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# **Development of WHO Biological Reference Preparations for Blood Safety- related *in vitro* Diagnostic Tests**

*Report of a meeting with the WHO Collaborating Centres for Biological  
Standards and Standardization, 29-30 January 2007*



**World Health  
Organization**



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## ABBREVIATIONS

<b>Anti-HBc</b>	antibodies to hepatitis B core antigen
<b>Anti-HBs</b>	antibodies to hepatitis B surface antigen
<b>B19V</b>	human parvovirus B19
<b>BRP</b>	biological reference preparation
<b>BSE</b>	bovine spongiform encephalopathy
<b>CBER</b>	Center for Biologics Evaluation and Research (USA)
<b>CC</b>	Collaborating Centre (WHO)
<b>CDC</b>	Centers for Disease Control (USA)
<b>CJD</b>	Creutzfeldt-Jakob disease
<b>CMV</b>	cytomegalovirus
<b>CRF</b>	circulating recombinant form
<b>DNA</b>	deoxyribonucleic acid
<b>ECBS</b>	Expert Committee on Biological Standardization
<b>ELISA</b>	enzyme-linked immunosorbent assay
<b>FDA</b>	Food and Drug Administration (USA)
<b>HA</b>	haemagglutination assay
<b>HBsAg</b>	hepatitis B surface antigen
<b>HAV</b>	hepatitis A virus
<b>HBV</b>	hepatitis B virus
<b>HCV</b>	hepatitis C virus
<b>HHV-8</b>	human herpes virus type 8
<b>HIV</b>	human immunodeficiency virus
<b>HTLV-1/2</b>	human T-cell lymphotropic virus types 1 and 2
<b>IFA</b>	immunofluorescence assay
<b>IgG</b>	immunoglobulin G
<b>IgM</b>	immunoglobulin M
<b>IVD</b>	<i>in vitro</i> diagnostic
<b>NAT</b>	nucleic acid amplification technique
<b>NIBSC</b>	National Institute for Biological Standards and Control (UK)
<b>NS</b>	non-structural
<b>p24</b>	nucleocapsid protein of HIV
<b>PEI</b>	Paul-Ehrlich-Institut (Germany)
<b>RNA</b>	ribonucleic acid
<b><i>T. cruzi</i></b>	<i>Trypanosoma cruzi</i>
<b>TPPA</b>	<i>Treponema pallidum</i> particle agglutination
<b>TSE</b>	transmissible spongiform encephalopathies
<b>WHO</b>	World Health Organization
<b>WNV</b>	West Nile virus

## 1. OPENING REMARKS

The Quality Assurance and Safety: Blood Products and Related Biologicals (QSD) team in the Department of Medicines Policy and Standards (PSM), World Health Organization (WHO) convened a two days meeting with the WHO Collaborating Centres for Biological Standards and Standardization (WHO CCs), namely the National Institute of Biological Standards and Control (NIBSC), the Paul-Ehrlich-Institut (PEI), and the Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA). The meeting was kindly hosted, from 29 to 30 January 2007, by the FDA/CBER in Rockville, Maryland, USA.

Dr Epstein, Director, Office of Blood Research and Review (CBER) opened the meeting and welcomed the participants. Dr Padilla (PSM/QSD) highlighted the objectives of the meeting which were to foster cooperation among the WHO Collaborating Centres for Biological Standards and Standardization, as well as to strengthen the development of WHO International Biological Reference Preparations (BRPs) for the control of *in vitro* diagnostic (IVD) tests related to blood safety. A discussion with experts of the WHO CCs with regard to the scientific issues involved in this work and on the major priorities and prospects of interest for each of the Centres was considered essential to support the establishment of WHO reference preparations in the *in vitro* biological diagnostic field.

Dr Epstein was elected Chairman of the meeting, and Drs Chudy and Mied were nominated as Rapporteurs. The list of participants is attached to this Report.

## 2. WHO IVD STRATEGIC PLAN

Dr Chudy informed the WHO CCs about WHO activities to establish a five-year strategic plan to prioritize development of WHO BRPs for the control of blood safety-related IVD tests. The development and appropriate use of BRPs relevant to safety testing of blood-borne agents are important for the regulation and control of IVD tests and contribute to a harmonized regulation of blood and blood products. Such global measurement standards are a useful tool for the validation, quality control and comparability of diagnostic tests at global level. The intended use of the BRPs (International Standards, Reference Reagents and International Reference Panels) is to provide an indication of the analytical sensitivity of IVD test kits. The WHO CCs represent the greatest know-how and experience in the preparation and calibration of WHO biological reference standards.

The agenda of the meeting covered the following high-risk microbiological agents which have an impact on blood safety: human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV, HCV) and other hepatitis viruses, human parvovirus B19 (B19V), human T-cell lymphotropic virus types 1 and 2 (HTLV-1/2), cytomegalovirus (CMV), arthropod-borne viruses (West Nile virus (WNV), dengue virus), and human herpes virus type 8 (HHV-8). The causative agents for bacterial and parasitic diseases such as syphilis, malaria, Chagas disease and leishmaniasis were also included in the agenda together with an update on the need for reference materials to validate candidate tests for detection of prion agents in blood. An overview of the

current established WHO BRPs, together with new proposals for development, was intended for all microbiological agents mentioned above.

The following additional topics were included in the agenda for discussion by the WHO CCs:

- a) collection and exchange of worldwide epidemiological information with impact on blood safety;
- b) new technologies for the detection of infectious agents;
- c) ways forward for collaboration between the WHO CCs and between the WHO CCs and WHO.

The recommendations and proposals from the meeting of WHO CCs would be included in the proposed five-year IVD strategic plan and conclusions with regard to the priority projects for the establishment of BRPs submitted to the Expert Committee on Biological Standardization (ECBS) for consideration and endorsement. The strategic plan should assure the establishment of the required BRPs for the detection of microbiological agents with an impact on the regulation and control of the safety of blood and blood products.

### **3. INFECTIOUS AGENTS - VIRUSES**

#### **3.1 Human immunodeficiency virus**

Dr Holmes gave a brief overview of the current WHO BRPs:

- a) 1<sup>st</sup> International Reference Reagent (IRR) for HIV-1 p24 antigen (90/636), lyophilized. The virus-derived non-recombinant p24 standard (from US source, strain not known, likely to be an early subtype B isolate such as IIIB) is expected to be representative of all HIV-1 strains and subtypes. It shows a good stability. There is a low usage which may be due to a lack of awareness about the existence of the preparation. 3000 vials remain.
- b) 1<sup>st</sup> International Reference Panel (IRP) for anti-HIV antibodies (02/210), lyophilized. Six panel members constituted by 6 HIV subtypes/groups/types (A, B, C, CRF\_01AE, group O, HIV-2). It was established by the ECBS in 2006 with no unitage. Information available shows good stability. Insufficient information is available about antibodies to circulating recombinant forms (CRFs) and non-B subtypes to recommend expansion of the Panel at the present time.
- c) 2<sup>nd</sup> International Standard (IS) for HIV-1 RNA (97/650), lyophilized. HIV-1 subtype B, cell-culture propagated and diluted in human plasma cryosupernatant. Preparation established by the ECBS in 2006.
- d) 1<sup>st</sup> International Reference Panel (IRP) for HIV-1 RNA genotypes (01/466). Set of 10 genotypes (A, B, C, D, CRF\_01AE, F, G, AG-GH, groups N and O). Viruses propagated and diluted in pool human plasma. Liquid preparation established by the ECBS in 2003. Information available shows good stability.

On the basis of the genetic diversity of HIV and the diagnostic implications, Dr Hewlett presented two proposals for development of new WHO BRPs. The first proposal is an extension of the 1<sup>st</sup> IRP for HIV-1 RNA genotypes by including the main CRFs, e.g. CRF\_01AE and CRF\_02AG. This panel is needed to assess the impact of new variants on tests sensitivity. CBER can provide characterized materials from its HIV-1 panel stocks and HIV-2 isolates.

