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**WHO Working Group on
Reference Preparations for testing
Diagnostic Kits used for detection
of HBsAg, Anti-HCV and Anti-HIV
antibodies in blood screening**

**Report of the third meeting:
Geneva, Switzerland
17-19 January 2000**



WORLD HEALTH ORGANIZATION
Blood Safety and Clinical Technology

**WHO WORKING GROUP ON REFERENCE PREPARATIONS FOR
TESTING DIAGNOSTIC KITS USED FOR DETECTION OF
HBsAg, Anti-HCV and Anti-HIV ANTIBODIES IN BLOOD SCREENING
Third Meeting
WHO Headquarters, Geneva
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OPENING REMARKS

The meeting was opened by Dr Jean Emmanuel, Director of Blood Safety and Clinical Technology Department and Dr Elwyn Griffiths, Coordinator of Quality Assurance and Safety: Biologicals. Dr Emmanuel reminded participants of the mandate of the Group which included setting specifications for appropriate candidate materials and manufacturing characteristics for the development of WHO Reference Preparations relevant in safety testing of blood-borne viruses. This meeting was called to review progress in developing reference panels for viral serology tests as well as to review the WHO studies done to support the establishment of the International Standards applied to the standardization of Nucleic Acid Amplification Tests (NAT). He stated that such reference materials are important for global public health and need to be made available to kit manufacturers and National Control Laboratories.

Dr Griffiths emphasized that the development of the reference preparations to be discussed by the Working Group is not easy but is of great importance and is an essential part of the biological standardization programme in WHO. He also reminded the Group that the final oversight on the establishment of all the International Biological Reference Preparations lies with the WHO Expert Committee on Biological Standardization (ECBS).

Dr Decker was invited to chair the Working Group and Drs Ferguson and Padilla agreed to serve as joint rapporteurs.

BACKGROUND

Dr Decker commented that the success of the meeting relied on the contributions of members of the Group. He noted that the specifications for the WHO Reference Preparations and Panels had been discussed in December 1996 and March 1998 but that the timetable proposed at the latter meeting had slipped. He therefore proposed that the Group should review whether the preparations considered previously are still required and then refine the goals and objectives.

Dr Padilla reviewed the discussions of the previous meetings and reminded members that they were charged with identifying the kind of preparations which would best assist Member States in identifying suitable and unsuitable diagnostic kits and how these preparations would be used. They should also define the criteria to be considered in the selection of candidate materials for its development.

Dr Padilla also informed the Group of the establishment by the ECBS of the International Standard for HIV-1 RNA and HBV DNA in 1999 which supplemented the International Standard for HCV RNA, genotype 1, established in 1997. She informed the Group that the

Catalogue of International Standards for Biologicals was now published on the WHO Website at the following address: <http://www.who.int/technology/biological.html>

A. REFERENCE PREPARATIONS/PANELS FOR VIRAL SEROLOGY TESTS

A.1 Reference Panel for HBsAg

Dr Ferguson reminded the Group of the identification, at the previous meeting, of a suitable donation for the preparation of the HBsAg reference panel and also of the production of a batch of re-calcified plasma which was anti-HBs negative. Members of this panel would not be inactivated. The panel had not been produced.

The Group considered the increasing problems associated with shipment and distribution of infectious reference materials and reviewed the proposed characteristics of the dilution panel. The Group discussed the offer of semi-purified inactivated antigen by Dr Lelie, from the Central Laboratory of the Netherlands Red Cross (CLB). This antigen was prepared by a method that had been used to produce a plasma-derived hepatitis B vaccine at the CLB in the early 1980s. The method involved PEG precipitation and heating at 101-103 degrees for 90 seconds followed by pasteurization at 65 degrees for 10 hours. Dr Lelie had indicated that in his experience there was little loss of reactivity on freeze-drying. Data on the reactivity of this antigen, subtype adw, with a number of commercial kits were reviewed, and the Group agreed that this material should be used for the production of the panel. As discussed previously, the Group agreed that anti-HBs negative re-calcified plasma should be used as diluent, with merthiolate added as a preservative.

