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

*Report of the 2<sup>nd</sup> meeting with the WHO Collaborating Centres for Biological Standards and Standardization, 17-18 February 2009*



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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>CDRH</b>	Center for Devices and Radiological Health (USA)
<b>CC</b>	Collaborating Centre (WHO)
<b>CDC</b>	Centers for Disease Control (USA)
<b>CFU</b>	colony forming unit
<b>CJD</b>	Creutzfeldt-Jakob disease
<b>CMV</b>	cytomegalovirus
<b>CRF</b>	circulating recombinant form
<b>DNA</b>	deoxyribonucleic acid
<b>EBV</b>	Epstein-Barr virus
<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>HDV</b>	hepatitis D virus
<b>HEV</b>	hepatitis E virus
<b>HHV-8</b>	human herpes virus type 8
<b>HIV</b>	human immunodeficiency virus
<b>HTLV</b>	human T-cell lymphotropic virus types
<b>IFA</b>	immunofluorescence assay
<b>IgG</b>	immunoglobulin G
<b>IgM</b>	immunoglobulin M
<b>IVD</b>	<i>in vitro</i> diagnostic
<b>JE virus</b>	Japanese encephalitis virus
<b>NAT</b>	nucleic acid amplification technique
<b>NIBSC</b>	National Institute for Biological Standards and Control (UK)
<b>p24</b>	nucleocapsid protein of HIV
<b>PEI</b>	Paul-Ehrlich-Institut (Germany)
<b><i>P. ssp.</i></b>	<i>Plasmodium species</i>
<b>RNA</b>	ribonucleic acid
<b>SoGAT</b>	Standardization of Genome Amplification Techniques
<b><i>T. cruzi</i></b>	<i>Trypanosoma cruzi</i>
<b><i>T. cruzi</i> I</b>	<i>Trypanosoma cruzi</i> group I
<b><i>T. cruzi</i> I</b>	<i>Trypanosoma cruzi</i> group I
<b>TSE</b>	transmissible spongiform encephalopathy
<b>WHO</b>	World Health Organization
<b>WNV</b>	West Nile virus



## 1. OPENING REMARKS

The Blood Products and Related Biologicals programme in the Quality and Safety of Medicines team (QSM), Department of Essential Medicines and Pharmaceutical Policies (EMP), World Health Organization (WHO) convened the 2<sup>nd</sup> meeting with the WHO Collaborating Centres for Biological Standards and Standardization (WHO CCs), i.e. the National Institute of Biological Standards and Control (NIBSC), UK, the Paul-Ehrlich-Institute (PEI), Germany, and the Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), USA. The meeting was kindly hosted, from 17 to 18 February 2009, by the Paul-Ehrlich-Institute in Langen, Germany.

Professor Seitz, Head of the WHO Collaborating Centre for Quality Assurance of Blood Products and in vitro Diagnostic (IVD) Devices opened the meeting and welcomed the participants. Professor Löwer, President of the PEI, gave an overview of the history of the PEI and its current responsibilities, duties and research activities. Changes in the regulation of medicines and IVDs in the European Community have influenced the future responsibilities and duties of the institute. He outlined how new products, such as gene transfer, cell therapy and tissue engineered products had become new areas of responsibility of the PEI.

Dr Padilla (EMP/QSM) explained the global importance of Biological Reference Preparations (BRPs) and underlined their role for the control of IVD tests related to blood safety and the control of infectious disease markers. She highlighted the objectives of the 2<sup>nd</sup> meeting which were to strengthen the cooperation among the WHO Collaborating Centres for Biological Standards and Standardization, and between the Centres and WHO, to continue the development of the IVD plan, and to strengthen the development of BRPs.

Prof Seitz was elected Chairman of the meeting, and Dr Chudy was nominated as Rapporteur. The list of participants is attached to this Report.