The second proposal is based on the extension of the established 1<sup>st</sup> IRP for anti-HIV-1/2 antibodies reference panel. This panel is needed for the control of enzyme-linked immunosorbent assay (ELISA) tests, as well as rapid tests, combined antigen/antibody tests, and new assays based on synthetic peptides. Samples from CBER comprising different HIV-1 and HIV-2 subtypes from different geographical regions could be provided as candidate materials.

Dr Holmes informed about two new proposals already endorsed by the ECBS in 2006. The first one is on an extension of the 1<sup>st</sup> International Reference Panel for HIV-1 RNA by including a range of CRFs and representatives of less common subtypes (G, H, J and K, groups N and O). This is similar to and incorporates part of Dr Hewlett's first proposal. The second proposal was made to develop an international standard for HIV-2 RNA following evaluation of cultured subtypes A and B.

Further to discussion of the above proposals, the group agreed on the following priority projects for HIV, with Dr Hewlett and Dr Holmes as the coordinators of all the agreed projects and tasks:

- a) Development of a 2<sup>nd</sup> International Reference Panel for HIV-1 RNA Genotypes
  - CBER and NIBSC to collaborate to identify useful subtypes and CRFs (extension of the 1<sup>st</sup> Reference Panel); to organize the exchange and testing of the materials as well as the evaluation/validation of heat inactivation for HIV materials; to evaluate the feasibility of freeze-drying panel members
  - development of a plan and discussion at a WHO coordinated workshop
  - report of the WHO collaborative study to ECBS in 2009.
- b) Development of an International Standard for HIV-2 RNA
  - CBER and NIBSC to collaborate to exchange information on available candidate HIV-2 strains (A and B) and their sequences;
  - development of a plan to evaluate/validate heat inactivation for HIV materials and to undertake freeze-drying and testing of candidate standards; discussion of a plan at a WHO coordinated workshop;
  - report of the WHO collaborative study to ECBS in 2009.
- c) Development of an HIV-2 RNA Genotype Reference Panel

CBER and NIBSC to determine the need for such a panel, to identify potentially useful strains and materials, to discuss the proposal at a WHO coordinated workshop
- d) Development of a 2<sup>nd</sup> Anti-HIV Antibody International Reference Panel
  - minimally diluted, extension of the 1<sup>st</sup> Reference Panel;
  - project proposal to be submitted to ECBS by 2008 for endorsement
- e) HIV-1 nucleocapsid protein (p24) antigen Reference Reagent

(see chapter New Tests and Technologies, pages 21-22).

WHO secretariat was asked to coordinate a workshop covering the following topics: survey of CCs for the availability of less common materials; prevalence studies to determine the significance of less common strains and CRFs and studies on current kit sensitivities; strategies for panel development; the possible need for further anti-HIV-2 types.

## **3.2 Hepatitis B virus**

### **3.2.1 HBsAg and anti-HBs**

Dr Ferguson presented a brief overview of the current BRPs for hepatitis B surface antigen (HBsAg) and for antibodies to hepatitis B surface antigen (anti-HBs):

2<sup>nd</sup> International Standard for HBsAg, adw2 (00/588) and International Reference Panel for HBsAg (03/262). The Reference Panel is a series of 4-fold dilutions of the 2<sup>nd</sup> International Standard. Both preparations were established by the ECBS in 2003 and no further action is required.

1<sup>st</sup> International Reference Preparation for anti-HBs immunoglobulin (W1042). It was established in 1977 and is now close to exhaustion (about 250 vials available). Dr Ferguson made a proposal for replacement of the reference preparation and suggestions for the design of the collaborative study. The development of a 2<sup>nd</sup> International Reference Preparation for Anti-HBs Immunoglobulin was agreed by the group and considered of highest priority. Dr Ferguson will be the coordinator of the project with the collaboration of Dr Yu, CBER and Dr Nick, PEI. The selected candidate material will be evaluated in a WHO collaborative study along with the current PEI standard and the proposed BRP for antibodies to hepatitis B core antigen (anti-HBc) which is additionally anti-HBs positive (see below). Clarification is needed on whether further positive human sera/plasma materials should be included in the study to demonstrate the usefulness of the candidate material for use with IVDs. The Report of the WHO collaborative study is expected to be submitted to the ECBS by October 2008.

### **3.2.2 Anti-HBc**

The assessment of the sensitivity of anti-HBc assays is required to ensure the detection of true low-level reactive samples. The standardization of anti-HBc testing is an important feature, particularly for blood screening. The proposal for the development of an Anti-HBc International Standard was outlined by Dr Scheiblauer, PEI. This project will be done in cooperation with NIBSC. Dr Scheiblauer presented data from the feasibility study with the candidate materials from NIBSC (95/522, already lyophilized) and from PEI (old standard material #82, liquid) compared by limiting dilution procedure in 14 different tests.

Furthermore, low anti-HBc positive materials without any other detectable HBV markers were included in the study to evaluate analytical versus diagnostic sensitivity. The outcome of the study demonstrated clearly that both materials, from PEI and NIBSC, showed comparable results and appeared to be adequate as candidate materials for the proposed WHO International Standard.

Because of the limited sample size of the PEI standard, the NIBSC material is proposed as the candidate material for the collaborative study to develop the proposed WHO International Standard. Dr Scheiblaue presented the protocol for this study. The inclusion of the low-reactive PEI samples (N3907/N3908) could be useful as an additional sensitivity index (e.g. benchmark control).

The group agreed on the conclusions from the feasibility study to prepare a WHO Anti-HBc International Standard and recommended to include the CBER standard material (bulk or dilution, to be determined) in the collaborative study. Dr Scheiblaue will be the project leader with the collaboration of Dr Ferguson, NIBSC and Dr Biswas, CBER. The timeline of the project will be as follows:

- a) characterize immunoglobulin G and M(IgG and IgM) potency of candidate and the other study materials using anti-HBc total and HBc-IgM assays;
- b) conduct the international collaborative study;
- c) collate results and perform statistical analysis at PEI by March/April 2008;
- d) submit the study report to ECBS in 2008.

### **3.2.3 HBV DNA**

Dr Baylis summarized the data of the WHO collaborative studies supporting the establishment of the 2<sup>nd</sup> International Standard for the HBV DNA (97/750). This preparation was first calibrated in the same collaborative study in 1999 as the 1<sup>st</sup> International Standard (97/746). A comparison analysis was performed in a second study. The results confirmed those of the original study, with no significant potency difference being found in the estimated International Units (IU/mL) for 97/746 and 97/750. Degradation studies indicated that 97/750 is very stable and suitable for long-term use. The 2<sup>nd</sup> International Standard was established by the ECBS in October 2006. No further action is required.

### **3.2.4 HBV genotypes**

Owing to its genetic variability, eight different genotypes are known for HBV, A to H, representing different subtypes related to the 'a' determinant of the HBsAg. Based on the results of studies on the HBV molecular epidemiology in different countries, some genotypes could be subdivided into sub-genotypes typically clustered in different geographical regions. Many of them are prevalent at high frequency and have therefore an impact on the blood safety.

The current WHO BRPs for HBsAg and HBV DNA, both generated from genotype A2/subtype *adw2*, represent only 1% of the worldwide HBV-infected population. During the "WHO Consultation on Global Measurement Standards and their Use in the *in vitro* Biological Diagnostic Field", held on 7-8 June 2004 in Geneva, it was agreed that the impact of other HBV genotypes on the sensitivity of HBsAg tests should be investigated further. Dr Nübling pointed out the need for the development of HBV genotype reference panels to evaluate HBsAg- and nucleic acid amplification technique (NAT)-tests in terms of their ability to detect those genotypes/subtypes prevalent in the regions where the tests are on the market.

The project was presented in 2005 and endorsed by ECBS. PEI is making efforts to collect plasma units worldwide. Materials from Russia, Germany and Japan, representing genotypes A, B, C, D and mixed genotypes, have already arrived at PEI. Ongoing efforts are being made to collect materials of the genotypes E (Africa), F and H (Latin America). The aim is to develop two genotype panels (one for HBsAg tests and one for NAT assays) originating from the same HBV high positive plasma units. The project is being performed in close collaboration with Prof Gerlich, Justus Liebig University Giessen, Germany.

