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Report

Review and Update of the WHO International Standards for Blood Grouping Reagents

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REVIEW AND UPDATE OF THE WHO INTERNATIONAL STANDARDS FOR BLOOD GROUPING REAGENTS

**Central Laboratory of the Netherlands Blood Transfusion Service,
Amsterdam October 18, 1999**

Report

Introduction

Professor W.G. Van Aken, Director of the WHO Collaborating Centre for Biological Standards at the Central Laboratory of the Netherlands Transfusion Service (CLB), welcomed the participants to the CLB facilities.

This meeting of representatives of the WHO Collaborating Centres and other interested parties was held as a follow-up to the September 9, 1998 consultation held in Washington, D.C. The primary aims of the meeting were to review the testing already performed on the Anti-D (RUM-1) (IgM Monoclonal) material donated by Serologicals, Inc. for use in the preparation of a new Anti-D Blood Grouping Reagent (Monoclonal) International Standard, to determine the schedule and protocol for a WHO Collaborative study to calibrate the candidate, and to discuss other pertinent issues. These minutes will be presented to the 50th meeting of the Expert Committee on Biological Standardization (ECBS), on 25-29 October, 1999. The proposed agenda, distributed on September 30, 1999, served as the meeting format with Dr. Phillips as the facilitator.

Proposals for discontinuation of Reference Materials (to be submitted at the ECBS Meeting on 25-29 October, 1999)

At its previous meeting in 1998, the ECBS deferred the decision regarding discontinuation of the Anti-A,B, Anti-C (complete), Anti-E (complete) and Anti-c (incomplete) reference preparations to the 1999 meeting. The consensus of the group was that the Anti-A,B reference preparation is clearly obsolete and can be discontinued. The utility of the three Rh-system reference preparations was discussed at length. This discussion included an evaluation of the effect discontinuation would have on developing countries; none was noted. It was also noted that the distribution of these reference preparations had been at very low levels and it was suggested that this was an indication of the lack of need of the reference preparations. It was also noted that these reference preparations are really no longer relevant in today's immunohematology community. On the other hand, it was pointed out that these specificities are on List A of the new European Medical Device Directive and that it might be premature to discontinue them at this time. It was proposed that a decision on these three reference preparations be deferred for an additional year.

Handling of remaining inventory of discontinued reference preparations was also discussed. It was pointed out that there should be WHO notification of the decision to discontinue any reference preparation so that those centers holding the reference preparations are not responsible for disseminating that information. It was agreed that the International Society for Blood Transfusion (ISBT) newsletter would be an appropriate place for such an announcement to be made. It was also agreed that at least 30 ampoules of any reference

preparations be held in retention for historical purposes and for future studies. In the case of a discontinued reference preparation, the remaining inventory in excess of the 30 retention ampoules can be destroyed or disposed of as the holder sees fit.

NIBSC, CBER, CLB, and IBGRL preliminary testing of candidate material for Anti-D Blood Typing Serum (Monoclonal) International Standard

This discussion began with a review of the intended use of the new Anti-D Blood Grouping Reagent (Monoclonal) International Standard. It was agreed that while it is important to ensure that all anti-D reagents appropriately detect weak D cells, the function of this reference preparation will not be for determining specificity but for determining the minimum potency necessary for any and all anti-D reagents. It was agreed that specificity is best addressed by a panel of weak D cells with cells of various D-site densities representing the spectrum of weak D cells.

Based on the testing performed at the three test sites (CBER, CLB, and IBGRL), it was also agreed up front that the reference preparation will be used at a dilution. Further discussion of the differences between reagents of monoclonal origin and polyclonal origin led to the conclusion that two different dilutions may be necessary. This will need to be decided as part of the evaluation protocol. It was also pointed out that WHO reference preparations are customarily labeled in International Units (I.U.) and attempts should be made to determine the concentration of anti-D in I.U./vial.