## 2. GENERAL TOPICS

### 2.1. *New ELISA systems and platforms*

Dr Nick gave an update on new developments of serological test systems for the detection of HBV, HIV and HCV infection. In the European Union all in-vitro diagnostic tests must comply with the requirements of the directive 98/79/EC and Common Technical Specifications (CTS) defining the minimum requirements for the so-called high-risk devices, such as HBV, HIV and HCV tests. Dr Nick informed about the ability of CE-marked HBsAg tests to detect escape mutations. There were several tests on the market which showed weaknesses in detecting well-described escape mutations. Consequently, some kit manufactures have improved mutant recognition by using a combination of monoclonal antibodies. With respect to clinical sensitivity significant differences were observed for HBsAg screening tests. In 2008 two new HBsAg tests with improved sensitivity were CE-marked. One assay can detect HBV infection on average 5.3 days earlier than the PRISM HBsAg test. Generally HBsAg tests show a high correlation between analytical and clinical sensitivity. The revised CTS now define more strict criteria for HBsAg tests with respect to analytical sensitivity (0.130 IU/ml based upon to the 2<sup>nd</sup> International Standard for HBsAg 00/588) and require recognition of mutants. Furthermore Dr Nick gave an

update on the sensitivity of HIV and HCV antibody, antigen and combined antigen/antibody test systems. Recently new HIV combination assays with enhanced sensitivity have been introduced into the market. In addition, a new highly sensitive HCV core assay was CE-marked.

## **2.2. *New NAT systems and platforms***

A short review of the evolution of NAT assays was given by Dr Nübling. Currently, there are two automated multiplex (HCV, HIV, HBV) NAT tests for screening of blood donation on the market: Procleix TIGRIS System with the Ultrio Assay (Gen-Probe) and cobas s201 with TaqScreen MPX test (Roche Molecular Systems). Results from a “Head-to head comparison study” of the two automated NAT systems performed in France and Australia were presented. Overall both systems appear similar with regard to different quality features, e.g. genotype sensitivity or analytical sensitivity. The TaqScreen assay might be slightly more sensitive for HBV, while Ultrio detects lower levels of HCV or HIV-1. A study performed at PEI on detection of HBV DNA by several commercial NAT assays in anti-HBc reactive specimens was presented. Most NAT assays (both quantitative and qualitative) consistently failed to detect HBV DNA by replicate testing in these low level positive specimens of chronic HBV carriers. Therefore screening for anti-HBc may be an option in low prevalence countries until sensitivity of assays assures consistent detection of HBV DNA in these specimens. High sensitivity of HBsAg assays is still an important issue to cover the early phase of HBV infection.

## **2.3. *Emerging/Re-emerging infectious pathogens and their impact on blood safety***

Numerous emerging and newly recognized infections have the potential of transmission through transfusion. In many cases little is known about their pathogenesis such as infectious period or risk factors. In addition, there is a paucity of information on the effective interventions for such cases.

Dr Nakhasi raised three basic questions in this context and provided CBER’s view:

- Is it in the blood supply?
- Development of assays for direct or indirect detection of the agent is needed. In addition, there is a need for estimation of the donor and blood component risks as well as estimation of residual risk (incidence and window period) from the donation.
- Is it transfusion-transmitted?  
Follow-up of case reports and performing look-back investigations is needed. In some cases data from animal studies could be helpful. Information from prospective and retrospective (repository specimens) studies with linked donation-recipient bio-specimens or databases is essential.
- If transmissible by transfusion, does it have a clinical impact i.e., do recipients of infected products have significant clinical manifestations?  
Follow-up of case reports and evaluation of their clinical outcomes such as severity (morbidity, mortality, case-fatality rate) should be performed

The FDA’s approach to optimize blood safety by following the so called ‘Five layers of blood safety’ was also explained. These include donor screening and deferral based on various risk

factors identified through questionnaire, laboratory testing and deferral, maintaining deferral registries to prevent use of blood from deferred donors, quarantine controls to prevent unit release pending verification of donor suitability, and investigation and corrections of deviations.

Dr Nakhasi presented an update on the following emerging and re-emerging pathogens and their potential impact on blood safety and availability. These include: Dengue virus, Chikungunya virus, West Nile virus (WNV), *Plasmodium ssp.*, *Babesia ssp.*, *Leishmania ssp.*, *Trypanosoma cruzi* (*T. cruzi*), HIV drug resistant and recombinant variants, HBV mutants, agents of bioterrorism (class A). The regulatory and scientific challenges posed by such agents on the blood safety and availability were emphasized such as non availability of screening assays, significant donor loss on exposure based deferral, inability to reentry donor who are not infected and its impact on blood availability, and lastly whether the existing tests can identify the variants of agents which are being currently screened for.