Efforts are ongoing at PEI to characterize the already available candidate materials (HBV quantification by NAT, sequencing, genotyping, determination of HBsAg content). In Giessen, pilot experiments are being performed to separate mainly the 20 nm HBsAg particle fraction from plasma samples without denaturing procedures. This purification method will reduce the infectivity by up to 99.9%. The plasma units are pelleted by sucrose ultracentrifugation. The supernatant (virus-depleted plasma) still contains 80-90% of the original HBsAg and will be further subjected to an isopycnic sucrose gradient centrifugation and subsequently to an isopycnic flotation in CsCl. HBsAg quantity will be monitored and purity controlled by different methods. The pelleted material from the ultracentrifugation can serve as stock material to generate the genotype panel for NAT assays.

The group agreed that the development of the Genotype Reference Panels for HBsAg and HBV DNA should be considered high-priority projects. Because of the difficulties to identify the appropriate candidate materials, the development of this project has been delayed. HBV positive plasma units, representing the genotypes E, F and H, are currently being identified with the collaboration of the WHO QSD team. The timeline of the project will be as follows:

- a) availability of all candidate materials at PEI by the end of April 2007;
- b) characterize and prepare candidate stock panel members;
- c) conduct a feasibility study to assess the collected candidate panel members; this will be performed at PEI using the available HBsAg tests and NAT assays (limiting dilutions versus the respective WHO BRPs);
- d) with the support of a working group, select the final panel members and discuss the next steps including the protocol of the collaborative study by September 2007;
- e) process the materials, e.g. freeze-drying (only panel for NAT tests) and prepare the panels;
- f) collaborative study to investigate the impact of the different genotypes on the sensitivity of the HBsAg and NAT tests
- g) Report of the collaborative study to be submitted to ECBS in 2008.

### **3.3 Hepatitis C virus**

#### **3.3.1 HCV RNA**

Dr Baylis, NIBSC reported about the ongoing collaborative study to replace the 2<sup>nd</sup> International Standard for HCV RNA (96/798). The candidate materials were generated from three anti-HCV negative window period genotype 1a donations. The absence of other viral markers was confirmed in these stocks. HCV RNA concentrations were determined by in-house quantitative PCR and Roche' COBAS Amplicor HCV Monitor Test, version 2.0 by comparison to the 2<sup>nd</sup> International Standard (96/798).

The materials were pooled and diluted in HCV RNA- and anti-HCV-negative plasma, and have been freeze-dried in two batches, batch 1 (2079 vials) and batch 2 (2257 vials). Accelerated degradation studies will be performed at NIBSC on both batches after 10-11 months storage at +45° C, +37° C, +20° C, +4° C, -20° C and -70° C.

The collaborative study materials (two lyophilized candidate materials, 2<sup>nd</sup> International Standard 96/798, and an unprocessed liquid bulk) were dispatched in November 2006 and January 2007.

Materials have been distributed to 32 laboratories, covering the main commercially available quantitative NAT tests as well as having been validated by quantitative real-time PCR in-house assays. The statistical analysis of the data collected will be performed at NIBSC. The results of the collaborative study will be presented at the SoGAT meeting in Warsaw in June 2007. It is expected to submit the proposal for establishment of the 3<sup>rd</sup> International Standard for HCV RNA at the 58<sup>th</sup> Meeting of the ECBS in October 2007.

#### **3.3.2 HCV genotypes**

Data concerning a genotype panel for HCV RNA, prepared at NIBSC, were presented by Dr Baylis. Plasma samples were procured for the HCV genotypes 2-6, specifically 2b, 3a, 4a, 5 and 6a. Genotypes 2-6 were calibrated against the 1<sup>st</sup> International Standard for HCV RNA (96/790) in a collaborative study. The calculated mean titres of the stock viruses were used to prepare the genotype panel (02/202, liquid frozen samples) with an HCV RNA concentration of 1,000 IU/mL for each genotype.

The expert group and the participants in the study agreed that the expression of the genotypes 2-6 in International Units should be taken with caution due to the genetic variability of the virus and the fact that the calibration had been made against the International Standard representing the genotype 1a. It was agreed that this panel had not the status of a WHO International Reference Panel. However, the panel is suitable for use in NAT assay validation which is well documented by the frequent use of the panel.

The stock materials are available for manufacturing replacement panels. Approximately 140 panels are currently held at NIBSC.

There was consensus by the group that there is no priority for the development of an HCV RNA Genotype Reference Panel. However, scientific studies are needed to assess the global variation of HCV and to evaluate the impact of HCV variants on the sensitivity of NAT tests. Further discussion will be needed at the next WHO CCs meeting.

### 3.3.3 Anti-HCV

The outcome of the ECBS discussion in October 2000 and the discussion during the "Meeting with WHO Collaborating Centres for Biological Standards" in February 2001 in Amsterdam strongly supported the development of an anti-HCV reference panel containing antibodies directed against single HCV antigens. This reference panel would be used in the quality control of anti-HCV tests.

In 2001 the "WHO Working Group on International Reference Preparations for testing Diagnostic Kits used in the detection of HBsAg and anti-HCV Antibodies" proposed the preparation of a reference panel for each of the four major antibodies suitable for detection by commercial anti-HCV test kits: anti-core and anti-bodies to non-structural proteins (NS)3, 4, and 5. WHO led discussions with manufacturers of diagnostic kits to identify appropriate candidate materials. In March 2003, Chiron offered to help in preparing "monospecific" anti-HCV antibodies. On the basis of a WHO Materials Transfer Agreement, a panel was prepared from two pooled HCV positive plasma units (genotype 1a) with high titres against each of the four RIBA HCV 3.0 antigens: c22P (core), c33c (NS3), c100P (containing two different NS4 peptides: 5-1-1p and c100p), and NS5. The method of choice for preparing the antibodies was a sequential depletion of four immune affinity columns loaded with the RIBA antigens.

A feasibility study was conducted by the WHO CCs with the above candidate materials. Dr Nick gave an update on the project and presented the results from the feasibility study. The main objectives of this study were:

- a) to assess sensitivity of diagnostic kits for antibodies to the antigens above mentioned
- b) to allow standardization of test kits;
- c) to compare the titres of the purified monospecific anti-HCV antibodies in different test kits with those of natural monospecific anti-HCV samples from different genotypes (commutability of the candidate standard materials).

The results and conclusions were summarized as follows:

- a) Anti-HCV antibodies specific to the RIBA antigens core, NS3, NS4, and NS5 were prepared
- b) Single bands to one antigen only are visible on Chiron RIBA HCV 3.0 strips.
- c) Preparations are, however, not real "monospecific" since they still contain antibodies to HCV epitopes or antigens other than those used for depletion or on the strips.
- d) Nevertheless, the samples are highly suitable to control the reactivity of the referred antigens in immunoassays and blots.
- e) Samples are commutable and may be used to assess test quality in terms of the analytical sensitivity (e.g. by manufacturers and control authorities).

- f) The next step is to complete an analysis of all the results of the feasibility study with the help of a statistician.

Information on the "monospecific" materials:

Sample	No. tubes	Total volume (mL)	Possible dilution factor	Total volume after dilution (mL)
anti-core	20	100	100	10,000
anti-NS3	22	110	100	11,000
anti-NS4	22	110	12.5	1,375
anti-NS5	26	130	100	13,000

Further discussion on real-time stability data on these materials is needed. A complete assessment of correlation of clinical and analytical sensitivity is still to be performed. New HCV antigens (NS3/4) have been prepared and published by Chiron in the meantime. The question remains as to whether a further depletion of antibodies on these antigens is necessary. Recently, Abbott reported the development of humanized recombinant anti-HCV chimeric monoclonal antibodies. The group considered these materials of interest for the WHO project. Dr Padilla, WHO will discuss with Abbott the possibility to obtain samples of the recombinant anti-HCV chimeric monoclonal antibody preparations for the WHO studies.

Following discussion on the above information and proposals made by Dr Nick, the group confirmed the need for an International Reference Panel for Anti-HCV Antibodies. Because of the limited amount of these materials, this panel was considered relevant for use by regulatory authorities to determine the potencies of the tests, rather than to be used for the control of the batch consistency by manufacturers. On this basis, there is no further need for purification to obtain more specific antibody preparations for the planned collaborative study. The group also agreed to describe accurately the reagents as "monospecific" versus the antigens against which they were absorbed.