Proposed specifications and protocol for a WHO Collaborative Study to develop an Anti-D Blood Grouping Reagent (Monoclonal) International Standard

The scope of the collaborative study will be to include as many different kinds of currently available anti-D reagents as possible. This can be achieved by including reagent manufacturers, national control authorities, and selected users in blood transfusion laboratories.

With these details in mind, the specifics regarding continuation of processing of the candidate material were discussed. Ten liters of material were donated by Serologicals; approximately 10,000 ampoules can be prepared from this amount. It was initially suggested that the volume to be filled and freeze-dried should be based on the distribution records for the WHO and FDA reference preparations. However, it was pointed out that the distribution records do not really reflect the actual need for the reference preparation. Historically, availability has been limited because of the difficulties associated with procuring and processing large volumes of material, which were previously polyclonal. Because of this limited availability, it has always been expected that manufacturers maintain a secondary standard to be used during in-process testing thus reserving the primary reference preparation for lot-release purposes. It was agreed that reagent manufacturing could be further standardized if manufacturers, regulatory authorities and reference laboratories were able to use the primary reference preparation for more of their testing needs. Therefore, it is proposed that the entire batch of 10 liters be dispensed in 1.0 ml volumes in European standard glass ampoules with a ceramic ring and associated safety devices.

Next, the design and coordination of the study was discussed. It was agreed that the functions of the study coordinator(s) would be design, distribution, receipt of results, collation and statistical analysis of results, preparation of the report for the ECBS, and ensuring adherence to timelines. Because of the specific expertise in some centres and specific limitations in other centres, identifying one coordinator proved to be difficult. For example, it was agreed that success of the project relies heavily on the involvement of statisticians in the analysis of the

study design and the data collected. It was noted that NIBSC probably has statisticians with the most experience in reviewing and analyzing this kind of study and data. Dr. Phillips agreed to pursue approval for the use of NIBSC statisticians in this project. Therefore, it was proposed that there be three coordinators; Peter Phillips will identify a coordinator from NIBSC, Masja de Haas will serve as the coordinator for CLB, and Sheryl Kochman will serve as the coordinator for CBER. CBER will distribute the samples for the study within the USA for the purpose of concurrently establishing an FDA working reference preparation.

Questions regarding funding were brought up but could not really be addressed except to say that it has historically been accepted that WHO Collaborating Centres are expected to fund their own contribution to the effort. In this particular case, the candidate material for the reference preparation has been obtained via donation and NIBSC has offered to handle the filling and freeze-drying. As usual for other reference preparations, the individual laboratories will cover the costs for the evaluation studies.

A preliminary protocol was discussed. Based on a review of data generated by the three test sites, it was agreed that the candidate material and other monoclonal anti-D reagents will be tested by a specified low-protein method in which the titration diluent will be 2% BSA. This will be considered the reference method for the reference preparation and all low-protein Anti-D reagents. Because of concerns that potency of high-protein polyclonal Anti-D reagents may not be accurately represented if cells in a low-protein diluent are introduced into the test system, it was agreed that the titration diluent for high-protein reagents should be the manufacturer's corresponding Rh control reagent. This will be considered the reference method for all high-protein Anti-D reagents. It was recommended that the red blood cell diluent also be 2% BSA. Incubation time and temperature will be 5 minutes at room temperature for low-protein anti-D and 15 minutes at 37 °C for high-protein anti-D. "Room temperature" will be defined in the protocol as 19 °C to 25 °C. It was agreed that the protocol would specify that equal volumes of reagent/dilution and red blood cell dilution must be used, that glass test tubes must be used, and that the titration endpoint must be described as the last tube in which a macroscopic 1+ reaction is seen. It was also agreed that the standard 12-point scoring method be included in the protocol. Equal volumes of reagent and red blood cells will be stipulated instead of specified volumes to allow use of dropper pipettes in laboratories that may not have automated pipettes. The use of alternative methods, such as gel and microplate, were considered but were ruled out due to their complex nature. The use of the slide test was ruled out because of the inherent difficulties involved in performing titrations on a slide. It was also agreed that a standardized questionnaire for use in determining the specific directions for use of the reagents under test by study participants would be mailed along with the study protocol. Worksheets on which study participants can record their test results will be prepared and mailed along with the study protocol. It was pointed out that inclusion of a closing date for reporting of study results would be crucial in meeting our tight timelines. A draft study protocol, questionnaire, and worksheets will be developed by CBER and will be circulated to the Members in the Working Group for approval.