Recommendations agreed by the Group

Hepatitis B virus would be inactivated by a validated heat inactivation method. A five-member HBsAg panel would consist of a series of four-fold dilutions approximately equivalent to 16, 4, 1, 0.25 and 0.0625 IU/ml. These materials will be provided to the National Institute for Biological Standards and Control (NIBSC) by Dr Lelie during the first term of the year 2000. They will be diluted in re-calcified plasma that is negative for HBsAg, anti-HCV, anti-HIV 1+2 and anti-HBs, and is non-reactive for HCV RNA, HIV RNA and HBV DNA by NAT methods. Filling and freeze-drying will be performed at NIBSC. 3000 1ml vials of each member of the dilution series, freeze-dried, was proposed. The Group agreed that a sample of the plasma diluent (non-reactive for all markers including anti-HBs) be made available for distribution with each reference panel. The unitage of HBsAg in each panel member would be assigned in a WHO International Collaborative study.

A.2 Anti-HCV Reference Panel

Dr Ferguson reminded the Group of agreement at the previous meeting to develop a reference preparation comprised of characterized genotype 1 donations. The preparation would not be inactivated. It was considered unlikely to be able to source an adequate supply of plasma units having antibodies to individual HCV proteins, e.g., core, NS3 and NS4 antigens, as originally proposed by the Group.

The Group reviewed data of the preliminary report of the WHO collaborative study to assess the suitability of the candidate anti-HCV genotype 1 reference material. The candidate material consists of 2 batches of 2000 vials produced from the same bulk. The vials contain the freeze-dried residue of re-calcified plasma having antibodies to hepatitis C virus, genotype 1 with 0.05% Bronidox as preservative. Each vial is reconstituted in 1ml distilled water.

13 laboratories in 12 countries participated in the study and submitted data from the use of 9 screening assays and 5 immunoblot assays. The approximate dilution equivalent to the cut-off value in enzyme immunoassays (EIA) was calculated from dose response curves obtained from the assay of a series of dilutions. Dr Ferguson reported that additional data had been received from 3 participants and would be incorporated into the final report, which will be circulated to all participants prior to its submission for consideration by the WHO ECBS in October 2000.

Although published data indicate that there is significant serological cross-reactivity with antisera from different HCV genotypes when tested with assay systems containing only genotype 1 antigens, the Group discussed further whether a panel containing members of other genotypes should be developed. Dr Ferguson indicated that three donations which contained antibodies to HCV genotype 2 and four that contained antibodies to HCV genotype 3 were already characterized and available for use. In addition, the Group agreed to source additional genotype 4, 5 and 6 donations. Dr Komuro and Dr Fields agreed to investigate the possibility of obtaining a genotype 6 donation through contacts in Vietnam. Dr York agreed to supply some candidate type 4 and type 5 donations, and Dr Fields agreed to genotype them. The Group also agreed to investigate the availability of additional genotype 1, 2 and 3 donations in case the number available could not be diluted to the required volume.

The Group proposed that if additional genotype panel members were to be produced, these panel members should be inactivated. Although Dr Fields expressed concern that disruption of virus might result in complexing of core antigen with antibodies and thus reduce the level of anti-core antibodies in the preparation, other members of the Group did not perceive this as a practical problem. The Group agreed on the use of a validated solvent-detergent method already applied in the production of reference panels by Dr Lelie in the Netherlands. Dr Lelie will provide the protocol used in the preparation of CLB reference materials.

Members of the Group who represent the different WHO Regions welcomed and encouraged the development of a WHO genotype panel that would include the relevant HCV genotypes circulating in the Regions. Dr Lavanchy indicated that the panel as proposed would be of great help to the countries, and every effort should be made to produce it. It was agreed that the characterization of genotype reference panels in established kits would enable control authorities to know what kind of responses to expect with locally produced kits and imported kits with which they had no experience. Similar to the proposal for the HBsAg reference panel, the Group recommended that a vial of non-reactive plasma diluent be made available for distribution with the HCV reference panel.

Although the genotype 1 panel member which has already been produced and characterized is infectious, the Group agreed that the collaborative study report should be submitted to the ECBS with a view to establishing this material as a WHO reference reagent, but that this material should not have an assigned unitage. However, the Group proposed that this panel member should be replaced with an inactivated preparation in due course.

