	05	1.58740
TLA	06	1.41421	SCA	09	2.00000
GCA	11	1.41421	DMA	13	2.00000
CSLA	07	1.58740	DGA	13	2.24492
DGA	13	2.24492			

Note: reagent NIIDA was not tested against A<sub>2</sub> cells

**Table 2b** Ratios of the mean titres of anti-A reagents to the mean titres of 03/188 (ascending order) for A<sub>2</sub>B cells

<i>A<sub>2</sub>B cells (2 donors)</i>					
<i>Reagent</i>	<i>Lab</i>	<i>Ratio</i>	<i>Reagent</i>	<i>Lab</i>	<i>Ratio</i>
CBERA	03	0.07875	BCA	16	0.66742
CBERA	03	0.08839	NIIDA	08	0.70711
LOA	09	0.12500	DEVA	02	0.70711
MTA	05	0.12500	94A	03	0.70711
131	06	0.12500	DEVA	02	0.79370
P/CA	12	0.13243	GCA	11	0.79370
131	06	0.14031	94A	03	0.79370
P/CA	12	0.15749	TLA	09	0.79370
110	06	0.17678	TLA	06	0.89090
LOA	09	0.17678	TLA	09	0.89090
110	06	0.18729	TLA	01	0.89090
BCA	14	0.22272	PKA	12	0.89090
S1A	04	0.25000	NOA	01	1.00000
MTA	05	0.25000	GCA	11	1.00000
S1A	10	0.28062	DSA	15	1.00000
ICA	11	0.28062	TLA	05	1.00000
ICA	05	0.28062	TLA	13	1.00000
S1A	10	0.28062	DSA	15	1.00000
S1A	04	0.35355	PKA	12	1.05946
ICA	11	0.39685	TLA	13	1.12246
ICA	05	0.39685	TLA	06	1.12246
26A	12	0.42045	SCA	12	1.18921
LOA	13	0.50000	NOA	01	1.25992
BCA	16	0.50000	TLA	01	1.25992
LOA	13	0.50000	SCA	12	1.25992
DSA	14	0.50000	DMA	13	1.41421
26A	12	0.52973	DSA	07	1.58740
DCLA	16	0.52973	TLA	05	1.58740
DCLA	16	0.52973	DGA	13	1.78180
BCA	02	0.56123	TLA	07	1.78180
LOA	14	0.56123	DGA	13	1.78180
BCA	02	0.62996	SCA	09	1.78180
DMA	13	0.62996	CSLA	07	2.24492
TLA	14	0.62996	SCA	09	3.56359

**Table 3a Laboratory mean titres using anti-B reagents with B red cells  
(first donor)**

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
03/164	575	813	181	912	1024	724	1625	645	2580	2048	912	1534	1825	575	813	362
112						228										
138B												2734				
143B			645													
250						64										
BCB		512												456		342
CBERB			81													
CSLB							2048									
DCLB																256
DEVB		512														
DGB													2048			
DMB													2048			
DSB							813							813	645	
GCB											1625					
ICB					1024						1149					
LOB									1024				512	813		
MTB					3649											
NCB	813															
NIIDB								362								
P/CB												645				
PKB												2896				
S1B				1448												
S3B				575						1825						
SCB									3251			1290				
TNB	912				2048	813	3251						3251	1825		

**Table 3b Laboratory mean titres using anti-B reagents with B red cells  
(second donor)**

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
03/164	645	813	228	645	1290	575	NT	NT	2299	2048	645	1534	2048	575	575	304
112						287										
138B												2048				
143B			1024													
250						51										
BCB		512												406		287
CBERB			128													
CSLB							NT									
DCLB																192
DEVB		575														
DGB													2580			
DMB													2048			
DSB							NT							575	512	
GCB											1448					
ICB					1024						912					
LOB									323				512	512		
MTB					2896											
NCB	813															
NIIDB								NT								
P/CB												431				
PKB												1825				
S1B				1290												
S3B				645						2048						
SCB									912			912				
TNB	1024				2048	813	NT						4096	2048		

NT = not tested

**Table 3c Laboratory mean titres using anti-B reagents with A<sub>1</sub>B red cells  
(first donor)**

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
03/164	256	512	181	456	813	161	912	456	912	1448	512	542	1024	406	287	256
112						144										
138B												1290				
143B			813													
250						9										
BCB		456												287		144
CBERB			114													
CSLB							1825									
DCLB																152
DEVB		456														
DGB													1448			
DMB													2048			
DSB							575							512	287	
GCB											1149					
ICB					512						575					
LOB									323				256	575		
MTB					2896											
NCB	362															
NIIDB								287								
P/CB												362				
PKB												1290				
S1B				645												
S3B				144						1448						
SCB									406			767				
TNB	512				1625	323	2299						2048	1024		

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
<b>03/164</b>	<b>256</b>	<b>512</b>	<b>181</b>	<b>456</b>	<b>813</b>	<b>161</b>	<b>912</b>	<b>456</b>	<b>912</b>	<b>1448</b>	<b>512</b>	<b>542</b>	<b>1024</b>	<b>406</b>	<b>287</b>	<b>256</b>
<b>112</b>						144										
<b>138B</b>												1290				
<b>143B</b>			813													
<b>250</b>						9										
<b>BCB</b>		456												287		144
<b>CBERB</b>			114													
<b>CSLB</b>							1825									
<b>DCLB</b>																152
<b>DEVB</b>		456														
<b>DGB</b>													1448			
<b>DMB</b>													2048			
<b>DSB</b>							575							512	287	
<b>GCB</b>											1149					
<b>ICB</b>					512						575					
<b>LOB</b>									323				256	575		
<b>MTB</b>					2896											
<b>NCB</b>	362															
<b>NIIDB</b>								287								
<b>P/CB</b>												362				
<b>PKB</b>												1290				
<b>S1B</b>				645												
<b>S3B</b>				144						1448						
<b>SCB</b>									406			767				
<b>TNB</b>	512				1625	323	2299						2048	1024		

**Table 3d Laboratory mean titres using anti-B reagents with A<sub>1</sub>B red cells  
(second donor)**

<i>Reagent (NIBSC code)</i>	<i>Laboratory</i>															
	<i>01</i>	<i>02</i>	<i>03</i>	<i>04</i>	<i>05</i>	<i>06</i>	<i>07</i>	<i>08</i>	<i>09</i>	<i>10</i>	<i>11</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>16</i>
<b>03/164</b>	512	645	256	287	813	456	NT	NT	912	724	575	767	912	NT	256	384
<b>112</b>						203										
<b>138B</b>												1722				
<b>143B</b>			1024													
<b>250</b>						4										
<b>BCB</b>		323												NT		192
<b>CBERB</b>			128													
<b>CSLB</b>							NT									
<b>DCLB</b>																203
<b>DEVB</b>		406														
<b>DGB</b>													1825			
<b>DMB</b>													2048			
<b>DSB</b>							NT							NT	203	
<b>GCB</b>											1024					
<b>ICB</b>					575						575					
<b>LOB</b>									203				256	NT		
<b>MTB</b>					2580											
<b>NCB</b>	645															
<b>NIIDB</b>								NT								
<b>P/CB</b>												362				
<b>PKB</b>												1367				
<b>S1B</b>				456												
<b>S3B</b>				91						813						
<b>SCB</b>									287			813				
<b>TNB</b>	813				1825	645	NT						2580	NT		