The following actions are to be taken:

- a) the transfer of the "monospecific" materials to PEI will be arranged following the Materials Transfer Agreement between WHO and Chiron
- b) PEI will discuss the stability data with Chiron
- c) PEI will finalize the analysis of all data from the feasibility study
- d) Progress report to be presented to the ECBS in October 2007
- e) It is expected that the WHO collaborative study will start in 2008

### 3.4 Other Hepatitis viruses

#### 3.4.1 HAV RNA

Dr Baylis informed the group about a stability review carried out on the 1<sup>st</sup> International Standard for HAV RNA (00/560), established by the ECBS in 2003. Approximately a 2 log<sub>10</sub> drop was observed for accelerated degradation samples, stored at +20° C for 1 year, and about 1 log<sub>10</sub> drop was observed for samples stored at +4° C for 1 year. Further acceleration degradation studies for this preparation are to be performed in the second quarter of 2007 and will be presented at the XX<sup>th</sup> SoGAT meeting in Warsaw, Poland, in June 2007.

Approximately 1200 vials of the 1<sup>st</sup> International Standard (00/560) remain and the usage is 100 vials per year. Upon exhaustion, about 1650 vials of a second freeze-dried batch (00/562) are available. This batch was also calibrated in the collaborative study (BS/03.1959) to establish the 1<sup>st</sup> International Standard.

Dr Baylis will provide a summary on the interim review of stability data for the 1<sup>st</sup> International Standard to the 58<sup>th</sup> ECBS Meeting in October 2007.

### **3.4.2 Anti-HAV and Anti-HEV**

Dr Ferguson reviewed the WHO reference preparations for anti-HAV immunoglobulin and anti-HEV serum, currently available. The anti-HAV immunoglobulin preparation was established as the 2<sup>nd</sup> International Standard (97/646) in 1998. There are about 2000 ampoules in stock. The anti-HEV preparation was established as a Reference Reagent (95/584) in 1997. This is a batch of freeze-dried ampoules containing a pool of equal volumes of serum obtained on five separate dates over a 45-day period (4-5 months after onset of illness) from a patient in the USA who developed acute hepatitis E following travel to India. There are more than 1000 ampoules in stock, and in 2006 only 12 ampoules were sent out.

No further action was proposed for these preparations.

### **3.5 Human parvovirus B19**

#### **3.5.1 Anti-B19V**

The 2<sup>nd</sup> International standard for B19V antibodies (01/602) was established by the ECBS in 2003. Dr Ferguson explained that differences in the detection of the standard material and the individual plasmas had been observed in the collaborative study, with tests based on different antigens, VP1, VP2, or VP1/VP2. However, since VP1 is a minor capsid protein and on its own does not assemble into virus particles, and the dominant response in individuals appears to be against VP2, it was considered reasonable to utilize only the data from tests based on VP2 antigen for the calibration of the standard material. There are over 2000 ampoules held at the NIBSC, about 100 having been issued. Dr Ferguson raised the question of a possible need for a proposed IgM standard preparation.

The group agreed that such a preparation would be relevant to general diagnostic rather than to blood safety. No further action was planned.

#### **3.5.2 B19V DNA and B19V genotypes**

Dr Baylis pointed out that the 1<sup>st</sup> International Standard for B19V DNA (99/800), established in 2000, would be nearly exhausted by 2009 at the current rate of usage. A second freeze-dried preparation (99/802) showed no significant difference in potency in the WHO collaborative study. The ECBS had noted that this batch could be reserved for potential future use as a replacement standard but that additional stability studies would be required.

A small collaborative study was proposed by Dr Baylis, similar to that performed for the replacement of the 1<sup>st</sup> International Standard for HBV DNA. The aim is to demonstrate the equivalence of the candidate replacement material to the 1<sup>st</sup> International Standard for B19V DNA based on real-time data and accelerated degradation data.

Dr Baylis noted that the VIII<sup>th</sup> Report of the International Committee on Taxonomy of Viruses had recently classified the A6- and V9-like viral strains as genotypes 2 and 3 of B19V<sup>1</sup>, respectively. In this regard, a question was raised concerning the preparation of a B19V genotype panel for NAT tests. She outlined the reasons for which standardized plasma samples representing the different B19V genotypes are required, as well as the need to meet regulatory requirements for testing plasma pools. Regulators want to be sure that all three genotypes (1, 2 and 3 with two subgroups) are detected and that appropriate standards are available to validate NAT assays worldwide.

The group agreed to the proposed replacement procedure of the 1<sup>st</sup> International Standard for B19V DNA and to submit the report of the WHO collaborative study to the ECBS in 2008 for establishment of the 2<sup>nd</sup> International Standard.

With regard to a Genotype Reference Panel for B19V DNA, a consensus was reached to:

- a) discuss ways of taking the standardization issues forward and identify source plasma materials of the genotypes 2 and 3 at a pre-SoGAT meeting at NIBSC in March 2007;
- b) present a report of this meeting and its progress at the XX<sup>th</sup> SoGAT meeting in Warsaw in June 2007.
- c) NIBSC to present an update of discussions at the SoGAT meetings to the ECBS in 2007

### **3.6 Human T-cell lymphotropic virus**

The CBER proposal for an Anti-HTLV-1/2 Reference Panel was presented by Dr Cowan. He pointed out that HTLV-1 and -2 are infectious agents that pose significant risks to the blood supplies in specific areas: Africa (HTLV-1/2), South America (HTLV-2), the Caribbean (HTLV-1) and Japan (HTLV-1). Some HTLV-2 subtypes may escape detection by currently available technology. There is now mandatory screening in many countries around the world.

The lack of reference panels hinders the ability to evaluate new tests with improved sensitivity and to assure that they are robust in detecting the antigenic variants. For the development of a reference panel, the candidate materials should include sera/plasma samples from HTLV-infected individuals from areas where HTLV-1 and -2 are endemic, including special samples that represent the HTLV-2 subtypes. CBER proposed to form a working group to discuss the various issues and steps in the development of such a panel.

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<sup>1</sup> Virus Taxonomy: VIII<sup>th</sup> Report of the International Committee on Taxonomy of Viruses. Academic Press, USA (2005).

The following actions were proposed by the group:

- a) WHO will contact the WHO Regional Offices where HTLV is endemic to discuss this project and to identify appropriate materials from the regions (timeline 4 months).
- b) WHO will consult with the three CCs to prepare a workplan for the development of the Anti-HTLV-1/2 Reference Panel.
- c) CBER will coordinate the feasibility study by collecting and testing serum/plasma from diverse geographical areas. The WHO Collaborating Centre for Quality Control of Serology in Blood Banks from Sao Paulo, Brazil will contribute by offering anti-HTLV-positive plasma units. NIBSC is willing to participate and may be able to contribute HTLV-positive plasma samples.

### **3.7 Cytomegalovirus**

Dr Chudy gave a short overview of the current situation concerning *in vitro* diagnostic tests for the detection of CMV infection. CMV testing is a major issue in the transplantation field, as well as for blood products transfused to CMV-susceptible individuals (e.g. neonates, pregnant woman, immunosuppressed patients) and for allogeneic cord blood stem cells.

Molecular CMV testing for both transplantation/transfusion safety and clinical diagnostic purposes requires the standardization of the test kits. Anti-CMV antibodies IgG and IgM are usually the initial testing parameter for the detection of virus infection. Currently, there are no international reference preparations available.

Dr Ferguson summarized the past activities for establishing a WHO anti-CMV immunoglobulin standard. This preparation was not accepted by ECBS in 1995 as a WHO reference preparation (WHO Technical Report Series, No 872, 1995, p 15). However, ECBS noted that reference materials would be required for evaluating diagnostic kits used for the detection of anti-CMV antibodies (IgG and IgM). Dr Ferguson raised the question of whether another collaborative study to assess the available material should be undertaken.

The view of the WHO CCs is that there could be a need for an Anti-CMV International Standard, but it was not considered an urgent priority. This topic will be re-discussed at the next meeting.