Recommendations agreed by the Group

Prepare an anti-HCV genotype reference panel. All components of this panel will be inactivated and will be composed of sera of genotypes 2 and 3, followed in order of priority by sera of genotypes 4, 5 and 6. An additional panel member of genotype 1 that has been inactivated will be prepared. Inactivated by a validated solvent detergent method, diluted as little as possible in re-calcified plasma negative for HBsAg, anti-HCV and anti-HIV 1+2 and

tested by NAT and found non-reactive for HCV RNA, HIV RNA and HBV DNA. Dr Lelie, from CLB will provide the solvent-detergent inactivation protocol that will be applied. 3000 0.5ml vials, freeze-dried, per panel member will be prepared at NIBSC.

A.3 Anti-HIV Reference Panel

Dr Holmes reminded the Group of the diverse nature of HIV and of the large number of genetic subtypes forming HIV-1 group M, with a smaller number of even more diverse genotypes falling into the outlying N + O genotypes. Most diagnostic kits in Europe and the US are based on subtype B strains and detect antibodies to a wide range of subtypes. Many of these kits have been modified to detect antibodies to type O more effectively. However, there have been few studies which evaluated the sensitivity of screening assays to different HIV subtypes. Dr Osmanov updated the Group on the distribution of the various HIV-1 subtypes around the world and highlighted the need for a broad panel to meet the needs of Member States from all regions.

At the meeting held in 1998 the Working Group had endorsed a proposal for the preparation of an anti-HIV-1 reference panel containing antibodies to a range of subtypes and that these reference materials should be inactivated and contain preservative. Several members had since contributed candidate materials to the coordinator of the study, Dr Holmes, NIBSC. A number of candidate materials from the UK Public Health Laboratory Service which had not been genotyped were to be sent to CDC, Atlanta where typing was performed. A pilot panel consisting of 13 candidate materials pre-diluted in serum negative for anti-HIV 1+2, anti-HCV and HBsAg was distributed to 5 members of the Group who prepared dilutions of each material in the proposed production diluent and assayed them in a range of EIA, passive particle agglutination and Western blot assays. Seventeen different kits were used in these studies, and the results indicated that the analytical activity of anti-HIV kits used in the laboratories varied widely. The two HIV-1 subtype O samples were detected quite poorly by many kits, and the reactivity of the two HIV-2 samples varied considerably.

Recommendations agreed by the Group

The Group proposed that a panel of anti-HIV-1 subtypes A, B, C, D, E, F and O should be produced along with an anti-HIV-2 reference preparation in the following order of priority: anti-HIV-1 subtypes C, B, O and A followed by HIV 2; then subtypes E, D and F. Each material would contain a 1 in 40 dilution of a single donation, except for the subtype O. Subtype O will be a pool of 2 donations. The diluent for all panel members will be serum negative for HBsAg, anti-HCV and anti-HIV 1+2 and found non-reactive for HCV RNA, HIV RNA and HBV DNA by NAT. All the panel members will be inactivated by a validated heat inactivation method. It was proposed that 3000 vials per panel member be filled with 0.5ml and freeze-dried. A similar number of vials will be prepared for the non-reactive diluent to be included with the panel. Dr Hewlett agreed to supply a validated procedure for heat-inactivation of anti-HIV infected serum.

A.4 Diluent

The Working Group endorsed the previous decision to use serum or re-calcified plasma as diluent and that freeze-dried fills of these materials should be prepared for issue with each panel.

B. REFERENCE PREPARATIONS/PANELS FOR STANDARDIZATION OF NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT)

B.1 HIV-1 RNA

Dr Holmes described the development of candidate reference materials for HIV-1 RNA for use in NAT assays. The suitability of 3 candidate materials, two freeze-dried and one liquid preparation (stored at -70 degrees), was assessed in an international collaborative study in which 25 laboratories participated. Copy number was determined using 4 commercial assays and two in-house quantitative assays, and consistent results were obtained in all assays. The stability of the freeze-dried preparations was assessed in accelerated degradation tests and was predicted to be approximately <0.2% per year when stored at -20 degrees, at 0.4% per month when stored at 2-8 degrees and 4% when stored at +20 degrees. Further real time stability studies were planned using the same kits as initially used in the quantitation of the copy number. This stability was considered acceptable for shipping materials at ambient temperatures. This material was established as the First International Standard for HIV RNA with an assigned unitage of 100,000 IU per vial.