NT = not tested

**Table 4a Ratios of the mean titres of anti-B reagents to the mean titres of 03/164 (ascending order) for B cells**

<i>B cells (2 donors)</i>					
<i>Reagent</i>	<i>Lab</i>	<i>Ratio</i>	<i>Reagent</i>	<i>Lab</i>	<i>Ratio</i>
250	06	0.08839	DMB	13	1.00000
250	06	0.08839	DSB	14	1.00000
LOB	09	0.14031	TNB	06	1.12246
LOB	13	0.25000	DGB	13	1.12246
P/CB	12	0.28062	DMB	13	1.12246
LOB	13	0.28062	PKB	12	1.18921
112	06	0.31498	SCB	09	1.25992
LOB	09	0.39685	NCB	01	1.25992
SCB	09	0.39685	DGB	13	1.25992
P/CB	12	0.42045	CSLB	07	1.25992
CBERB	03	0.44545	ICB	11	1.25992
DSB	07	0.50000	138B	12	1.33484
112	06	0.50000	ICB	11	1.41421
CBERB	03	0.56123	DSB	14	1.41421
NIIDB	08	0.56123	LOB	14	1.41421
SCB	12	0.59460	NCB	01	1.41421
S3B	04	0.62996	TNB	06	1.41421
BCB	02	0.62996	S1B	04	1.58740
DCLB	16	0.62996	TNB	01	1.58740
BCB	02	0.62996	TNB	01	1.58740
DEVB	02	0.62996	TNB	05	1.58740
DEVB	02	0.70711	138B	12	1.78180
DCLB	16	0.70711	TNB	13	1.78180
BCB	14	0.70711	GCB	11	1.78180
DSB	15	0.79370	PKB	12	1.88775
BCB	14	0.79370	S1B	04	2.00000
ICB	05	0.79370	TNB	07	2.00000
SCB	12	0.84090	TNB	13	2.00000
S3B	10	0.89090	TNB	05	2.00000
LOB	14	0.89090	GCB	11	2.24492
DSB	15	0.89090	MTB	05	2.24492
BCB	16	0.94387	TNB	14	3.17480
BCB	16	0.94387	MTB	05	3.56359
S3B	04	1.00000	TNB	14	3.56359
ICB	05	1.00000	143B	03	3.56359
S3B	10	1.00000	143B	03	4.48985

**Table 4b Ratios of the mean titres of anti-B reagents to the mean titres of 03/164 (ascending order) for A<sub>1</sub>B cells**

<i>A<sub>1</sub>B cells (2 donors)</i>					
<i>Reagent</i>	<i>Lab</i>	<i>Ratio</i>	<i>Reagent</i>	<i>Lab</i>	<i>Ratio</i>
250	06	0.00984	S3B	10	1.12246
250	06	0.05568	ICB	11	1.12246
LOB	09	0.22272	DSB	14	1.25992
LOB	13	0.25000	NCB	01	1.25992
LOB	13	0.28062	DGB	13	1.41421
SCB	09	0.31498	LOB	14	1.41421
S3B	04	0.31498	NCB	01	1.41421
S3B	04	0.31498	S1B	04	1.41421
LOB	09	0.35355	SCB	12	1.41421
SCB	09	0.44545	TNB	06	1.41421
112	06	0.44545	S1B	04	1.58740
P/CB	12	0.47194	TNB	01	1.58740
BCB	16	0.50000	GCB	11	1.78180
CBERB	03	0.50000	PKB	12	1.78180
BCB	02	0.50000	CSLB	07	2.00000
DCLB	16	0.52973	DMB	13	2.00000
BCB	16	0.56123	TNB	13	2.00000
DCLB	16	0.59460	DGB	13	2.00000
NIIDB	08	0.62996	TNB	01	2.00000
DSB	07	0.62996	TNB	05	2.00000
DEVB	02	0.62996	TNB	06	2.00000
CBERB	03	0.62996	DMB	13	2.24492
ICB	05	0.62996	GCB	11	2.24492
P/CB	12	0.66742	TNB	05	2.24492
BCB	14	0.70711	138B	12	2.24492
ICB	05	0.70711	PKB	12	2.37841
DSB	15	0.79370	138B	12	2.37841
BCB	02	0.89090	TNB	07	2.51984
DEVB	02	0.89090	TNB	14	2.51984
112	06	0.89090	TNB	13	2.82843
S3B	10	1.00000	MTB	05	3.17480
DSB	15	1.00000	MTB	05	3.56359
ICB	11	1.00000	143B	03	4.00000
SCB	12	1.05946	143B	03	4.48985

Table 5

Stability of the candidate IS for minimum potency of anti-A grouping reagents, 03/188: test results after storage at varying temperatures for 10 months.

<i>Storage temperature</i>	<i>Titre with blood group A red cells</i>									
	<i>-70°C</i>		<i>-20°C</i>		<i>+4°C</i>		<i>+20°C</i>		<i>+37°C</i>	
<i>Assay number</i>	<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>	
	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>
1	1024	1024	1024	1024	1024	1024	1024	1024	1024	1024
2	1024	1024	1024	1024	1024	1024	1024	1024	1024	1024
3	1024	1024	1024	1024	1024	1024	1024	1024	1024	1024
4	1024	1024	1024	1024	1024	1024	1024	1024	1024	1024
5	1024	1024	1024	1024	1024	1024	1024	1024	1024	1024
6	1024	1024	1024	1024	1024	1024	1024	1024	1024	1024
Overall Geometric Mean Titre	1024		1024		1024		1024		1024	

Table 6

Stability of the candidate IS for minimum potency of anti-B grouping reagents, 03/164: test results after storage at varying temperatures for nearly 23 months.

<i>Storage temperature</i>	<i>Titre with blood group B red cells</i>									
	<i>-70°C</i>		<i>-20°C</i>		<i>+4°C</i>		<i>+20°C</i>		<i>+37°C*</i>	
<i>Assay number</i>	<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>	
	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>
1	512	256	512	256	256	512	256	512	-	-
2	256	512	512	256	512	512	256	256	-	-
3	512	512	256	256	256	256	256	512	-	-
4	256	512	512	512	256	512	512	512	-	-
5	512	512	512	512	512	512	512	256	-	-
6	512	512	512	512	512	256	512	512	-	-
Geometric mean titre	406	456	456	362	362	406	362	406		
Overall Geometric Mean Titre	431		406		384		384			

\*would not reconstitute

Table 7

Stability of the British Minimum Potency Preparation for anti-A, 88/722: test results after storage at varying temperatures for nearly 16 years.