### **3.8 Arthropod-borne viruses: West Nile virus and Dengue virus**

Owing to the increasing seasonal WNV endemics in the USA, starting from October 1999, and the documented WNV transmissions by blood in 2002, the FDA accepted the use of NAT systems beginning in July 2003. These tests were developed to detect WNV RNA as the most promising tool for the identification of infectious blood donations. Dr Rios pointed out the need for a WNV standard preparation for these assays and presented the results from the collaborative study to evaluate the CBER WNV panel.

The CBER panel was composed of 14 coded members (1000, 500, 100, 50, 10 and 5 viral copies/mL, prepared from NY99-FDA and FDA-Hu2002 strains, and two negative samples) and was distributed to seven independent laboratories. The outcome of the study showed that there was great variability of results with low copy number panel members. Generally, the performance of the qualitative assays was better than those of the quantitative assays.

Dr Rios also presented some facts related to the dengue virus situation. This virus causes the most common disease transmitted by arthropod vectors endemic in tropic and subtropic regions (*Aedes aegypti* and *Aedes albopictus*). About 50-100 million cases were reported worldwide annually. Four serotypes (DEN-1 to DEN-4) are known. No prevalence data on dengue virus infection in blood donor populations are currently available. However, there has been one documented case of transmission of dengue virus by transfusion reported by the Department of Health in Hong Kong in 2002.

Dr Ferguson gave an overview of the available 1<sup>st</sup> Reference Reagent for Anti-dengue virus type 1, 2, 3 and 4 serum (02/186) and of the NIBSC Anti-dengue reference panel (05/248). The material 02/186 had been evaluated as a candidate standard for anti-DEN 1+2+3+4 for use in studies to evaluate vaccines. It was not established as an International Standard because too few ampoules were available. Further WHO-coordinated studies are under way to develop a larger panel for use in the standardization of dengue virus antibody neutralization assays in laboratories involved in the vaccine development. The Anti-DEN reference panel (05/248) contains 4 panel members: anti-DEN1, anti-DEN2, anti-DEN3, anti-DEN4.

Further to discussion of the above proposals, CBER and PEI experts considered it worthwhile to develop a WHO BRP for WNV RNA. WHO was asked to convene a consensus meeting of all interested parties, including flavivirus experts and IVD manufacturers to discuss the need, feasibility and support for development of the BRP. Another topic of discussion should be the usefulness of a multiplex NAT test broadly reacting against several members of the *Flaviviridae* family group and the possible development of a suitable reference preparation for NAT assays. Stocks of the strains used to formulate the CBER panel were made available for use in preparing WHO standard materials.

The diagnostic value of anti-dengue standard preparations was considered limited due to the cross-reactivity with other members of the arbovirus group.

## **4. INFECTIOUS AGENTS - BACTERIA AND PARASITIS**

### **4.1 Treponema pallidum (causative agent for syphilis)**

The current anti-syphilitic reference serum (1<sup>st</sup> International Standard, code HS) was established in 1957. The assigned value (49 IU per ampoule) was determined on the basis of the cardiolipin and Kahn assays that were routinely in use at that time. This standard is used by reference laboratories, diagnostic laboratories and manufacturers of diagnostic immunoassays.

Dr Ferguson presented an update of the WHO international collaborative study to evaluate two human plasma pools preparations, representing active (IgG/IgM) and latent syphilis (IgG), that had been selected as replacement candidates for the 1<sup>st</sup> International Standard (project coordinator: Dr Rijpkema, NIBSC). The aims of the collaborative study are as follows:

- a) to assess the suitability of the two freeze-dried preparations as candidate reference standards for *Treponema pallidum* particle agglutination (TPPA) tests and cardiolipin assays;
- b) to assess the reactivity of the two candidate reference standards and the current 1<sup>st</sup> International Standard in various immunoassays.

Test data from TPPA and cardiolipin assays have been received from the study participants, along with additional data from immunoassays (recombinant ELISA, FTA) at NIBSC. Some of these assays detect specific IgM and IgG. The data are currently being analysed. It is expected that the study report will be submitted to the 58<sup>th</sup> ECBS Meeting in October 2007, proposing the establishment of the 2<sup>nd</sup> Anti-syphilitic International Standard.

A representative from CDC, Dr Ballard, was linked to the meeting via teleconference. He confirmed the interest of CDC in participating in the above mentioned collaborative study. The group agreed that NIBSC would send the CDC a set of test samples to perform the assays requested in the protocol of the study. It was also agreed that the forthcoming results would be included in the report of the WHO collaborative study that would be submitted to the ECBS in October 2007.

#### **4.2 Plasmodium species (causative agent for malaria)**

Dr Kumar presented a summary of the "FDA workshop on testing for malarial infections in blood donors" held on 12 July 2006. The major issues discussed at the workshop were:

- a) Exposure-based donor deferral: The majority of donors who caused transfusion-transmitted malaria were either born, lived or travelled in Africa. Representatives from blood banking expressed scepticism for the one-year deferral policy for travellers who visit resorts in Mexico and the Caribbean.
- b) Detecting malaria infections in blood donors: The technology of DNA-based tests is deemed to be unready, owing to its inability to detect a few parasites in a unit of blood. The experiences with antibody testing in the United Kingdom, France and Australia were found to be satisfactory, based on the detection of 2 out of 4 species (*Plasmodium falciparum* and *Plasmodium vivax*).
- c) Future prospects for testing in the USA: In the near future, antibody-based tests offer the best prospect. The number of *Plasmodium* species to be represented in the test (geographical species distribution) is debatable. The algorithms for testing are under discussion (testing of all donors versus testing donors in at-risk populations).

#### 4.2.1 *Plasmodium falciparum* DNA

The results from the collaborative study conducted in 14 laboratories to establish the 1<sup>st</sup> International Standard for *Plasmodium falciparum* DNA (04/176) for NAT assays (whole blood) were presented by Dr Baylis. The intended use of this reference material is for the standardization of qualitative and quantitative DNA NAT assays. The standard is freeze-dried and the potency is expressed in IU. Stability data showed no loss in potency, and it is stable and suitable for long-term use. Out of 2,000 vials manufactured, 1,800 remain. The ECBS adopted the material as the 1<sup>st</sup> International Standard at their 57<sup>th</sup> Meeting in October 2006.

The group agreed that no further action was needed.

#### 4.2.2 Anti-*Plasmodium* species

Drs Ferguson and Kumar presented the NIBSC and CBER proposals, respectively, for the development of reference preparations for anti-*Plasmodium* species. Dr Ferguson reported on NIBSC ongoing activities in the vaccine development area. The aim of this WHO project is to develop a candidate International Standard for use in the evaluation of responses in vaccinees. From this background different positive plasma/serum samples of blood donors from different regions were characterized and archived at NIBSC, which could serve as potential candidate materials for the collaborative study, especially the already freeze-dried materials for anti-*Plasmodium falciparum* (Vietnam) and for anti-*Plasmodium vivax* (Ethiopia), currently being used as working materials.

Dr Kumar pointed out the need for an antibody reference panel to define the sensitivity and specificity of serology assays both for clinical diagnostic and blood donor screening to detect malaria infection. The panel would be used for the validation of ELISA test kits or other high throughput screening tests and to compare the efficacy of commercial test kits by regulatory agencies and by the user. Additionally, these antibody standard preparations would be a useful tool for assays to measure the safety and efficacy in the development of malaria vaccines. Owing to the lack of cross-reactivity between the *Plasmodium* species, the proposed malaria antibody reference panel should cover the recognition of all *Plasmodium* species rather than being an anti-pan-plasmodium preparation. It would be ideal to have monospecific sera. There is consensus between the experts in this field that observed cross-reactivity is based on multiple infections with different *Plasmodium* species.

