THE INTERNATIONAL STANDARD FOR INCOMPLETE ANTI-D BLOOD GROUPING SERUM

by

K. L. G. Goldsmith
A. E. Mourant
International Blood Group Reference Laboratory, London
and
D. R. Bangham
International Laboratory for Biological Standards
National Institute for Medical Research, London

I. HISTORICAL INTRODUCTION

In 1953 500 ml of saline-agglutinating or "complete" anti-D (anti-Rho) serum were collected by the International Blood Group Reference Laboratory in order to produce a standard. Tests of this material, freeze-dried by a method then in common use, at intervals over the next three years showed that the potency fell considerably and the preparation was therefore unsuitable for use as a standard. In 1956, Dr. Mourant reported his findings to the Expert Committee on Biological Standardization, stating in his memorandum that in view of the instability and shortage of "complete" anti-D (anti-Rho), it might be necessary to prepare an "incomplete" anti-D standard, the material for which was more freely available.

---

This draft memorandum on the International Standard for incomplete anti-D blood grouping serum, subject to amendments proposed by participants in the collaborative assay, will be submitted for publication in the Bulletin of the World Health Organization in the normal way.

Reprints will then be available to users of the International Standard, and to others interested.

Director since 1965.

Director until 1965.

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.
The Expert Committee on Biological Standardization asked the International Blood Group Reference Laboratory, London, to carry out extensive collaborative studies in order to prepare the ground for the establishment of an international standard for albumin-potentiated anti-D (anti-Rho) blood typing serum.²

A collaborative investigation was then carried out in which ten expert laboratories studied the effect of titration techniques upon the observed titres of six different freeze-dried preparations of "incomplete" anti-Rho (anti-D).³,⁴ The object of this investigation was to determine whether if one "incomplete" anti-D preparation was designated as a standard and was assigned an arbitrary strength expressed either as a titre or as a number of units per millilitre, the relative strengths deduced for other sera would depend upon the method of titration.

It is well known that when a particular serum is titrated against a particular red cell sample or genotype, the results obtained by different laboratories vary very considerably, but it is generally assumed that in comparing the potency of two or more sera, the results obtained by two laboratories will usually show the same ratio between the titres of one serum as with those of another. When, however, the results from the ten laboratories were compared, their estimated titres differed by as much as 500-fold for a given serum by a given method. When the results for five sera were compared with a sixth, arbitrarily designated as the standard, differences between laboratories, although still considerable, were appreciably reduced. Furthermore, differences occurred according to the methods employed; those methods involving colloid media and those involving enzyme-treatment of red cells gave somewhat similar results but the results by each method differed markedly from estimates made with the anti-human-globulin technique in this respect.

Mourant concluded that the anti-human-globulin technique might be dependent at least in part on a component of serum other than that responsible for cell agglutination in the presence of colloids or proteolytic enzymes. A statistical analysis of the data of this collaborative investigation was produced by Uemura.⁵

Despite the differences in estimates by different methods it was decided that work towards the preparation of a standard should continue. In consultation with the International Laboratory for Biological Standards at the National Institute for Medical Research, the International Blood Group Reference Laboratory took steps to
collect a large number of samples of "incomplete" anti-D (anti-Rho) to be pooled in an attempt to prepare a standard for "incomplete" anti-D (anti-Rho) serum. This paper reports the collection, selection and pooling of individual sera and of tests carried out on the serum when it had been pooled and subsequently after it had been freeze-dried in ampoules.

II. THE PREPARATION OF THE STANDARD

(a) The collection of the material

Through the collaboration of expert blood group and transfusion laboratories (listed in Appendix A) in eleven countries, quantities of raw sera containing potent "incomplete" anti-D (anti-Rho) were collected. Certain laboratories sent the whole serum supply in the first instance, while others sent at first a small sample so that it might be tested at the International Blood Group Reference Laboratory and its suitability for inclusion in an anti-D standard pool assessed. If the results were satisfactory, the bulk was then sent to the International Blood Group Reference Laboratory.

Serum sent from laboratories within the United Kingdom was transmitted by post, rail or by road transport, while that from abroad was sent by air, by means of postal or freight services. Samples were taken from each contribution of serum on arrival and all sera stored thereafter at -25°C until required.