#### **2.4. Standardization of clinical diagnostics**

In particular, real-time NAT assays are replacing traditional diagnostic methods in clinical microbiology laboratories. Dr Minor pointed out that many of the assays are in-house developed and therefore the lack of standardized reference materials could lead to a variability of results within and between laboratories. He presented the approach by the Clinical Virology Network in collaboration with NIBSC/HPA to develop working reagents for clinical NAT assays. The reagents will be prepared at NIBSC and evaluated in two phases. Proposals for the development of International Standards for CMV DNA and EBV DNA for NAT-based assays were accepted by the ECBS in 2008. Targets for future standardization are BK virus and JC virus.

### **3. PARASITES**

#### **3.1. Anti-*Trypanosoma cruzi* antibody panel**

*T. cruzi* is the causative agent for Chagas disease, which is a global health problem due to population mobility between endemic and non-endemic areas. Appropriate control of IVD tests for the detection of anti- *T. cruzi* antibodies is essential, for both screening and clinical diagnostics. There is a global need for the regulation and control of diagnostic tests by appropriate international BRPs. One of the outcomes of the 1<sup>st</sup> Collaborating Centers Meeting in January 2007 was the need for the development of a Biological Reference Panel for Anti- *T. cruzi* antibodies. Dr Scheiblaue presented an update of the activities in this area. QSM/EMP/WHO convened the 1<sup>st</sup> Consultation on the Development of a WHO Reference Panel for the Control of Chagas diagnostic tests, held from 2 to 3 July 2007, in Geneva. The participants of the meeting elected Prof. Luquetti (Brazil), Dr Otani (Brazil) and Dr Guzman (Mexico) to coordinate this project. NIBSC agreed to process the batches of the candidate materials. The report of this meeting is available at the website [http://www.who.int/bloodproducts/ref\\_materials/WHO\\_Report\\_1st\\_Chagas\\_BRP\\_consultation\\_7-2007\\_final.pdf](http://www.who.int/bloodproducts/ref_materials/WHO_Report_1st_Chagas_BRP_consultation_7-2007_final.pdf). The ECBS endorsed the proposal for the development of a WHO Reference Panel in October 2007. The 2<sup>nd</sup> Consultation was held in Geneva from 27-28 January 2009. Participants included the coordinating group, regulatory authorities, reference laboratories, diagnostic laboratories, blood banks, organizations representing *in vitro* diagnostic tests manufacturers and WHO. The outcome of the meeting was to define the criteria for the proposed candidate materials and setting future time-lines for the project.

The following recommendations were proposed:

- Need for sera from two geographical origins: territories where *T. cruzi* group I and *T. cruzi* group II are predominant: Samples from Mexico and Brazil were selected to represent *T. cruzi* I (TCI) and *T. cruzi* II (TCII) respectively.
- De-fibrinated plasma from blood donors will be used as source material.
- Pools of 2-3 samples for each of the above *T. cruzi* groups will be considered
- Pilot study will be performed to determine the antibody titer of the selected samples before proceeding for the collaborative study (CS).
- Antibody level of the two candidate materials selected should be at the lower positive range so that no positive results are obtained when used with poor sensitivity tests.
- Effect of lyophilisation on the antibody titer of the samples will be determined.
- Completion of the WHO collaborative study is planned in 2009-2010. The study will include using large number of tests and various technologies such as EIA, IHA, IFA, RIPA, IB, TESA- Blot, rapid, supplemental tests etc. The tests used will be commercially available or licensed, no in-house tests will be used for validation. The CS will represent labs from both endemic and non-endemic countries, as well as both diagnostic labs and blood banks.
- Intended use: quality control (analytical sensitivity) of anti- *T. cruzi* diagnostic tests.
- Batch size approx. 3000 freeze-dried ampoules per panel member, 1 ml/ampoule (August 2009).
- The Report of the WHO collaborative study is expected to be finalized for submission to the Expert Committee at their annual meeting in October 2010.

### 3.2. *Plasmodium* ssp. reference preparations

*Plasmodium* species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*) are the causative agent for malaria disease. Half of the world's population is at risk of malaria, and there were an estimated 247 million cases of malaria and 881 000 deaths from the disease in 2006, mostly among children in Africa, making it one of the world's leading killers.<sup>1</sup> *P. falciparum* and *P. vivax* are the most common and *P. falciparum* is the most deadly.