Dr Kumar emphasized that the panel should include sera from individuals who were exposed to only one species of *Plasmodium*. These samples will be useful in distinguishing *Plasmodium* epitope cross-reactivity versus recognition due to exposure to multiple *Plasmodium* species. The following plan was agreed by the group:

- a) CBER and NIBSC expressed interest in establishing an Anti-*Plasmodium* Species Reference Panel and agreed to collaborate as follows: NIBSC will send plasma/sera from positive donations to CBER for further characterization;
- b) CBER will determine the reactivity of the samples to different monospecific recombinant antigens;

- c) CBER and NIBSC will select a pilot panel of sera;
- d) CBER and NIBSC will hold a discussion regarding the protocol of the collaborative study.
- e) Update of these discussions will be reported to the ECBS

### 4.3 Trypanosoma cruzi (causative agent for Chagas disease)

Dr Duncan gave an update on the situation of Chagas testing in the USA. Although the USA is not a country of high prevalence of Chagas disease, the immigration of people from high endemic areas is an issue in relation to blood safety. In December 2006 the FDA approved the ORTHO *T. cruzi* ELISA Test System for use as a blood screening assay. Large blood centres in the USA have started to implement testing since the end of January 2007. CBER is responsible for the pre-release testing of manufacturers lots. A CBER lot release panel is under development (10 panel members: 4 positives, two dilutions each and two negatives). Although variability in the antibody response throughout the endemic range does not appear to be a large problem, there is enough concern in the field that a reference panel of reactive sera should have representatives of multiple geographical areas. The various issues and steps in the development of this panel should be discussed in a working group: e.g. number of sera, pooling of sera, sera from different geographical areas, antibody titres, and stability.

Dr Otani from the WHO Collaborating Centre for Quality Control of Serology in Blood Banks in Sao Paulo, Brazil, presented prevalence data of Chagas disease from Sao Paulo. The prevalence of infection with *T. cruzi* among first-time blood donors decreased from 48 to 35 cases per 10,000 donors between 1995 and 2001. The blood donor screening for anti-*T. cruzi* antibodies has been implemented for several years in most countries in Latin America and tests strategies have been changed over time. Starting the testing with ELISA, haemagglutination assay (HA) and indirect immunofluorescence assays (IFA), over 4% of the units were discarded in 1991. This ratio was drastically decreased (to under 1%) by testing with currently available ELISA tests.

Since 1995 positive plasma units have been archived for the following different purposes:

- a) to establish the national 'External Quality Assessment Schemes' for the Latin American region;
- b) for tests evaluation;
- c) for laboratory internal control;
- d) for research

From the Brazilian REDS Study<sup>1</sup> 463 plasma units are available: 20% have weak or median reactivity for both ELISA, HA, and IFA; 80% are strong positives. All samples will be retested with the FDA-licensed ORTHO *T. cruzi* ELISA test system.

The participants in the meeting agreed that the development of an Anti-*T. cruzi* Reference Preparation/Panel was a high priority. Several countries in Latin America,

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<sup>1</sup> The Retrovirus Epidemiology Donor Study (REDS) funded by the National Heart, Lung, and Blood Institute, which is part of the National Institutes of Health, USA.

representing the highest endemic region for Chagas disease, have implemented the mandatory testing of blood donors for anti-*T. cruzi* antibodies. Additionally, owing to the emigration of people from high-endemic areas to other countries, the testing of such blood donors for Chagas infection is performed in other countries (e.g. in Spain). Some countries are developing prevalence studies (e.g. France).

The following actions are to be taken:

- a) CBER proposed the development of an International Reference Panel for Anti-*T. cruzi* antibodies. WHO will form a working group involving participation of the WHO Collaborating Centres for Biological Standards, the WHO Collaborating Centre for Quality Control of Serology in Blood Banks, Sao Paulo, Brazil and of representatives from regulatory agencies, blood establishments, manufacturers and research institutions. The WHO AMRO/PAHO will be part of this working group.
- b) The working group will discuss issues related to the development of a WHO anti-*T. cruzi* BRP, including the need for the establishment of a panel of reactive sera representing multiple geographical areas.

## 5. INFECTIOUS AGENTS - TSE AGENTS

Dr Asher gave an update on TSE caused by unconventional agents (TSE agents or prions) and measures to reduce the possible risk of transmission of Creutzfeldt-Jakob Disease (CJD) and variant CJD (vCJD) by blood and blood products.

TSE infectivity in blood has been demonstrated by different experiments with rodents, sheep and non-human primates. Up to January 2007, four transfusion-transmitted cases of vCJD infection in the United Kingdom have been reported; all cases involved transmission by transfusion of non-leukoreduced red blood cells. These cases showed shorter incubation periods than in food-borne cases.

A general approach to reducing the risk of transmitting vCJD by blood products is the deferral policy for donors at increased risk of exposure to the agent of the bovine spongiform encephalopathy. However, most deferred donors are probably not infected and not all potentially infected donors can be deferred by this approach. Dr Asher summarized information on current developments to develop and validate reliable methods to remove infectious TSE agents from the products and, to develop and validate screening tests to detect infected donors. Six candidate blood screening tests for detecting TSE agents were presented to a WHO consultation in September 2005 and later to an open meeting of the FDA TSE Advisory Committee. So far, none of these candidate tests have been evaluated independently by using reference materials. Proposals for the development of such materials, previously discussed by the WHO Working Group on TSE Reference Materials, were outlined.

Dr Minor presented an overview of the available WHO CJD biological reference materials held at NIBSC. In relation to the blood screening tests, he raised three issues that should be taken into consideration: sensitivity/specificity of the tests; infectivity implied by a positive result (positive predictive value of a reactive test result); and questions related to consequences for the donor with a reactive test result (living with uncertainty about possible development of the disease).

With regard to the TSE Blood Reference Preparations, infected animal and human blood would be needed; however, there is no adequate source of funding to develop and characterize these materials. Only brain-based and spleen-based materials are currently available. There are ethical issues involved in obtaining large amounts of blood from living patients. The possibility of obtaining human postmortem blood samples to establish reference materials was discussed. Postmortem blood could be included in the autopsy protocol. A further possible option could be to use reactive plasma units, identified during the pilot screening studies currently under discussion study in the United Kingdom. Alternatively, the suitability of TSE-infected animal blood to serve as a surrogate reference material should be further discussed. The group decided to play an advocacy role to facilitate the obtaining of postmortem blood. A follow-up teleconference of experts coordinated by Drs Asher and Minor will address the protocol for bringing forward a joint letter addressed to the recommended target audience.

## **6. INFECTIOUS AGENTS - OTHER BLOOD-BORNE PATHOGENS**

### **6.1 Blood-borne Bacteria**

Dr Unger presented the PEI proposal for the establishment of blood-borne bacterial reference materials to be used in the comparison and validation of methods for platelets bacteria screening and pathogen reduction. Despite improvements in blood donor screening procedures, the risk of transmitting bacterially contaminated blood products has been estimated at between 1 in 2,000 and 1 in 3,000 units of platelet concentrates. Severe septic reaction after platelet transfusion has been estimated at approximately 1 in 50,000, and fatal cases were observed at a ratio of 1 in 100,000 to 200,000.

To improve blood safety there is a need for methods applicable in platelet bacterial screening and in pathogen inactivation/reduction of cellular blood components. These methods need to be validated by standards representing the suitable bacterial strains. At PEI, standard materials were prepared from different blood-borne bacteria. These materials were characterized for their growth in blood and kept frozen. PEI is offering six defined, stable and shippable bacterial standards (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Bacillus cereus*) for a WHO feasibility study.

The group proposed that PEI organize a collaborative study involving CBER and other interested parties to evaluate the feasibility of the PEI bacterial strains as candidate materials for WHO BRPs. A further discussion is needed to clarify which additional bacterial strains are to be studied, especially those that cause fatalities from sepsis.

### **6.2 Relevant Leishmania species**

The parasite disease leishmaniasis is a global health problem with 12 million people infected and 350 million people at risk worldwide. Visceral leishmaniasis (VL) is the most severe form of the disease and can be fatal if left untreated. About 500,000

new cases of VL per year are reported (90% in India, Bangladesh, Nepal, Sudan, Brazil). VL infection is asymptomatic in healthy individuals such as blood donors. As Dr Duncan outlined 15 probable or confirmed cases of *Leishmania* transmission by blood transfusion that have been reported worldwide.

Most countries currently follow a deferral policy for blood donors, e.g. in the USA, lifetime deferral for a history of any type of leishmaniasis, and a one-year deferral for travel to Iraq only. The USA blood supply is currently not screened for anti-*Leishmania* antibodies. No FDA-approved test to screen blood donations is available. Because no countries perform regular screening of blood donors, there is no immediate need to prepare an anti-*Leishmania* reference material. However, CBER can have access to material for future panel development through its collaboration with the US Department of Defence.