(b) Selection and pooling of sera

All the tests on the serum samples and the pool at the International Blood Group Centre were carried out by the same person throughout all stages of the preparation. The titre of each initial sample was estimated using saline and albumin agglutination techniques. All titrations were performed in test-tubes; in the saline technique a 2.5% red cell suspension was prepared in 0.85% physiological saline solution, the same solution being used as diluent for the antibody-containing serum. In the albumin technique a 5% red cell suspension was prepared in 20% bovine albumin solution (Armours); the diluting fluid for the antibody-containing serum was inert AB serum. Group O cells of the Rh genotypes CDe/CDe(R_1 R_1), cDe/cde (R_2^+), Cde/cde(R' r), cD/cde(R''r) and cde/cde (rr) were used in each case with both saline and albumin techniques. In addition, ficin-treated Group O red cells of the Rh
genotypes Cde/cde(R' r) and cdE/cde(R'' r) were used to exclude the presence of Rh antibodies other than anti-D (anti-Rho) in each serum sample.

Because they are more easily obtainable throughout the world than any other Rh genotype, red cells of the genotype CcDee (R_1 r) were included, at Dr. Mourant's suggestion, in tests on the standard. Although they were not used in the initial screening of individual contributions, they were included in all tests after the sera were pooled.

Only those serum samples that failed to agglutinate red cells in saline suspension, and in which other Rh antibodies were not detected by means of the ficin technique and which agglutinated the Rh D positive red cells to a titre of at least 64 were considered suitable to include in the standard preparation.

Unwanted anti-A and anti-B agglutinins were removed from sera that fulfilled above criteria. These agglutinins were removed by the addition of an equal volume of washed packed Group A_1 B Rhesus-negative cdE/cde(rr) red cells to the unabsorbed sera. The cell-serum mixtures were left at 4°C for approximately 18 hours. Following this, the red cells were separated from the serum by centrifugation and the supernatant serum removed.

At this stage, each individual serum was fully tested, both for adequate potency as before, and also to exclude the presence of any unwanted antibodies, including anti-A, anti-B, anti-M, anti-N, anti-S, anti-s, anti-Ww, anti-P, Rh antibodies other than "incomplete" anti-D (anti-Rho), anti-Lu^b, anti-K, anti-k, anti-Le^a, anti-Le^b, anti-Fy^a, anti-Wr^a and anti-Xg^a. Despite all the precautions listed above, a trace of anti-C, detectable only by the use of ficin-treated red cells, appeared in the final pool, as reported in Section IV (i) below.

Sera which satisfied the required criteria were contributed by the laboratories listed in Appendix A. Tests for potency and specificity of a trial pool of these sera gave acceptable results, so a definitive pool of approximately 2000 ml was prepared, on which tests for potency (using CcDee (R_1 r) red cells) and for specificity gave satisfactory results.

(c) Distribution of serum into ampoules and freeze-drying

In June 1964, approximately 1.8 litres of the pooled sera were sent to the
Division of Biological Standards, National Institute for Medical Research, London, where the material was centrifuged at 450 g for 30 minutes at 2°C. The supernate was then filtered through successive glass fibre prefilters and Millipore membranes of decreasing pore diameter until the solution passed a sterilizing membrane (average pore diameter 0.45 μ).

After storage overnight at 2°C the material was dispensed from an ice-cooled reservoir in 0.5 ml amounts into 3350 sterile neutral glass ampoules. Check weighings showed the mean weight of serum per ampoule to be 0.4560 g ± 2.15%. The ampoules were cooled in liquid nitrogen, transferred to the freeze drier shelves at -30°C and freeze-dried as a single batch during three days. The ampoules were placed over fresh P₂O₅ in evacuated desiccators for 14 days. At the end of this period, the ampoules were filled with pure dry nitrogen and sealed. After being tested for cracks and pinholes, they were stored at -10°C in the dark.