NIBSC presented the proposal to develop an International Standard for anti-*Plasmodium* antibodies used to investigate the efficacy of vaccines. The proposal will be submitted to ECBS in 2009. High titre multivalent serum is to be used and assessed reactivity against a range of individual antigens of interest in vaccine development. Serum samples from African blood donors will be sourced through an international project in Kyushu, Kenya.

Currently NIBSC has no intention to establish reference materials fit for diagnostic purposes. Nevertheless there are activities initiated and led by CBER to develop an anti-*Plasmodium* ssp. reference panel preferentially used for blood screening purposes. Different recombinant antigens were used in ELISA studies with three lyophilized samples containing the serum or plasma from humans with malaria infections provided by NIBSC. Two of the samples were from *P. falciparum* and one from *P. vivax* exposed individual. In summary, blood stage *P. falciparum* antigens MSP1<sub>42</sub> and AMA-1 were recognized by the *P. falciparum* infected samples, but not by the *P. vivax* infected sample. Reactivity of the *P. falciparum* infected samples with the recombinant antigen CSP from *P. malariae* suggests mixed infections in the samples.

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<sup>1</sup> World Malaria Report; Geneva, UNICEF/World Health Organization, 2008

Combination of antigens from *P. falciparum*, *P. vivax*, and *P. malariae* provides a broad recognition and higher sensitivity to detect multi-species infections. Although there is no proposal yet for an international reference panel to be submitted to ECBS, further activities in this area are ongoing. NIBSC will provide further malaria positive samples for the ELISA studies at CBER. Both CCs want to discuss the scope of their collaboration in the development of a WHO reference panel for anti-*Plasmodium spec* antibodies. Additionally, CDC, NIH and the US Department of Defence will collaborate in this project.

Dr Cunningham from WHO gave a presentation about malaria diagnosis and quality assurance of rapid diagnostic tests. The program implemented for the testing of malaria diagnostic products under the coordination of WHO and partners provides an independent assessment of the quality of rapid diagnostic tests. The development of a recombinant antigen reference panel to evaluate the performance and stability of the rapid IVDs is planned for the future. The CCs will discuss possible ways to support this project.

### **3.3. *Anti-Babesia antibody reference panel***

Babesiosis, a malaria-like illness, is caused by infection of erythrocytes by several species of protozoan parasites of the *Babesia* genus. *Babesia* infections are endemic in several parts of the United States. In these areas, *Babesia* infected blood donors, especially asymptomatic carriers, present a serious risk of transfusion-transmitted infections. Therefore CBER has an interest for the development of an anti-*Babesia* antibody reference panel. Dr Kumar described the ways how the panel can be prepared. The collection of suitable serum or plasma samples will be done in collaboration with Dr Krause from Yale University. The next step will be the production of recombinant proteins to develop suitable ELISA tests. The WHO CCs agreed that further consultation is needed to determine the global need.

### **3.4. *Anti-Toxoplasma immunoglobulin standard and Toxoplasma gondii DNA international standard***

Toxoplasmosis is an infectious disease caused by the parasitic protozoan *Toxoplasma gondii*. It is endemic worldwide and, depending on geographical location, 15 to 90% of the human population are asymptotically infected. Routine diagnosis is carried out by serology. However, the parasite is a major opportunistic pathogen of immunocompromised patients. Also, if pregnant women develop a primary infection it can be transmitted to the fetus and cause serious damage. For these two subpopulations rapid and accurate diagnosis is required for treatment.

Dr Rijpkema gave an overview of the existing anti-*Toxoplasma* antibody standards. The 1<sup>st</sup> IS was already established in 1968 and was replaced by the 2<sup>nd</sup> IS (TOXS) in 1980. The 3<sup>rd</sup> IS (TOXM), established by the ECBS in 1994, is an anti-*Toxoplasma* serum containing both high levels of IgM as well as IgG, which make the standardization of assays detecting IgG only or IgM/IgG difficult. In 2003, the 1<sup>st</sup> IS for anti-*Toxoplasma* IgG (01/600) was established. Also in 2003 the ECBS recommended to develop a standard preparation with calibrated IgM and IgG levels to replace TOXM). The usefulness of an IgG/IgM standard preparation was discussed. For diagnostic purposes IgG is the most relevant parameter and hence an IgG standard is most important. Nevertheless, the WHO CCs agreed to make proposal for a separate IgM only standard, because currently the technical know how is available to prepare this type of standard material. A technical consultation group will be formed to make efforts in creating a subset of IgM-purified preparations. The need of an IgM standard should also be discussed with the kit manufacturers. Candidate materials from the UK reference centre are available. Also discussions with Center for Devices and Radiological Health (CDRH)/FDA and CDC would be helpful to

clarify the availability of other materials. A proposal for an IgM only standard will be submitted to the ECBS in 2009.