<i>Storage temperature</i>	<i>Titre with blood group A red cells</i>					
	<i>-20°C</i>		<i>+4°C</i>		<i>+20°C</i>	
<i>Assay number</i>	<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>	
	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>
1	1024	2048	1024	2048	1024	1024
2	1024	1024	1024	1024	1024	1024
3	1024	2048	1024	1024	1024	1024
4	1024	1024	1024	1024	512	1024
5	2048	1024	1024	1024	1024	1024
6	1024	1024	1024	1024	1024	1024
Overall Geometric Mean Titre	1218		1085		967	

Table 8

Stability of the British Minimum Potency Preparation for anti-B, 88/724: test results after storage at varying temperatures for nearly 16 years.

<i>Storage temperature</i>	<i>Titre with blood group B cells</i>							
	<i>-20°C</i>		<i>+4°C</i>		<i>+20°C</i>		<i>+37°C</i>	
<i>Assay number</i>	<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>		<i>Ampoule</i>	
	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>
1	1024	512	512	512	512	256	512	512
2	512	512	512	512	512	256	512	256
3	512	256	512	512	512	512	512	512
4	512	512	512	512	512	512	512	512
5	512	512	512	512	512	512	512	512
6	512	256	256	512	512	512	256	512
Overall Geometric Mean Titre	483		483		456		456	

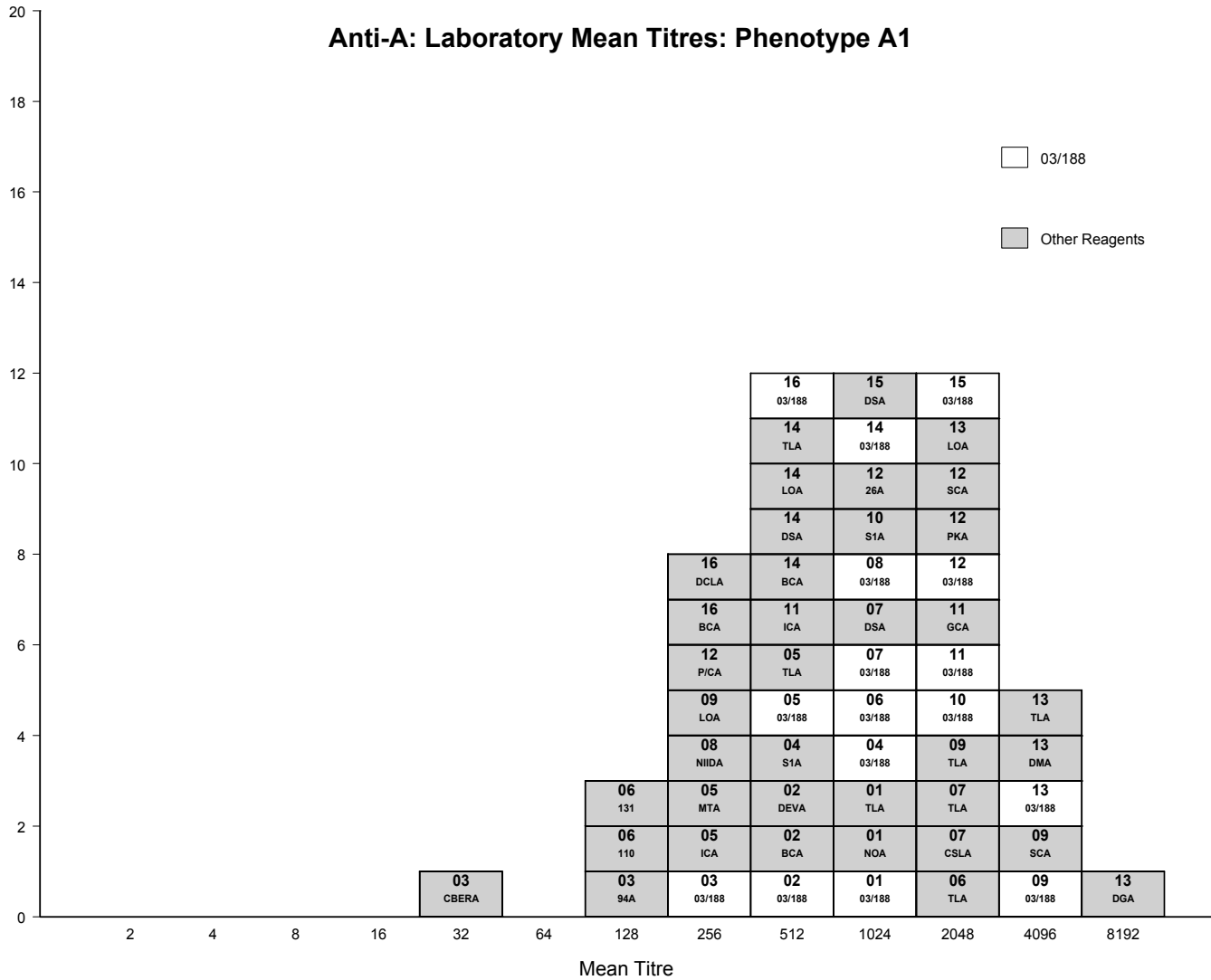
Table 9

Questionnaire results summary for initial dilutions of candidate International Minimum Potency Reference Reagents for anti-A (03/188) and anti-B (03/164) following the main collaborative study in 2004.