(d) Stability tests

Tests carried out at the International Blood Group Reference Laboratory showed a lower estimated titre on the pooled serum after freeze-drying. Since the samples were compared directly against the same substrate and on the same day it is probable that this represented a true small loss of potency. Estimates by direct comparison of ampoules of the standard (i.e., in the final form) kept at -10°C with ampoules kept at 4°C, 20°C and 37°C for 10 months showed no loss of potency in the latter, at least with R₂+h cells (Table I). These observations suggest that at the temperature at which the standard will be kept (-20°C) the material is unlikely to show a detectable loss of potency for many years. Similar tests using R₁+h cells gave more irregular figures which because they showed no trend correlating with the temperature of storage probably represent a borderline endpoint between dilution steps.

III. COLLABORATIVE STUDY OF THE "INCOMPLETE" ANTI-D (ANTI-Rho) POOL

Ampoules of the proposed standard were tested by 29 specialist laboratories (listed in Appendix B) in 21 different countries. Each laboratory was asked to test the serum

(1) To exclude the presence in it of any unwanted antibodies, especially of anti-A, anti-B, and of Rh anti-C and saline agglutinating anti-D.
(2) To titrate the serum with cells of type CcDee (R_1 ^r) by the following methods, the techniques of which are described in full in Appendix C.

(i) Albumin suspension method
(ii) Albumin displacement method
(iii) Enzyme treatment method using Papain
(iv) Indirect anti-human-globulin test

(3) In addition, each laboratory was asked to test the serum against the widest range of D^u cells available, and, if possible, with red cells found to carry particular Rh antigens, or combinations of antigens, of the series described by L. J. Unger and A. S. Wiener as A, B, C and D. ⁶

IV. RESULTS OF THE COLLABORATIVE STUDY OF THE INTERNATIONAL STANDARD

(1) Qualitative

No laboratory detected anti-A or anti-B antibody. Anti-C was detected in trace amounts only in certain laboratories, but not by either of the albumin techniques or by the anti-human-globulin technique. The presence of certain other blood group antibodies was excluded, and at the International Blood Group Reference Laboratory the absence of anti-M, anti-N, anti-S, anti-s, anti-Vw, anti-P_1 , anti-E, anti-c, anti-e, anti-f, anti-Lu_a, anti-Lu_b, anti-K, anti-k, anti-Le_a, anti-Le_b, anti-Fy_a, anti-Fy_b, anti-Jk_a, anti-Jk_b, anti-Xg_a and anti-Wr_a was confirmed.

Tests with D^u cells were performed by 15 laboratories using albumin or anti-human-globulin techniques. All 15 laboratories reported positive results using one or other technique. Three laboratories reported titration results using D^u cells of various antigen strengths but these have not been included here because of the small numbers of results available.

Tests with red cells carrying Rh antigens or combinations of antigens of the series described by Drs L. J. Unger and A. S. Wiener ⁶ were performed by only two laboratories but in each case the anti-D was shown to react with D^b red cells.
Results of titrating the serum with red cells of type CoDee (R₁r) by four techniques as reported by participating laboratories are shown in Table II, as well as in Figures I and II.

Certain laboratories reported their findings using enzyme techniques, namely Bromelin, Ficin and Löw's papain, but again the number of results available is too small to warrant inclusion.

(2) Quantitive

Titres reported by the 29 laboratories using red cells of the type CoDee (R₁r) estimated by each of the four techniques are listed in Table II, and histograms of the frequency distribution of these titres for each method are shown in Figure I. The histogram in Figure II shows the frequency distribution of all estimates by all methods.

These results again show that titres estimated in the hitherto conventional way (without comparison with a standard) varied widely both between laboratories and between the methods used, thus confirming the findings of Mourant in a similar study reported in 1959. The geometric means calculated from these titres are listed in Table III, together with confidence limits (p = 0.95).

The results obtained with the albumin suspension and albumin displacement methods were closely similar and the results of t tests between mean log titres (Table IV) showed no significant difference in estimates by these two methods. The geometric means of titres by the antiglobulin and papain treatment methods, although not much different from each other, were however significantly higher than those obtained with the two albumin methods.