The WHO CCs shared the view that, before starting the project, an information exchange was needed with the *Leishmania* group in WHO.

### **6.3 HHV-8 Antibodies and HHV-8 DNA**

CBER proposed the development of WHO BRPs for HHV-8. Dr Hewlett informed the group that HHV-8 can be transmitted by blood transfusion, according to a linked study that followed donors and recipients in Uganda. If donor screening moves forward, one strategy would be to screen with an antibody assay and run NAT as a supplemental test (although the frequency of DNA-positive cells is low). There is a need to initiate efforts to develop reference preparations for standardization of HHV-8 assays for both antibody and DNA detection worldwide, particularly in West Africa. Candidate materials should be serum samples from HHV-8-infected individuals from regions where HHV-8 is highly prevalent.

Leukoreduction is presumed to be effective because the virus is cell-associated. CBER has ongoing activities to acquire materials in some of these areas and may be able to contribute relevant candidate sera.

Currently, NIBSC and PEI saw no priority need for the development of HHV-8 reference preparations. CBER expressed interest in collaborating in this area. Although mainly countries from West Africa are affected, the global issue is to provide better diagnostic tools. This project should be re-discussed in forthcoming WHO CCs meetings.

## **7. EPIDEMIOLOGICAL DATA**

The changing of the epidemiological situation of blood-borne agents has an influence on the efficacy of the blood screening systems. The capability of test kits to detect new sub- or genotypes prevalent in donor populations has to be evaluated. WHO BRPs, as a standardization tool for test kits, have to be fitted to the prevalent types of the infectious agents. Data on the presence of emerging/re-emerging agents and their relevance to blood safety are important to enable appropriate BRPs for the standardization of test kit developments to be established.

The meeting participants discussed the possibilities for obtaining relevant epidemiological data: global blood safety database; input from the WHO Regional Offices; better assessment of scientific literature; activities of, for instance, governmental laboratories and academic centres; closer links to blood centres. Exchange of information can take place in regularly held WHO CCs meetings and meetings of WHO Regional Offices and working groups. Additionally, information about urgent incidents could be shared via teleconferences.

## **8. NEW TESTS AND TECHNOLOGIES**

### **8.1 HIV-1 p24 antigen**

Dr Nick gave an update about the combined antigen/antibody test systems for HIV and HCV. The first combined HIV test kits entered the European market in 1998. In a study using seroconversion panels, HIV-1 p24 antigen tests became positive 4-5 days later than NAT, HIV antigen/antibody combo tests became positive 6-9 days later than NAT, and anti-HIV-1/2 tests became positive 10-11 days later than NAT. The results from the performance evaluations with HIV-1 p24 diagnostic kits demonstrated a good correlation between clinical sensitivity on seroconversion panels and analytical sensitivity. Three different HIV-1 p24 antigen standard preparations were used: for test validation of the analytical sensitivity:

- a) WHO 1<sup>st</sup> International Reference Reagent for HIV-1 p24 antigen (90/636), adopted by ECBS in 1992. This is a p24 peptide isolated from detergent-treated HIV-1, 1000 IU per vial.
- b) PEI p24 antigen, defined in ng/mL, recombinant p24 preparation.
- c) AFSSAPS HIV-1 Antigen Standard, inactivated HIV-1 virus pelleted from supernatant of HIV-1<sub>Bru</sub> cultures and diluted in human serum.

Comparative studies with these materials revealed differences in the analytical sensitivity of HIV-1 p24 antigen test kits.

The WHO 1<sup>st</sup> International Reference Reagent for HIV-1 p24 antigen is used for the evaluation of both screening and diagnostic tests in Europe. There is an emerging need in the USA for a combo assay reference material. Regarding the discussions about the use of different available HIV-1 p24 standard materials:

- a) CBER/NIBSC will coordinate the formation of a working group consisting of the three WHO CCs to develop a study protocol to investigate the commutability of the WHO Reference Reagent (90/636) and to determine how robust the reference reagent is with respect to assays designed to detect variants;
- b) Samples of the WHO Reference Reagent material will be provided to CBER;
- c) CBER will perform the study.

## 8.2 HCV core antigen

Since the implementation of the 1<sup>st</sup> generation anti-HCV assays in 1990, the tests followed major changes to improve their sensitivity. Recently HCV core antigen assays and the HCV antigen/antibody combination assays have been developed; some are already CE-marked and others are under evaluation. In developing countries, combo tests could be a good option to screen blood donations in the absence of HCV NAT assays. Since there are further HCV core antigen and antigen/antibody combination assays under development, there is a need for a commutable HCV core antigen reference material to assess the sensitivity of the assays. In addition, an HCV core antigen international standard would help manufacturers in the standardization of the assays and will assist regulatory authorities in controlling test kits.

PEI will coordinate the formation of a working group of interested parties, including the three WHO CCs, to discuss the approach and assess the feasibility of the development of HCV core antigen reference material using 'window period' units from plasma donors. The candidate material could be obtained from plasma fractionators.

## 8.3 WHO BRPs for NAT assays

A short review of the evolution of NAT assays was given by Dr Nübling. Many new commercial NAT tests based on real-time techniques are multiplex and/or based on automated systems. Further infectious markers were included, e.g. HIV-2, HAV, B19V, CMV, WNV, *Plasmodium spec.* In this context it was mentioned that NAT assays for the detection of genetic markers are also being developed (e.g. blood group and HLA typing).

WHO BRPs for NAT assays that detect the high-risk blood-borne agents are currently available. These materials are generated from positive plasma units or prepared via propagation in cell lines or animals and represent the whole infectious agent. However, to standardize the amplification/detection steps, the manufacturers have developed in-house synthetic standard materials. Such synthetic NAT standards can be traced to SI units by reference physical methods and a quantification of the uncertainty is possible.

The potential use of such materials is currently being evaluated by different NAT manufacturers. For 2007 a common feasibility study by the industry is planned, including *in vitro* HCV RNAs, armored HCV RNAs, and the PEI reference material that has been calibrated against the WHO BRP. Dr Nübling will inform the WHO CCs on the outcome of the study and further activities in this area.

The group commented that the discussion about synthetic versus biological standards was not new. It was agreed that the current available WHO BRPs are the most suitable standard materials for standardization of the whole NAT procedure, including the sample preparation step. However, if there are not sufficient biological materials from less prevalent sub- or genotypes of infectious agents available (e.g. B19V genotypes 2 and 3), the use of synthetic materials (e.g. plasmid DNA or RNA transcripts) could be an option. The group recognized the efforts in this field and requested to be informed of forthcoming activities.

#### 8.4 BRPs for microarrays and nanotechnology-based tests

Dr Hewlett outlined the gene chip- (microarray) and nanotechnology as the major emerging technologies for diagnostic tests. Microarrays are being increasingly evaluated for multiplex pathogen detection. Nanotechnologies are useful for both individual and multiplex detection, as well as for protein and nucleic acid detection. Ongoing efforts are taking place to combine both technologies to develop low-cost rapid molecular analysis with a high sensitivity based on simple devices.

Dr Hewlett presented examples of the development of microarrays (chip for blood-borne and bioterror pathogens) and nanoparticle assays (avian influenza viral RNA, HIV-1 p24) in which CBER is involved. Reference materials are needed to facilitate comparison of different microarray and nanoparticle assays. A core issue in standardization is whether the developed reference materials need to be further refined. Regardless of technology or platform, reference preparations would generally be the same as for an NAT pathogen detection assay, i.e. to allow accurate detection of the pathogen.

The group agreed that the established WHO BRPs were suitable to cover the new technologies. However, if such tests become available on the market, they would need to be evaluated in a collaborative study.

### 9. MEETING RECOMMENDATIONS: PRIORITY SETTING FOR DEVELOPMENT OF WHO BIOLOGICAL REFERENCE PREPARATIONS

The following priority projects were identified, both for the establishment of new WHO BRPs or for the replacement of existing BRP batches near exhaustion.