Dr Anderson presented the proposal for the development of an IS for *Toxoplasma gondii* NAT assays. Serological diagnosis is not always reliable as reactivation is not always accompanied by changes in antibody levels. The presence of IgM does not necessarily indicate recent infection either. For these reasons PCR is frequently used in the diagnosis of *Toxoplasma gondii* in these at-risk patients. PCR has been found to be sensitive, specific and a rapid method for the detection of *Toxoplasma gondii*. In the UK, approximately 2000 patients a year are tested for *Toxoplasma gondii* and in Europe at least 20,000 patients each year are tested for *Toxoplasma gondii*. Testing is also carried out heavily in other countries throughout the world. However no international reference materials exist so far for the detection of *Toxoplasma gondii* DNA by NAT assays. Several organizations (European Food Standards Agency, Royal College of Veterinary Surgeons and the Toxoplasma agency in UK) have recently expressed the need for standardization of NAT assays for detection of *Toxoplasma gondii* DNA. The candidate material (RH strain) was grown in mice, purified and inactivated (Dr Guy, HPA Toxoplasma Reference Laboratory, Swansea). Two bulk materials have been diluted in Tris buffer containing human serum albumin at NIBSC. For final formulation the bulk materials were pooled and further diluted with 10 mM Tris buffer containing 30 mg/ml Trehalose. 900 ml will be freeze-dried in 0.5 ml quantities yielding 1800 vials. A collaborative study will be initiated to evaluate the candidate freeze-dried material along with some frozen samples of the most common strains. The participants of the WHO CCs meeting agreed to submit the proposal for the development of the 1<sup>st</sup> IS for *Toxoplasma gondii* DNA to the ECBS in October 2009.

## **4. HEPATITIS VIRUSES SESSION**

### **4.1. *HBV epidemiology***

Dr Lavanchy gave an overview about the global HBV epidemiology, disease burden and treatment. He explained the WHO strategic plan of measures for current and emerging prevention, control and elimination of the disease worldwide.

### **4.2. *HBV genotype reference panel***

The current WHO IS materials for HBV DNA and HBsAg, both originate from genotype A2/HBsAg subtype *adw2*, which is mainly prevalent in Western Europe and in North America, representing only 1% of the worldwide HBV-infected population. The majority of the HBV-infected people coming from the Mediterranean area, Africa and Asia were the genotypes A1, E, B, C, and D are the most prevalent ones. During the 'WHO Consultation on Global Measurement Standards and their use in the in vitro Biological Diagnostic Field' in June 2004 concern was raised that HBsAg test kits and NAT test kits for the detection of HBV DNA might be less efficient for some HBV genotypes other than A2, which is represented by the current IS preparations. The PEI proposed projects to establish WHO International Reference Panels for HBV DNA and for HBsAg representing different genotypes of HBV. The projects were endorsed and assigned as a high priority by the WHO Expert Committee on Biological Standardization in October 2005. Dr Chudy presented an update of the development of the HBV genotype reference panels. HBsAg and HBV DNA high titre plasma units were collected worldwide. Two hundred and fifteen potential candidate materials were further characterized.

The proposed HBV genotype panel intended for use with HBV NAT assays consists of 15 samples and covers the most prevalent HBV genotypes (A-G). Unfortunately, at the time of preparing the panel, no genotype H material was available. The HBV DNA concentration of each sample was determined by four different NAT assays. For the preparation of the bulk materials, 12 samples were diluted with a negative plasma pool to a final HBV DNA concentration of approximately  $10^6$  IU/ml in a volume of each 1.2 litre. The volume and the titre of the original samples for 6/B, 14/F, and 15/G allowed only a dilution to a final concentration of  $10^4$  IU/ml,  $10^5$  IU/ml, and  $10^4$  IU/ml, respectively. The plasma diluent was negative tested for the following markers: HIV-1 RNA, HCV RNA, HBV DNA, HBsAg, anti-HBs, anti-HBc, anti-HIV-1/2, and anti-HCV. The filling and lyophilization were performed by a certified company. The filling volume per vial was 0.5 ml. Overall 30,000 vials, 2,000 vials per each panel sample, were produced. The initial stability results and the residual moisture content determined indicate that these preparations are suitable for long term use. The collaborative study was initiated to test the panel samples (15 lyophilized preparations) concurrently with the WHO IS (97/750). Where possible, laboratories were encouraged to use quantitative methods and the results are reported in IU of HBV DNA/ml. Nineteen laboratories (national control laboratories, diagnostic laboratories, and kit manufacturers) from 13 different countries were invited to participate in the study. The study will be completed by the end of May, 2009. The study report will be submitted to ECBS at the end of July, 2009.