The confidence limits of the combined estimates using the antiglobulin technique (Table III) were narrower than those of the other three techniques which were similar to one another. The albumin replacement method which was found to be the most precise one in the previous collaborative study, was not carried out in this study.
V. DISCUSSION

The benefits of using a standard for comparative estimation of antibody levels in anti-D sera are much the same as for the classical antitoxins for which the unit notation system has been in use for many years. Some of these benefits, such as the increased precision obtained within a laboratory from day to day, and the agreement made possible between all laboratories using it have already been demonstrated for anti-D antibodies in the collaborative study carried out in 1959.

The aims of the collaborative study on this occasion were therefore limited to testing the qualitative specificity of the international standard, and to obtaining a survey of titres estimated for it by the four commonly used methods, in a large number of expert laboratories. The specificity was found to be entirely satisfactory. The results of quantitative determinations showed that the potency (titre, strength) was satisfactory for use in the great majority of the laboratories which tested it. The minor differences in the patterns of the histograms (Figure I) are of unknown significance and possibly of little importance. However, the difference in mean estimates made with the albumin methods and the other two methods is statistically significant at a high level of probability (Table IV). Until the mechanism of these assay methods is better understood it seems that for the full characterization of a serum it may be necessary to employ three assays, one with albumin, one with an enzyme and one by means of the antiglobulin technique. Moreover, it will be necessary to specify the method when sera are assayed.

These points are of considerable practical importance and must be considered both when national and laboratory standards are calibrated in terms of the international standard - and also when, in due course, a replacement is prepared for the latter.

VI. ESTABLISHMENT OF THE INTERNATIONAL STANDARD AND DEFINITION OF THE INTERNATIONAL UNIT

Since this international standard is the first official standard for anti-D antibodies to be given a unitage, the figure selected for the value of the new International Unit could be regarded as essentially an arbitrary choice. For practical reasons it was decided to make it 32 I.U. per ampoule (of about 0.5 ml
serum, or 64 I.U. per ml) which is the same figure as the lowest commonly estimated titre (64 per ml) using the albumin assay methods (Table II, Figure I), although this is below the geometric mean for all titres estimated by these methods and which was nearer to 128 (Table III). This figure is the next lower dilution (on a doubling dilution scale) to the geometric mean titre of the albumin test results; the fact that it is substantially lower than the mean titre obtained with the other two methods should be borne in mind.

The figure of 32 I.U./ampoule was accepted by all laboratories participating in the collaborative assay, all of whom also agreed that the material was suitable to serve as the International Standard. In accordance with authorization given by the WHO Expert Committee on Biological Standardization at its eighteenth session (1965) the material was established as the International Standard. The mean dry weight of the contents of six ampoules was found to be 0.0304 g/ampoule. The International Unit of anti-D antibodies is thus defined as the activity contained in 0.95 mg of the First International Standard for incomplete anti-D serum, established in 1966.

VII. SUMMARY

The value of assaying incomplete anti-D (anti-Rho) antibodies in terms of units of a common standard serum was demonstrated in collaborative studies reported by Mourant and led to the decision to set up an international standard for antibodies specific for this blood group.

This paper describes the collection and testing of contributions of sera at the International Blood Group Reference Laboratory where unwanted antibodies in the sera were absorbed and tests carried out after the sera selected had been pooled. The pooled material was distributed in equal amounts among 3350 ampoules and freeze-dried. Ampoules were tested by 29 expert laboratories in 21 countries for qualitative specificity and the titre estimated by four widely-used methods involving suspension in albumin, replacement with albumin, papain-treated red cells and antiglobulin sera. The results of these and other tests for stability show that the material was suitable to serve as an international standard, and with the agreement of the laboratories in the collaborative study it was established and the International Unit defined.
ACKNOWLEDGEMENTS

All those whose names appear in Appendix A contributed sera for the standard. Those who check-tested the standard are listed in Appendix B. Dr E. W. Ikin performed all the serological investigations carried out at the International Blood Group Reference Laboratory.
### TABLE I. ESTIMATED TITRES OF AMPOULES OF THE INTERNATIONAL STANDARD WHICH HAD BEEN STORED AT ELEVATED TEMPERATURES