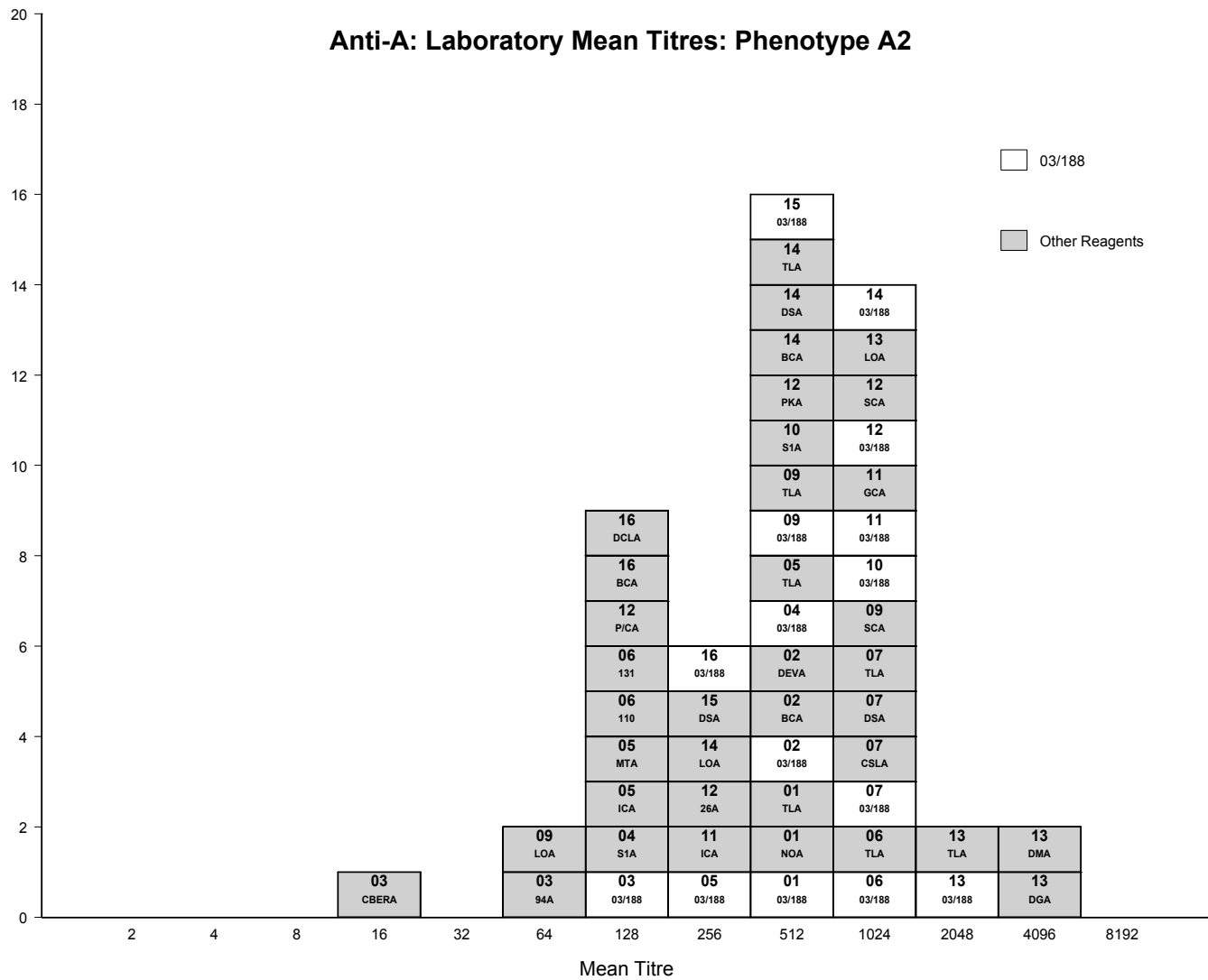
<i>Lab number</i>	<i>Anti-A</i>				<i>Anti-B</i>			
	<i>1 in 4</i>	<i>1 in 5</i>	<i>1 in 8</i>	<i>other</i>	<i>1 in 2</i>	<i>1 in 3</i>	<i>1 in 4</i>	<i>other</i>
1	x	x	x	1 in 16	x	x	x	1 in 16
2	x	yes	yes		x	yes	yes	
3	x	x	yes		x	x	yes	
4	x	x	yes		x	x	yes	
5								
6	(yes)*	yes	x		x	(yes)*	yes	
7	yes				yes			
8	yes	x	x		yes	x	x	
9	x	yes	x		x	x	yes	
10								
11	yes					yes		
12	yes	(yes)*	x		yes	(yes)*	x	
13	yes				yes			
14	(yes)*	Yes	x		yes	(yes)*	x	
15			yes				yes	
16	yes	?	x		yes	?	x	
<b>totals</b>	<b>6/8</b>	<b>4/5</b>	<b>4</b>	<b>1</b>	<b>6</b>	<b>2/5</b>	<b>6</b>	<b>1</b>

\*agree to this dilution if it is the consensus dilution

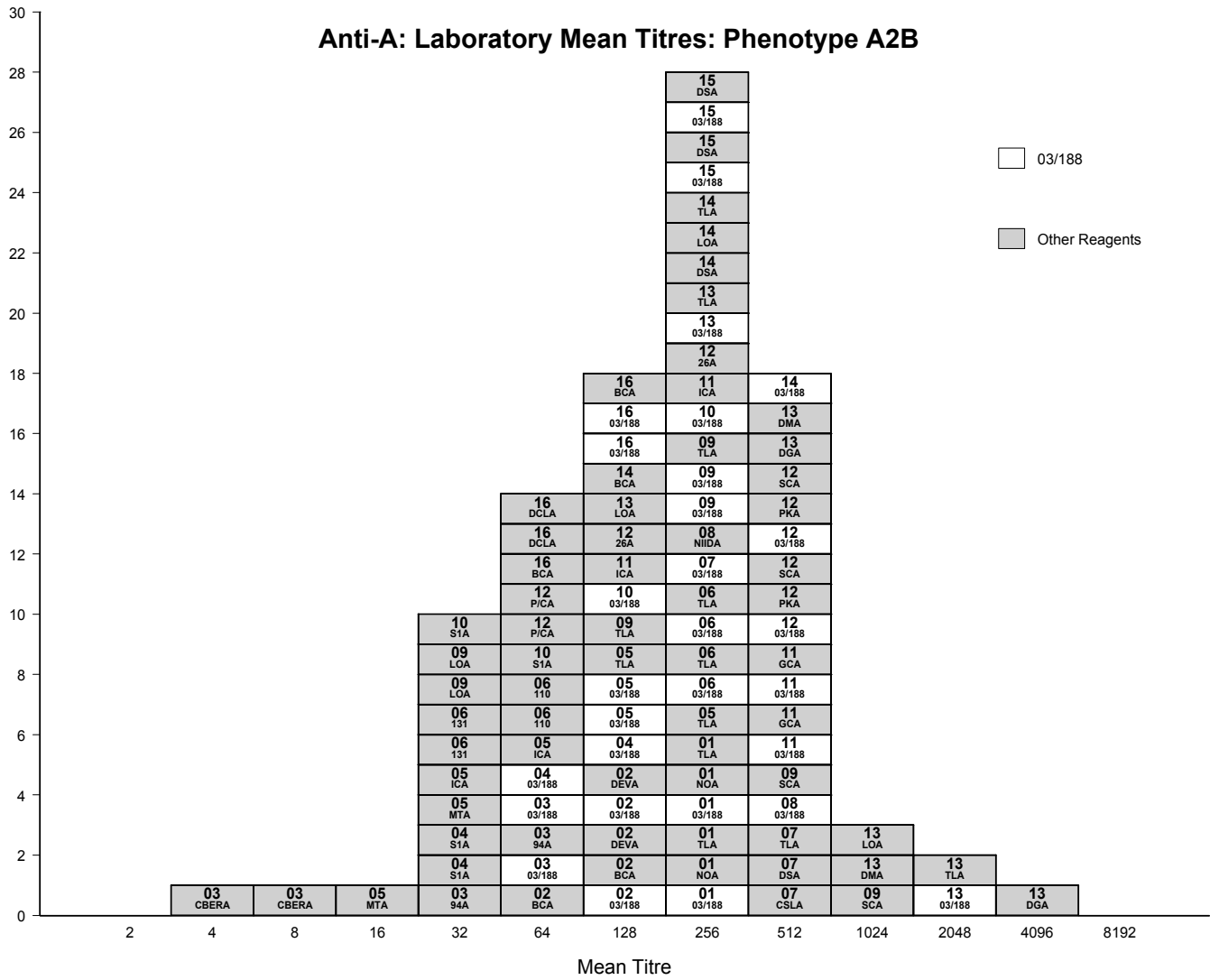
**Figure 1a Laboratory mean titres of the candidate International Standard for minimum potency, 03/188, and anti-A blood grouping reagents using A<sub>1</sub> red cells**