### **4.3. HBsAg**

The summary about the current HBsAg BRPs was given by Dr Minor presented on behalf of Dr Ferguson. Currently the 2<sup>nd</sup> IS (00/588) and the HBsAg reference panel (03/262) in place which represent genotype A2 and subtype *adw2*. The IS material was prepared by Sanquin from plasma derived HBsAg vaccine. HBsAg was purified by PEG precipitation and ultracentrifugation to remove Dane particles and inactivated by heating at 101°C-103°C for 90 seconds, followed by pasteurization at 65°C for 10 hours. The IS has an assigned unitage of 33 IU/vial. Currently 1461 vials are available. About 100 vials were shipped in 2008.

The panel comprises a series of fourfold dilutions of the IS and a negative control. The distribution of this panel aimed at users who may not have appropriate diluent with which to dilute the IS. Currently 1915 of the 2000 prepared panels are available. Although the current figures of the availability of these materials predict their use for at least some years the replacement of the BRPs is difficult since no further such material is available. This issue should be discussed by the collaborating centres to ensure that in future a solution for the establishment of the replacement preparations is found.

The participants agreed that further discussion is needed between the CCs, CLB, Prof Gerlich (Institute of Medical Virology, Justus Liebig University Giessen, Germany) and other interested parties to identify appropriate source materials for the replacement. NIBSC will check the availability of some material from Vietnam which is comparable to the current BRPs.

### **4.4. HCV RNA genotype panel**

Dr Anderson explained the need for an international reference panel for HCV RNA genotypes. Mandatory NAT testing for HCV RNA of plasma pools for the manufacturing of plasma derivatives and of blood donations in transfusion medicine was introduced by many countries for several years. An important requirement of HCV NAT is the ability to detect the six major genotypes with equal sensitivity. The NIBSC HCV genotype panel (Code 02/202) comprises the

genotypes 1-6 based on HCV positive plasma units. Sufficient stocks held at NIBSC to prepare a new panel with sufficient vials. NIBSC proposed a new collaborative study for the re-filled panel.

Although there was consensus by the group at the 1<sup>st</sup> CCs meeting in January 2007, that there is no priority for the development of such a reference panel, after re-discussion the experts now agreed that 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. For this purpose a proposal for the development of an HCV RNA Genotype Reference Panel should be submitted to the ECBS in October 2009.

#### **4.5. *Anti-HCV monospecific antibodies and HCV core antigen***

Dr Nick summarized briefly the situation regarding the project for the development of an anti-HCV monospecific antibody panel. So far, no progress was made. At the present time, no further activities will be done to continue this project. Since HCV core antigen tests as well HCV antigen/anti-HCV combination assays are becoming available. Dr Nick outlined that there is an interest and need for the development an international BRP for HCV core antigen. This standard preparation would be very useful for estimating the analytical sensitivity of these assays. It may serve for calibration of diagnostic kits by manufacturers and for calibration of secondary standards and used for the control of the batch consistency of the test kits by national control laboratories worldwide. HCV antigen-positive human plasma (ideally of HCV genotype 1) which is available at PEI could serve as candidate material. In a feasibility study this material should be further characterized in terms of the antigen content, HCV genotype and stability after lyophilisation. Once this study is approved by the participants and if the results confirm the suitability of the preparation, a collaborative study involving reference laboratories and test kit manufacturers (5 to 10 laboratories) will be initiated. The outcome will be the unitage assignment of this preparation and to propose it as the 1<sup>st</sup> IS.

The group agreed to submit the proposal for the development of the 1<sup>st</sup> IS for HCV Core Antigen to ECBS in October 2009 for endorsement.