<table>
<thead>
<tr>
<th>Temperature Stored</th>
<th>Tested with ccDEe red cells</th>
<th>Tested with CcDee red cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Months stored at each temperature</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>-10°C</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>4°C</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>10°C</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>20°C</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>37°C</td>
<td>128</td>
<td>64</td>
</tr>
</tbody>
</table>
TABLE II. TITRES OF AMPOULES OF THE INTERNATIONAL STANDARD ESTIMATED BY FOUR TECHNIQUES USING RED CELLS OF THE GENOTYPE CoDee IN 29 LABORATORIES

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Technique</th>
<th>Albumin Suspension</th>
<th>Albumin Displacement</th>
<th>Papain</th>
<th>Anti-Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>64</td>
<td>2048</td>
<td>1024</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>256</td>
<td>128</td>
<td>NT</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>64</td>
<td>256</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>64</td>
<td>NT</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>256</td>
<td>128</td>
<td>512</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>256</td>
<td>256</td>
<td>512</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>128</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>256</td>
<td>256</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>64</td>
<td>256</td>
<td>256</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>512</td>
<td>256</td>
<td>512</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>64</td>
<td>NT</td>
<td>128</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>64</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>256</td>
<td>256</td>
<td>1000</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>16</td>
<td>1024</td>
<td>1024</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1024</td>
<td>64</td>
<td>1024</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>128</td>
<td>256</td>
<td>1024</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1000</td>
<td>500</td>
<td>1000</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>32</td>
<td>128</td>
<td>NT</td>
<td>1024</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>64</td>
<td>64</td>
<td>NT</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>64</td>
<td>64</td>
<td>NT</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>256</td>
<td>256</td>
<td>256</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>128</td>
<td>64</td>
<td>256</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>128</td>
<td>512</td>
<td>32</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>32</td>
<td>4</td>
<td>1000</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>32</td>
<td>4</td>
<td>128</td>
<td>1024</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>512</td>
<td>18</td>
<td>512</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>256</td>
<td>512</td>
<td>1024</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>32</td>
<td>128</td>
<td>128</td>
<td>256</td>
<td></td>
</tr>
</tbody>
</table>

GM = Geometric Mean;  NT = Not tested

GM = 107.6
GM = 115.9
GM = 382.1
GM = 269.0
**TABLE III. GEOMETRIC MEAN OF TITRES OF INTERNATIONAL STANDARD ESTIMATED BY 29 LABORATORIES USING FOUR TECHNIQUES**

<table>
<thead>
<tr>
<th>Assay Method</th>
<th>Geometric Mean Titre</th>
<th>Confidence Limits (p = 0.95)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>as titre</td>
</tr>
<tr>
<td>Albumin suspension</td>
<td>107.6</td>
<td>67.9-170.3</td>
</tr>
<tr>
<td>Albumin displacement</td>
<td>115.9</td>
<td>69.1-194.3</td>
</tr>
<tr>
<td>Papain</td>
<td>382.1</td>
<td>235.0-621.1</td>
</tr>
<tr>
<td>Antiglobulin</td>
<td>269.0</td>
<td>199.1-363.5</td>
</tr>
</tbody>
</table>
TABLE IV. RESULTS OF t TESTS BETWEEN MEAN LOG TITRES ESTIMATED BY THE 29 LABORATORIES USING THE FOUR TECHNIQUES

<table>
<thead>
<tr>
<th></th>
<th>Albumin Displacement</th>
<th>Papain</th>
<th>Anti-Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin Suspension</td>
<td>t = 0.22 NS</td>
<td>t = 3.9 ***</td>
<td>t = 3.4 ***</td>
</tr>
<tr>
<td>Albumin Displacement</td>
<td>t := 3.4 **</td>
<td></td>
<td>t = 2.92 **</td>
</tr>
<tr>
<td>Papain</td>
<td></td>
<td>t = 1.3 NS</td>
<td></td>
</tr>
</tbody>
</table>

NS = Not significant (p > .05)
** = .01 > p > .001
*** = p < .001
LIST OF THOSE WHO CONTRIBUTED ANTI-D SERA FOR THE PROPOSED INTERNATIONAL STANDARD.