**Figure 1b Laboratory mean titres of the candidate International Standard for minimum potency, 03/188, and anti-A blood grouping reagents using A<sub>2</sub> red cells**

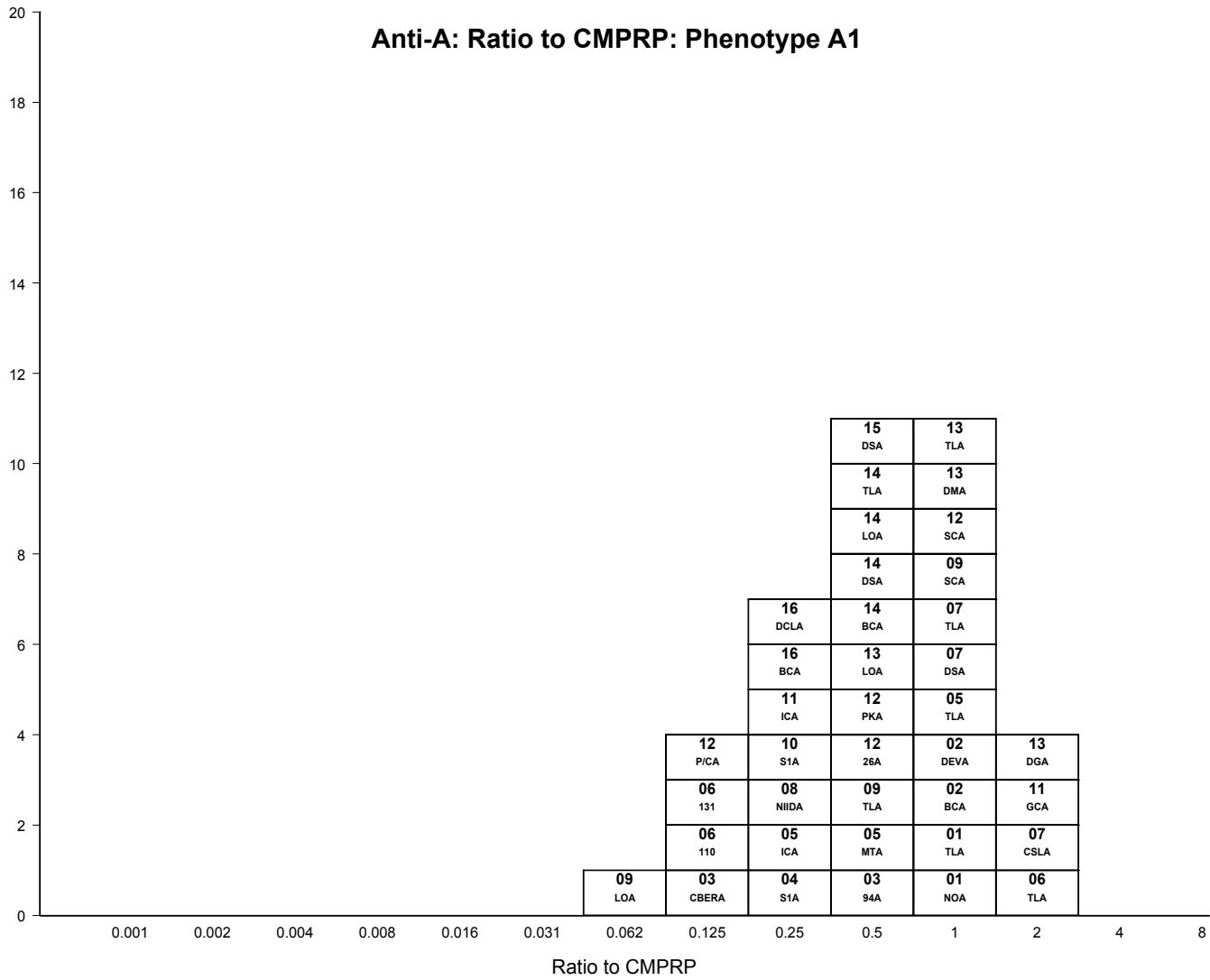


**Figure 1c** Laboratory mean titres of the candidate International Standard for minimum potency, 03/188, and anti-A blood grouping reagents using A<sub>2</sub>B red cells