- a) Replacement of existing WHO BRPs for:
  - Anti-HBs immunoglobulin (proposed 2<sup>nd</sup> International Reference Preparation)
  - HCV RNA (proposed 3<sup>rd</sup> International Standard)
  - B19V DNA (proposed 2<sup>nd</sup> International Standard)
  - Anti-Syphilitic Standard (proposed 2<sup>nd</sup> International Standard)
  
- b) New WHO BRPs required:
  - HIV-1 genotype panel (proposed 2<sup>nd</sup> International Reference Panel)
  - HIV-2 RNA International Standard
  - Anti-HIV antibody panel (proposed 2<sup>nd</sup> International Reference Panel)
  - HBsAg and HBV DNA genotype panels
  - Anti-HBc International Standard
  - Anti-HCV antibody panel
  - Anti-HTLV-1/2 antibody panel
  - Anti-*Plasmodium* species panel
  - Anti-*Trypanosoma cruzi* antibody preparation/panel

The following proposals for WHO BRPs need further discussion and will be included in the agenda of forthcoming WHO CCs meetings:

- HIV-2 RNA genotype panel
- HCV genotype panel
- B19V genotype panel
- Anti-CMV antibody standard
- WNV RNA preparation/pan panel for arthropod-borne flaviviruses RNA
- HCV core antigen preparation
- Preparations for HHV-8 antibodies and HHV-8 DNA
- TSE blood preparations
- Blood-borne bacteria panel
- Anti-*Leishmania* antibody panel

Other projects of interest to the PEI (e.g. anti-tetanus, vaccinia immunoglobulin) and the NIBSC (e.g. NAT standard for *Toxoplasma gondii*) were not discussed at this meeting. These together with other WHO BRP proposals for the detection of non-blood-borne infectious agents may also be discussed at the next meeting.

Furthermore, the group agreed on the collection and exchange by the WHO CCs of epidemiological information with an impact on blood safety. With regard to new technologies for the detection of infectious agents, there was consensus that the established WHO BRPs and those to be developed are suitable to cover the forthcoming new technologies.

## **10. PLAN OF ACTION**

The recommendations of the meeting will form a five-year IVD strategic plan. This plan will include the priority projects agreed to ensure establishment of WHO BRPs aimed to support international regulations for blood and blood products safety. It will be submitted to the ECBS for endorsement in October 2007. It was agreed that the IVD strategic plan will be used to support resource mobilization activities.

Improved opportunities for collaboration between WHO CCs and between the WHO CCs and WHO were agreed upon. Annual face-to-face meetings were considered necessary as well as teleconferences to monitor progress. A need to establish a network of WHO CCs for IVD-related biological standardization representing all the WHO Regions was emphasized, in order to ensure complementary and focused expertise at global level. The group agreed to use a master form sheet for future BRP proposals. This sheet should be completed by adding telephone and fax numbers, as well as the e-mail address of the contact person/coordinator.

## ANNEX 1: REFERENCES

Further information related to the development and establishment of WHO BRPs can be found in the following references:

- Recommendations for the preparation, characterization and establishment of international and other biological reference standards . WHO Expert Committee on Biological Standardization. Fifty-fifth report, 2004. (WHO Technical Report Series, No. 932)
- <https://www.who.int/bloodproducts/norms/en/>
- Catalogue of WHO International Biological Reference Preparations:  
<http://www.who.int/bloodproducts/catalogue/en/index.html>

Other references on previous WHO Consultations related to WHO BRPs for IVD tests can be found as follows:

- WHO Consultation on Global Measurement Standards and their use in the *in vitro* Biological Diagnostic Field. Geneva, Switzerland (Jun 2004)  
<http://www.who.int/bloodproducts/publications/en/Minutes-220804.pdf>
- WHO Working Group on International Reference Preparations for Testing Diagnostic Kits used in the Detection of HBsAg and anti-HCV Antibodies (Oct 2003)  
<http://www.who.int/bloodproducts/publications/en/HEPATITISOCT-2003.pdf>
- WHO Consultation on International Standards for *in vitro* Clinical Diagnostic Procedures based on Nucleic Acid Amplification Techniques (NAT). Geneva, Switzerland (Apr 2002)  
<http://www.who.int/bloodproducts/publications/en/BIVD02apr22.pdf>
- WHO Consultation on International Biological Standards for *in vitro* Diagnostic Procedures. Geneva, Switzerland (Sep 2000)  
<http://www.who.int/bloodproducts/publications/BIVD00Sep14.pdf>
- WHO Working Group on Reference Preparations for testing Diagnostic Kits used for detection of HBsAg, Anti-HCV and Anti-HIV antibodies in blood screening. Geneva, Switzerland (Jan 2000)  
<http://www.who.int/bloodproducts/publications/en/BSaf00Jan17.pdf>

## **ANNEX 2: LIST OF PARTICIPANTS**

**Dr David Asher**, Chief, Laboratory of Bacterial, Parasitic and Unconventional Agents (LBPUA/DETTD/OBRR/CBER), Center for Biologics Evaluation and Research, HFM-313, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Sally Baylis**, Division of Virology, National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG United Kingdom

**Dr Robin M. Biswas**, Chief, Product Testing Staff (DETTD/OBRR/CBER), Center for Biologics Evaluation and Research, HFM-310, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Elliot P. Cowan**, Chief, Product Review Branch (DETTD/OBRR/CBER), Center for Biologics Evaluation and Research, HFM-394, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Robert Duncan**, Laboratory of Bacterial, Parasitic and Unconventional Agents (LBPUA/DETTD/OBRR/CBER), Center for Biologics Evaluation and Research, HFM-313, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Jay S. Epstein**, Director, Office of Blood Research and Review (OBRR/CBER), Center for Biologics Evaluation and Research, HFM-300, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Morag Ferguson**, Division of Virology, National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG United Kingdom

**Dr Indira K. Hewlett**, Chief, Laboratory of Molecular Virology, (LMV/DETTD/OBRR/CBER), Center for Biologics Evaluation and Research, HFM-315, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Harvey Holmes**, National Institute for Biological Standards and Control (NIBSC) Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, United Kingdom

**Dr Sanjai Kumar**, Laboratory of Bacterial, Parasitic and Unconventional Agents (LBPUA/DETTD/OBRR/CBER), Center for Biologics Evaluation and Research, HFM-313, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Paul Mied**, Division of Emerging and Transfusion Transmitted Diseases (DETTD/OBRR/CBER), HFM-310, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Phil Minor**, Head, Division of Virology, National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, United Kingdom

**Dr Hira L. Nakhasi**, Director, Division of Emerging and Transfusion Transmitted Diseases (DETTD/OBRR/CBER), HFM-310, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Sigrid Nick**, Testing Laboratory for IVD, Paul-Ehrlich-Institut (PEI), Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany

**Dr C. Micha Nübling**, Division of Virology, Paul-Ehrlich-Institut (PEI), Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany

**Dra Márcia Otani**, Jefe Depto. Control de Calidad - Serologia, Fundação Pró-Sangue - Homocentro de São Paulo, Av. Dr. Enéas de Carvalho Aguiar 155, Cerqueira Cezar, São Paulo, Brazil

**Dr Maria Rios**, Laboratory of Molecular Virology, (LMV/DETTD/OBRR/CBER), Center for Biologics Evaluation and Research, HFM-315, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852, USA

**Dr Heinrich Scheiblaue**r, Testing Laboratory for IVD, Paul-Ehrlich-Institut (PEI), Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany

**Dr Gabriele Unger**, Coordinator, WHO Collaborating Centre for Quality Assurance of Blood Products and *in vitro* Diagnostic Devices, Paul-Ehrlich-Institut (PEI), Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany

#### **SECRETARIAT**

**Dr Michael Chudy**, Technical Officer, Quality Assurance and Safety: Blood Products and related Biologicals, WHO Headquarters, Geneva, Switzerland

**Dr José Ramiro Cruz**, Regional Adviser on Laboratory and Blood Services, WHO Region of the Americas, Washington DC, USA

**Dr Ana Padilla (Secretary)**, Scientist, Quality Assurance and Safety: Blood Products and related Biologicals, WHO Headquarters, Geneva, Switzerland