Dr Holmes informed the Group that a subtype panel including subtypes A-H + N and O had been sourced and that a collaborative study was being planned under the auspices of the WHO International Working Group on the Standardization of Gene Amplification Tests (SOGAT). Dr Holmes indicated that in order to reduce the chances of selecting a recombinant virus, where possible, viruses of known sequence had been selected as candidate panel members and that most of the viruses were field isolates from the UNAIDS collection which had been grown on PBMCs. The Group indicated that such a panel was likely to be of use to kit manufacturers and National Control Laboratories. Dr Hewlett also informed the Group that similar panels were under development at CBER and would be used in the batch release of licensed assay kits for the quantitation of HIV RNA. These panels would also be available for use in investigation of HIV vaccines under development, many of which are in trials in areas of the world where different subtypes are prevalent. Dr Osmanov suggested that such reference panels would be useful to evaluate samples from patients undergoing therapy. Although the Group was unclear as to what the likely demand would be for a WHO panel of such materials, they endorsed the production of a small-scale panel and its evaluation in a collaborative study to see whether detection of different genotypes justifies the development of further freeze-dried reference panels.

B.2 HBV DNA and HCV RNA

Dr Ferguson updated the Group with information on the International Standards for HCV RNA (genotype 1) and HBV DNA (subtype adw) that had been developed and characterized in WHO international collaborative studies organized by Dr John Saldanha, NIBSC, under the auspices of SOGAT. At present no further reference materials for HBV are being planned, and the Group agreed that this was reasonable due to the availability of universal primers suitable for use with all subtypes and genotypes.

Dr Ferguson also informed the Group that a WHO collaborative study was planned by the SOGAT group to investigate the detection of HCV RNA genotypes in NAT assays. The Group supported this initiative and indicated that it was likely that such panels would be required by National Control Laboratories for the assessment of new or improved assays. The

Group agreed that the need for a subtype panel should be evaluated when data from the collaborative study were available. The Group agreed to investigate the possibility of including serological assay studies in a proposed study on the sensitivity of NAT assays for detection of genotypes 1 to 6.

C. OVERALL PRIORITIES FOR PRODUCTION OF REFERENCE PANELS

Because approximately 20 preparations have been identified which are to be processed and freeze-dried, the Group considered priorities for their production. The order of production should be based on availability of materials as well as the order of priorities agreed upon. The Group considered that anti-HCV materials should be first priority, as many countries were rapidly moving towards implementation of screening programs. Anti-HIV serology panels have second priority with HBsAg last. However, consideration should be given to material availability. The Group considered that additional NAT reference materials are a lower priority than reference materials for serological testing.

D. USE OF REFERENCE PANELS

The Group reviewed a Draft of the Memorandum that will be issued with the anti-HCV genotype 1 material that has recently been produced and characterised. The Group agreed that in addition to indicating how the material could be used and the characteristics of the material, the limitations of the use of the material should also be highlighted. All the WHO International Reference Preparations will be distributed with Memoranda incorporating these discussions.

The Group asked Dr Padilla to promote appropriate dissemination of the available information to the WHO Regional Offices in order to ensure that relevant laboratories in the Regions are involved in the studies and to facilitate the distribution of the materials to the selected laboratories.

DATE OF NEXT MEETING: May/June 2001

This date was proposed in order to make possible the evaluation of the WHO Collaborative studies which would be submitted to the WHO Expert Committee on Biological Standardization by the end of the year 2001. It was agreed that efforts should be made to make available as many panel members as possible by the end of the year 2001.

**WORKING GROUP ON REFERENCE PREPARATIONS FOR TESTING
HEPATITIS B, HEPATITIS C AND HIV DIAGNOSTIC KITS
WHO Headquarters, Geneva: Room B
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