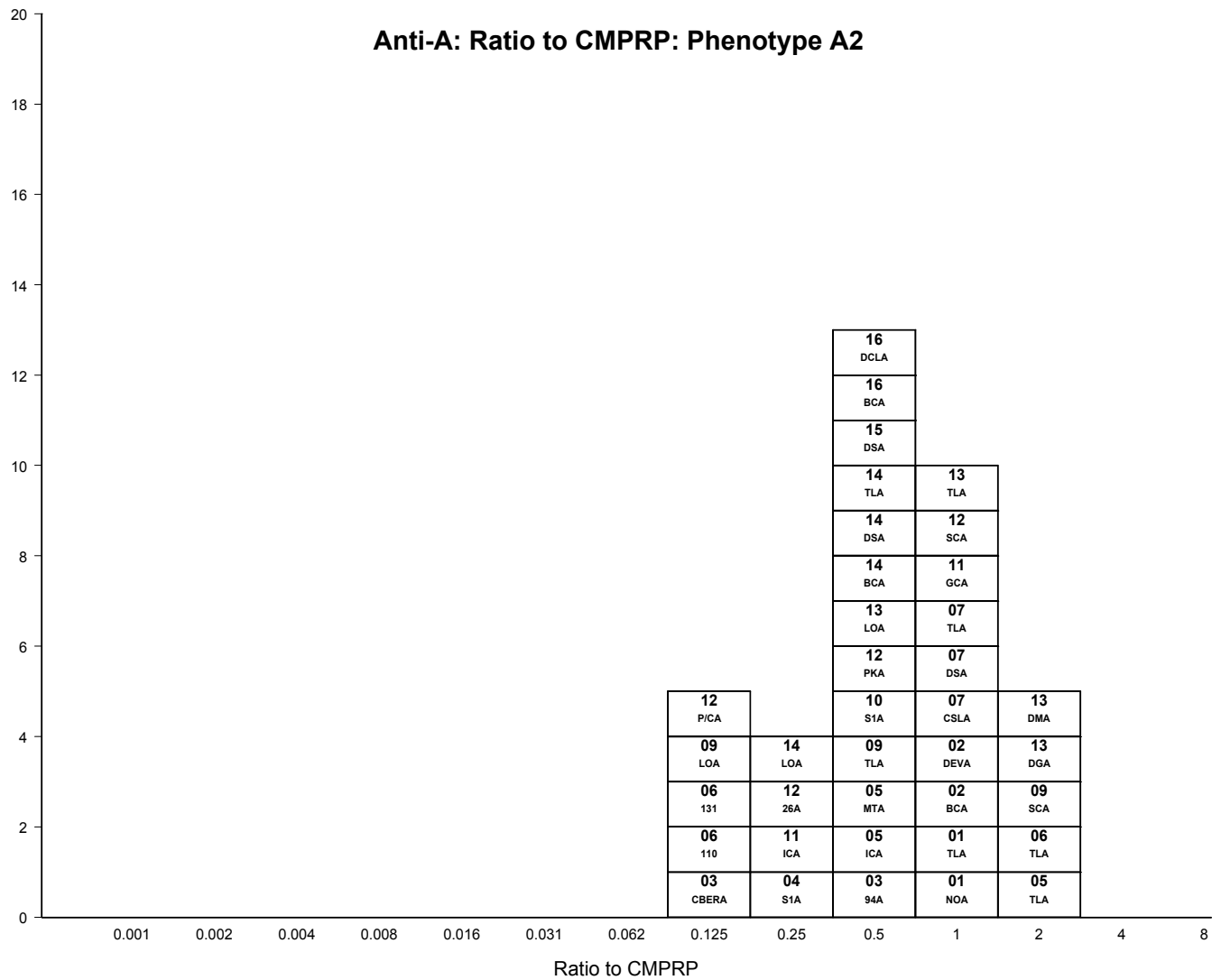


**Figure 1d Laboratory mean titres of the candidate International Standard for minimum potency, 03/188, and anti-A blood grouping reagents using all blood group A phenotypes**





**Figure 2b Ratios of the mean titres of anti-A reagents to the mean titres of the candidate International Standard for minimum potency, 03/188, using A<sub>2</sub> red cells (CMPRP = 03/188)**



**Figure 2c Ratios of the mean titres of anti-A reagents to the mean titres of the candidate International Standard for minimum potency, 03/188, using A<sub>2</sub>B red cells (CMPRP = 03/188)**







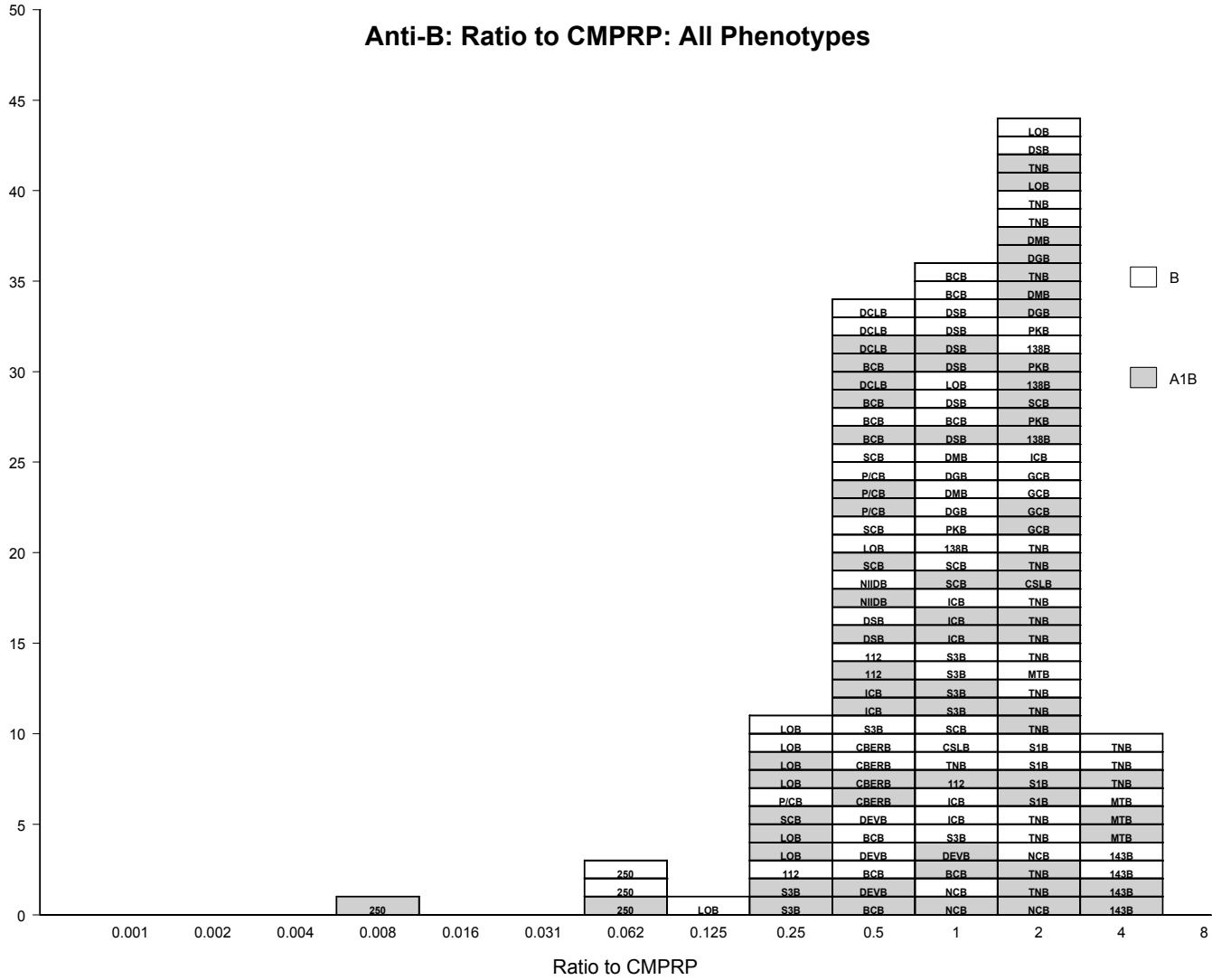








**Figure 4c Ratios of the mean titres of anti-B reagents to the mean titres of the candidate International Standard for minimum potency, 03/164, using all blood group B phenotypes (CMPRP = 03/164)**









**Appendix 1 Participants of the collaborative study**

(listed in alphabetical order of country)

P-Y Le Pennec, Centre National de Référence pour les Groupes Sanguins, Paris, France

JP Prasad, National Institute of Biologicals, Ministry of Health & Family Welfare, Noida, India

T Mizuochi, National Institute of Infectious Diseases, Tokyo, Japan

N Irshaid and J Merza Niquir, National Blood Bank, Amman, Jordan

M Overbeeke and M de Haas, Sanquin Blood Supply at CLB, Amsterdam, The Netherlands

F Carbonell Uberos, Centro Transfusion Comunidad Valenciana, Spain

P Horovitz and H Dieringer, Medion Diagnostics GmbH, Switzerland

R Knight, North London Blood Transfusion Centre, Colindale, London NW9, UK

M Scott, International Blood Group Reference Laboratory (IBGRL), Bristol, UK

B Fox and SJ Thorpe, National Institute for Biological Standards and Control, Potters Bar, UK