Dr J. Andersen
Statens Serum Institut
Copenhagen, Denmark

Professor P. Cazal
Centre de Transfusion Sanguine
Montpellier, France

Dr R. J. Drummond
Regional Transfusion Centre
Rhyd Lafar, Near Cardiff, Wales

Dr M. C. Huth
Northern Ireland Blood Transfusion Service
Belfast, Northern Ireland

Dr M. Kout
Institute of Haematology
Prague, Czechoslovakia

Dr I. Liotta
Centro Nazionale Transfusione Sangue
Rome, Italy

Dr S. Murray
Regional Transfusion Centre
Newcastle-upon-Tyne, England

Dr J. P. O'Riordan
Blood Transfusion Service Board
Dublin, Eire

Dr S. Pawelski
Instytut Hematologii
Warsaw, Poland

Dr P. Speiser
Pathologish-Anatomisches Institut der
Universitat Wien
Vienna, Austria

Dr G. H. Tovey
Regional Transfusion Centre
Bristol, England

Dr R. A. Zeitlin
South London Transfusion Centre
Sutton, England

Dr A. Zoutendyk
The South African Institute for Medical Research
Johannesburg, South Africa
**LIST OF THOSE WHO TESTED THE PROPOSED STANDARD**

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. J. N. Ashworth</td>
<td>National Institutes of Health, Bethesda, USA</td>
</tr>
<tr>
<td>Dr. C. C. Bowley</td>
<td>Regional Transfusion Centre, Sheffield, England</td>
</tr>
<tr>
<td>Dr. E. Broman</td>
<td>Statens Rattskemiska Laboratorium, Stockholm, Sweden</td>
</tr>
<tr>
<td>Dr. F. Cazal</td>
<td>Centre de Transfusion Sanguine, Montpellier, France</td>
</tr>
<tr>
<td>Dr. M. Danielescu</td>
<td>Centrul de Hematologie, Bucharest, Romania</td>
</tr>
<tr>
<td>Dr. R. J. Drummond</td>
<td>Regional Transfusion Centre, Rhyl, Near Cardiff, Wales</td>
</tr>
<tr>
<td>Dr. C. Elosegui</td>
<td>The Instituto Espanol de Hematologia, Madrid, Spain</td>
</tr>
<tr>
<td>Dr. O. Hartmann</td>
<td>Statens Institut for Folkshelse, Oslo, Norway</td>
</tr>
<tr>
<td>Dr. S. R. Hollan</td>
<td>National Blood Center, Budapest, Hungary</td>
</tr>
<tr>
<td>Dr. M. C. Huth</td>
<td>Northern Ireland Blood Transfusion Service, Belfast, Northern Ireland</td>
</tr>
<tr>
<td>Dr. E. W. Ikin</td>
<td>International Blood Group Reference Laboratory, London, England</td>
</tr>
<tr>
<td>Dr. M. Kout</td>
<td>Institute of Haematology, Prague, Czechoslovakia</td>
</tr>
<tr>
<td>Dr. I. Liotta</td>
<td>Centro Nazionale Transfusione Sanguine, Rome, Italy</td>
</tr>
<tr>
<td>Professor J. J. Van Loghem</td>
<td>Central Laboratory for Blood Transfusion of the Netherlands Red Cross, Amsterdam, Holland</td>
</tr>
<tr>
<td>Dr. A. Lundsgaard</td>
<td>Statens Seruminstitut, Copenhagen, Denmark</td>
</tr>
<tr>
<td>Dr. B. P. L. Moore</td>
<td>Canadian Blood Transfusion Service, Toronto, Canada</td>
</tr>
</tbody>
</table>
Professor P. Moureau
Universite de Liege
Liege, Belgium

Dr S. Murray
Regional Transfusion Centre
Newcastle-upon-Tyne, England

Dr J. P. O'Riordan
Blood Transfusion Service Board
Dublin, Eire

Dr H. Pettenkofer
Robert Koch Institute
Berlin, Germany

Professor W. Rudowski
Instytut Hematologii
Warsaw, Poland

Dr R. T. Simmons
Commonwealth Serum Laboratories
Parkville, Victoria, Australia

Professor P. Speiser
Pathologisch-Anatomisches Institut der
Universitat Wien
Vienna, Austria