It was also proposed that the WHO Anti-D (complete) reference preparation also be included in the study so that international units could be calculated, if necessary; however, this will require that the three test sites perform preliminary testing of that reference preparation by the same methods already used in the preliminary testing of the candidate material. As such, CLB will need to ship samples to each of the three testing sites for this testing.

A preliminary participant list was also discussed. As an international reference preparation, we must be certain to include an adequate geographical representation of

manufacturers, reference laboratories and national control authorities. WHO and ISBT will be the conduit to ensuring appropriate inclusion of laboratories from all the WHO Regions while CBER will request participation of all U.S.-licensed manufacturers.

Stability studies were also covered. It was agreed that some accelerated stability testing would have to be performed so that we need not wait for extended real-time testing to be completed. It was noted that the NIBSC standard protocol for accelerated stability testing includes incubation of samples at 56 °C, 45 °C, 37 °C, 20 °C, 4 °C, -20 °C, and -70 °C with testing being performed at three-month intervals. It was agreed that this is a standard, and therefore acceptable, protocol. Based on limitations of various centres, NIBSC will hold the samples at the various temperatures and then ship them for testing at the three test sites.

It was also agreed that a meeting to review the results of the collaborative study prior to preparation of the report for the WHO Collaborative study for submission to the WHO Expert Committee on Biological Standardization would be required. It was recommended that a follow-up meeting be scheduled to coincide with the ISBT meeting in Vienna in July 2000. There is a possibility that WHO could provide some funding for such a meeting. Since Dr. Scott will be making arrangements for meetings of the ISBT Expert Working Panel, she indicated that she could also make arrangements for a meeting of this committee.

Future projects

It was generally agreed that performance and completion of the work associated with the Anti-D Blood Grouping Reagent (Monoclonal) International Standard would be very time consuming and that we should not compromise that work by taking on too many projects at once. It was also agreed that the next most important work to be done would be replacement of the Anti-A and the Anti-B reference preparations and that these can and should be handled together. It was proposed that we consider beginning that project at this same time next year. It would be appropriate to pursue donations of source material at this time. The committee reiterated the policy that any manufacturer making a donation of material for use as a WHO reference preparation is not to make claims relating to the fact that their product is a WHO reference preparation. Therefore, Dr. Scott will contact Serologicals and ask them to submit a letter acknowledging these terms.

Timetable and Commitments

Task	Deadline	Responsibility
Obtain letter from Serologicals	ASAP	Dr Scott
Assign NIBSC coordinator	ASAP	Dr Phillips
Distribute Anti-D (chemically modified)	ASAP	CLB
Test Anti-D (chemically modified)	ASAP	CBER, CLB, IBGRL
Fill and freeze-dry Anti-D	Dec. 1999	NIBSC
Draft protocol, questionnaire, & worksheets	Nov. 19, 1999	CBER
Draft participant list	Nov. 19, 1999	CBER, IBGRL, WHO
Final protocol ready	Dec. 24, 1999	CBER with input from all
Ampoules ready to ship	Jan. 31, 2000	NIBSC
Testing	March/April 2000	Individual study participants
Results collation & review	May/June 2000	NIBSC, CLB
Meeting to review results	July 2000 @ ISBT	WHO with input from all
Prepare report of WHO Collaborative Study for ECBS	August 2000	CBER, CLB, NIBSC

**Review and Update of the WHO International Standards for
Blood Grouping Reagents: Proposed joint venture between the
WHO Collaborating Centres for Biological Standards**

**Venue: Central Laboratory of the Netherlands Red Cross (CLB),
125, Plesmanlaan. 1006 AD Amsterdam, the Netherlands
18 October 1999, 8:00 a.m.**

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