S Galloway, Serologicals, Livingston, UK

M Berry and J Allan, Alba Bioscience (formerly Diagnostics Scotland), Edinburgh, UK

J Rockwell and R Collins, American Red Cross, USA

S Kochman, Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration (CBER/FDA), Rockville, Maryland, USA

C Johnson and H Yorek, Gamma Biologicals, Inc., USA

D Stone and S Webber, Immucor, Inc., USA

K Reis and L Vellucci, Ortho-Clinical Diagnostics, USA

## Appendix 2

# REFERENCE METHOD FOR TESTING THE CANDIDATE MINIMUM POTENCY REFERENCE PREPARATIONS FOR ANTI- A AND ANTI-B

### 1. GENERAL INFORMATION

This method is to be used by participants in the WHO collaborative study to assess proposed new minimum potency reference preparations for anti-A and anti-B blood grouping reagents. This method should be used for parallel testing of the candidate minimum potency reference preparations 03/188 and 03/164 against **monoclonal anti-A and anti-B reagents**.

### 2. MATERIALS SUPPLIED BY WHO COLLABORATING CENTRES

- 9 ampoules of each of the candidate WHO minimum potency reference preparations for anti-A and anti-B, 03/188 and 03/164 respectively.

### 3. MATERIALS TO BE SUPPLIED BY PARTICIPANTS

- Water (deionized or distilled)
- Buffered saline (buffered 0.85 – 0.9% NaCl)
- Buffered saline containing 2% (w/v) bovine serum albumin (BSA) i.e. 2 grams BSA per dL of buffered saline, or 1 mL of 30% BSA plus 14 mL buffered saline, or 2 mL of 22% BSA plus 20 mL buffered saline

*NOTE: BSA should not be deliberately polymerized*

- Glass test tubes (10-12 mm diameter x 70-80 mm length)
- Pipettes (transfer, graduated, & semi-automatic with changeable tips)
- Red cell samples:

*For testing anti-A:*

1 x A<sub>1</sub>

1 x A<sub>2</sub>

2 x A<sub>2</sub>B (to be tested individually, do not pool)

1 x B (control)

1 x O (control)

*For testing anti-B:*

2 x B (to be tested individually, do not pool)

2 x A<sub>1</sub>B (to be tested individually, do not pool)

1 x A<sub>1</sub> (control)

1 x O (control)

The A<sub>2</sub> and A<sub>2</sub>B cells are to give unequivocal positive reactions with anti-H in order to exclude H-deficient A<sub>1</sub> (or A<sub>1</sub>B) samples.

- As many commercial monoclonal anti-A and anti-B blood grouping reagents as are available to you.

#### 4. PREPARATION OF DILUTIONS

Pool the reconstituted contents of 3 ampoules of each of the candidate minimum potency reference preparations 03/188 and 03/164 **each day** to ensure sufficient volumes for testing.

Prepare 2 independent series of two-fold serial (doubling) dilutions (1 in 2, 1 in 4, etc) from neat of each of the preparations 03/188 and 03/164, and each of the anti-A and anti-B reagents under test, continuing to 1 in 8192. **The dilutions should be such that there are at least two tests with negative results in the titration.**

- Use buffered saline containing 2% bovine serum albumin as diluent.
- Test tubes should be of a size that facilitates adequate mixing of the contents
- A separate, clean pipette or pipette tip should be used for each dilution to avoid carryover of higher reagent concentrations.

#### 5. PREPARATION OF RED BLOOD CELL SUSPENSIONS

- a) Wash the red cells at least three times in buffered saline or until a clear supernatant is obtained.
- b) Resuspend packed cells to a 2% (v/v) suspension in buffered saline.

#### 6. THE TEST

- a) Place 1 volume of neat and each of the dilution series, in replicate, of 03/188 or 03/164 and each of the anti-A or anti-B reagents, respectively, in a separate, clean test tube.
  - b) Add 1 volume of the 2% red cell suspension to each test tube.
  - c) Mix the contents of each tube thoroughly and incubate the test tubes at room temperature (19-25 °C) for 5 minutes.
  - d) Centrifuge for 1 minute at approximately 1000 rpm (100-125 rcf) or 15 seconds at approximately 3400 rpm (900-1000 rcf) or at a time and speed appropriate for the centrifuge being used or, in the case of a reagent, for the shortest period of time at the lowest speed recommended in the manufacturer's package insert. Ensure that the centrifugation does not result in an excessive force being necessary to dislodge the cell button prior to observation.
  - e) Resuspend the cell button by gentle agitation.
  - f) Macroscopically grade the reactions as described below and record the results on the accompanying results sheets. Record the red cell phenotype eg A<sub>2</sub>B (no 1) or A<sub>2</sub>B (no 2).
- **Perform the titrations with each red cell phenotype, always testing the candidate minimum potency reference preparations in parallel with the appropriate grouping reagents.**

## 7. INTERPRETATION OF THE TEST

REACTION STRENGTH	GRADE	OBSERVATION
++++	4+/C	Cell button remains in one clump
+++	3+	Cell button dislodges into several large clumps
++	2+	Cell button dislodges into many small clumps
+	1+	Cell button dislodges into granular but definite clumps
±/(+)	D	Cell button dislodges into fine small granules
0	0	Cell button dislodges to give a homogeneous suspension of red blood cells

NB The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.

## Appendix 3 Anti-A and anti-B reference preparations

Reference Preparation	Code	Type	Clone (if monoclonal)	Reconstitution	Recommended use for potency comparisons
2 <sup>nd</sup> WHO IS for anti-A blood typing serum; 470 IU/ampoule	W1001	Polyclonal	-	1.0 ml distilled water	Add a further 6.3 ml saline/BSA to give 64 IU/ml. Reagents tested in parallel must have a potency titre equal to or greater than the diluted reference.
3 <sup>rd</sup> WHO for anti-B blood typing serum; 860 IU/ampoule	W1002	Polyclonal	-	1.0 ml distilled water	Add a further 12.4 ml saline/BSA to give 64 IU/ml. Reagents tested in parallel must have a potency titre equal to or greater than the diluted reference.
US CBER/FDA reference anti-A blood grouping reagent	Lot 6a	Polyclonal	-	2.5 ml saline	Reagents tested in parallel must have a potency titre equal to or greater than the reference.
US CBER/FDA reference anti-B blood grouping reagent	Lot 7a-1	Polyclonal	-	1.0 ml distilled water and 2.5 ml saline	Reagents tested in parallel must have a potency titre equal to or greater than the reference.
British anti-A minimum potency reference preparation	88/722	Monoclonal	BRIC 131	1.0 ml distilled water	Reagents tested in parallel must have a potency titre equal to or greater than a 1 in 14 dilution of the reference.
British anti-B minimum potency reference preparation	88/724	Monoclonal	ES4	1.0 ml distilled water	Reagents tested in parallel must have a potency titre equal to or greater than a 1 in 4 dilution of the reference.