Dr J. M. Staveley
Auckland Blood Transfusion Service
Auckland, New Zealand

Dr F. Stratton
Regional Transfusion Centre
Manchester, England

Dr G. H. Tovey
Regional Transfusion Centre
Bristol, England

Dr J. Wallace
Blood Transfusion Service
Law Hospital, Carluke, Scotland

Dr R. A. Zeitlin
South London Transfusion Centre
Sutton, England

Dr A. Zoutendyk
South African Institute for Medical Research
Johannesburg, South Africa
METHODS USED IN TITRATING THE INCOMPLETE ANTI-D WITH TYPE CcDee (R_1^r) RED CELLS

1. **Albumin suspension method**

   5% cell suspension in 20% bovine albumin.
   Diluting fluid: inert human AB serum.

2. **Albumin displacement method**

   5% cell suspension in physiological saline.
   Diluting fluid: physiological saline. The mixture is incubated for 1-1/2 hours at 37°C. One standard titration volume of 20% albumin is then added without disturbing the contents of the tube, which is then incubated at 37°C for a further 1/2 hour.

   Read microscopically.

3. **Enzyme treatment method**

   2% suspension of papainised cells in physiological saline.
   Diluting fluid: physiological saline.
   The papainised cells are prepared as follows:

   (a) Papain solution prepared by suspending 1 g of papain powder in 100 ml of isotonic saline. This solution may be stored for one month or longer at 4°C.

   (b) Buffered saline solution prepared by adding one volume of 1/15 M Sorensen phosphate buffer pH 7.3 to nine volumes of isotonic saline.

   Sorensen buffer solution is prepared by adding 8.0 ml of disodium hydrogen phosphate solution (11.876 g Na_2HPO_4·2H_2O per litre) to 2.0 ml of potassium dihydrogen phosphate solution (9.078 g KH_2PO_4 per litre).
Preparation of papainised cells

(a) Add nine volumes of buffered saline to one volume of the papain solution.
(b) Add one volume of packed red cells to two volumes of this mixture. Mix well.
(c) Incubate the mixture in a 37°C water bath for 30 minutes.
(d) Centrifuge. Remove the supernatant and wash the cells three times with isotonic saline.

4. Anti-human-globulin test

Cell suspension in physiological saline, of usual strength used in the laboratory for this purpose.

Diluting fluid for serum: physiological saline. The mixture is incubated at 37°C for two hours. The cells are then thoroughly washed four times in physiological saline and mixed with anti-human-globulin serum at its optimal dilution for the detection of γ(7S) antibodies.

5. Other methods in regular use in each laboratory.
REFERENCES

1. Mourant, A.E. "Anti-Rhesus Serum Standards"
   WHO/BS/366, 25 October 1956

2. Tenth Report of the Expert Committee on Biological Standardization
   WHO/BS/374, 21 November 1956

3. "The Anti-Rhesus Serum Standards" from the Blood Group Reference
   Laboratory, Lister Institute, London, UK
   WHO/ES/448, 18 September 1958

4. Mourant, A. E. "International Comparison of six 'incomplete'
   or albumin-potentiated anti-D sera, titrated by several
   methods in ten laboratories"
   WHO/BS/453, 21 July 1959

5. Uemura, K. "Statistical analysis of the data presented in
   WHO/BS/453 Rev. 1 on six 'incomplete' anti-D sera titrated
   in ten laboratories"
   WHO/BS/453 Rev. 1, Add. 2, 11 July 1960

6. Unger, L. J. and Wiener, A. S. "Some observations on the blood
   factor RhA of the Rh-Hr blood group system" (1959)

7. Stratton, F. and Renton, P. H. "Practical Blood Grouping"
Fig. 1. Frequency distribution of the International Standard estimated by 29 laboratories by the 4 methods.

- **Albumin Suspension**
- **Albumin Displacement**
  - Mean titre
  - Confidence limits (p = .95)

- **Papain**
- **Antiglobulin**
Fig. 2. Frequency distribution of the International Standard estimated by all methods by 29 laboratories