**Appendix 4 Anti-A grouping reagents  
(in alphabetical order of code; details supplied by participants)**

Code	Manufacturer	Product name	Reagent type	Clone	Labs
110	Plasmatec Laboratory Products Ltd, Dorset, UK	Anti-A monoclonal ABO/110	Monoclonal	9113D10	6
131	IBGRL, Bristol, UK	BRIC 131 Anti-A	Monoclonal	BRIC 131	6
26A	Gamma Biologicals, Houston, TX, USA	Anti-A 6401 (murine monoclonal)	Monoclonal	BIRMA-1	12
94A	Gamma Biologicals, Houston, TX, USA	Anti-A (murine monoclonal) by Slide, Tube or Microwell test	Monoclonal	BIRMA-1	3
BCA	Ortho-Clinical Diagnostics, Raritan, NJ, USA	Anti-A BioClone	Monoclonal blend	MHO4 + A3D3	2, 14, 16
CBERA	CBER	#6a			3
CSLA	CSL Ltd, Australia	Rhesolve-A	Monoclonal		7
DCLA	Diamed, Switzerland	DiaClon anti-A	Monoclonal	LM297/628 (LA2)	16
DEVA	Ortho-Clinical Diagnostics, Raritan, NJ, USA	Anti-A under development to replace anti-A BioClone	Monoclonal blend	MHO4 + A3D3	2
DGA	Diagast, Cedex, France	Monoclonal anti-A (ABO1)	Monoclonal	9113D10	13
DMA	DiaMond, Jordan	Monoclonal anti-A	Monoclonal		13
DSA	Diagnostics Scotland, UK	Anti-A Z001	Monoclonal	LA2	7, 14, 15
GCA	Meridian, Rüsselsheim	Gull-Clone anti-A	Monoclonal	BIRMA-1	11
ICA	Immucor GmbH, Rüolemark	ImmuClone Anti-A monoclonal	Monoclonal	BIRMA-1	5, 11
LOA	Lorne	Anti-A	Monoclonal	9113D10	9,13, 14
MTA	Medion Diagnostics GmbH	Anti-A Mono-type	Monoclonal blend	A1 + A20	5
NIIDA	National Institute of Infectious Diseases, Tokyo, Japan	NIID control anti-A antibody (minimum potency 1:256)	Monoclonal blend		8
NOA	Serologicals Ltd, Livingston, UK	Anti-A (NO)	Monoclonal	BIRMA-1	1
P/CA	Diamed	Anti-A polyclonal	Polyclonal		12

	Switzerland				
PKA	Sanquin Reagents, Amsterdam, The Netherlands	Pelikloon anti-A (IgM) monoclonal	Monoclonal	BIRMA-1	12
S1A	Immucor Gamma, Norcross GA, USA	Anti-A monoclonal Series 1	Monoclonal	BIRMA-1	4, 10
SCA	Biotest, Dreieich	Seraclone anti-A	Monoclonal	A003	9, 12
TLA	Bioscot, Serologicals Ltd, Livingston, UK	Anti-A (NO)	Monoclonal	BIRMA-1	1, 5, 6, 7, 9, 13, 14

**Appendix 5 Anti-B grouping reagents  
(in alphabetical order of code; details supplied by participants)**

<b>Code</b>	<b>Manufacturer</b>	<b>Product name</b>	<b>Reagent type</b>	<b>Clone</b>	<b>Labs</b>
<b>112</b>	Plasmatec Laboratory Products Ltd, Dorset, UK	Anti-B monoclonal ABO/112	Monoclonal	9621A8	6
<b>138B</b>	Gamma Biologicals Inc, Houston, TX, USA (Immucor Gamma)	Anti-B (murine monoclonal) gamma clone 6407	Monoclonal	LB2	12
<b>143B</b>	Gamma Biologicals Inc, Houston, TX, USA (Immucor Gamma)	Anti-B (murine monoclonal) BM143-5	Monoclonal	GAMA-110	3
<b>250</b>	IBGRL, Bristol, UK	BRIC 250 Anti-B	Monoclonal	BRIC 250	6
<b>BCB</b>	Ortho-Clinical Diagnostics, Raritan, NJ, USA	Anti-B Bioclone	Monoclonal blend	NB1.19 + NB10.5A5 + NB10.3B4	2, 14, 16
<b>CBERB</b>	CBER 7a-1	Anti-B #7a-1			3
<b>CSLB</b>	CSL Ltd, Australia	Rhesolve-B	Monoclonal		7
<b>DCLB</b>	Diamed, Switzerland	DiaClon anti-B	Monoclonal	LM 306/686 (LB2)	16
<b>DEVB</b>	Ortho-Clinical Diagnostics, Raritan, NJ, USA	Anti-B in development (as BCB above with reformulation)	Monoclonal blend	NB1.19 + NB10.5A5 + NB10.3B4	2
<b>DGB</b>	Diagast, Cedex, France	Monoclonal anti-B (ABO2) S2	Monoclonal	9621A8	13
<b>DMB</b>	DiaMond, Jordan	Monoclonal anti-B (7B04020)	Monoclonal		13
<b>DSB</b>	Diagnostics Scotland, UK	Anti-B	Monoclonal	LB2	7, 14, 15
<b>GCB</b>	Meridian, Rüsselsheim	Gull Clone anti-B II	Monoclonal	LB2	11
<b>ICB</b>	Immucor GmbH	ImmuClone anti-B monoclonal	Monoclonal	LB2	5, 11
<b>LOB</b>	Lorne, Reading, UK	Anti-B	Monoclonal	9621A8	9, 13, 14
<b>MTB</b>	Medion Diagnostics GmbH	Anti-B Mono-type	Monoclonal	AB26	5
<b>NCB</b>	Serologicals Ltd, Livingston, UK (Bioscot)	Anti-B (NC)	Monoclonal	ES-4	1
<b>NIIDB</b>	National Institute of Infectious	NIID control anti-B antibody (minimum	Monoclonal blend		8

	Diseases, Tokyo, Japan	potency 1:256)			
<b>P/CB</b>	Diamed, Switzerland	Anti-B polyclonal	Polyclonal		12
<b>PKB</b>	Sanquin Reagents Amsterdam, The Netherlands	Pelikloon anti-B (IgM) monoclonal	Monoclonal	LB-1	12
<b>S1B</b>	Immucor Gamma, Norcross, GA, USA	Anti-B monoclonal Series 1	Monoclonal	ES-4	4
<b>S3B</b>	Immucor Gamma, Norcross, GA, USA	Anti-B monoclonal Series 3	Monoclonal	LB2	4, 10
<b>SCB</b>	Biotest, Dreieich	Seroclone anti-B	Monoclonal	B005	9
<b>TNB</b>	Serologicals Ltd, Livingston, UK (Bioscot)	Anti-B (TN)	Monoclonal	LB2	1, 5, 6, 7, 13, 14

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