#### **4.6. *HDV RNA***

Dr Kress presented a proposal for the establishment of the 1<sup>st</sup> International Standard for hepatitis D virus (HDV) RNA. HDV is highly endemic in several African countries, the northern parts of South America, the Mediterranean region, and in the Middle East. HDV can be acquired either as a co-infection with HBV or as a super-infection of persons with chronic HBV. 70 to 80% of super-infected HDV patients develop evidence of chronic liver disease with cirrhosis. A major problem in the treatment of chronic hepatitis D is monitoring the response to therapy because no standardized assays for the assessment of HDV viraemia are available. The HDV RNA International Standard will be used by clinical diagnostic laboratories, IVD manufacturers and NCLs for the development of standardized NAT assays and their calibration and, for the calibration of secondary and working standards used in quality control and quality assurance of NAT assays.

The HDV RNA-positive plasma samples (HDV-1) will be provided by the Institute of Hepatology, Ankara University, Turkey. If the characterization of these samples reveals suitable antibody titres, it will also be possible to establish an international anti-HDV antibody standard material.

The group agreed that such a preparation would be relevant especially to clinical microbiology laboratories. The project proposal will be submitted to ECBS in October 2009 for endorsement.

#### **4.7. HEV RNA**

Dr Baylis presented a proposal to develop standards and reference panels for hepatitis E virus (HEV) RNA for NAT-based assays. Together with hepatitis A virus (HAV), HEV is a major cause of acute hepatitis worldwide and appears to be an emerging infection. The virus is transmitted mainly via the faecal-oral route and is associated with large waterborne epidemics of hepatitis in the developing world. The consequences of HEV infection may be severe in pregnant women and individuals with underlying liver disease, with increasing evidence of persistent infection in immunocompromised patients. In industrialized countries HEV infection is usually associated with travel to endemic regions, however autochthonous infections are being reported more frequently in Europe, Japan and elsewhere. Phylogenetic analysis of full length strains of HEV has identified four main genotypes capable of infecting humans. Genotype 1 has been found in people throughout Asia and in other parts of the world. In Mexico, a second genotype has been reported. Viruses belonging to genotypes 3 and 4 been detected not only in humans but also in swine and other animal species such as wild boar and deer, with sequence analysis demonstrating that viruses originating from the same geographic regions are genetically very similar to one another regardless of whether they have been found in humans or animals. In the clinical setting it is widely recommended to test for HEV, when other causes of acute hepatitis have been eliminated.

Transmission of HEV by transfusion has been reported in several countries, and the effect on the recipient may be severe. Studies on the inactivation of HEV demonstrate that it is rather more resistant to virus inactivation procedures and may pose a risk in plasma-derived products that undergo very limited virus inactivation/removal steps such as solvent/detergent treated plasma. In the Hokkaido district in Japan, NAT testing has been introduced and approximately 1 in 8300 donations is positive for HEV RNA. ALT testing may act as a surrogate marker for HEV infection, but such testing is not consistently implemented and ALT levels may not always be elevated in cases of HEV infection. It was agreed to present the proposal at SoGAT (Standardization of Genomic Amplification Techniques) meeting in Brussels in May 2009 and to seek feedback from participants at the meeting. Dr Baylis described the availability and characterization of candidate HEV positive samples and presented the proposal to perform an initial study to investigate assays performance and evaluate a panel of materials as candidate standards. Data were also presented demonstrating the detection of HEV RNA (genotypes 3 and 4) in plasma fractionation pools from Europe, North America and South East Asia. This proposal was well received and the development of reference materials should be pursued to enable laboratories to set up testing for this virus as necessary.

The participants agreed that further studies are needed which demonstrate the relevance for blood safety. As this topic is on the agenda of the SoGAT meeting 2009 in Brussels, additional information from the discussion should be taken in consideration for the project proposal.

#### **5. PARVOVIRUS B19 DNA GENOTYPE PANEL**

Dr Baylis together with Dr Yu gave an update on the evaluation of a genotype panel for parvovirus B19. The panel members were prepared from window-period plasma donations positive for genotypes 1, 2 and 3 a. A negative plasma control member has also been included in the panel. Preliminary testing on the stock materials was performed at CBER/FDA, PEI, Talecris

Biotherapeutics and the National Genetics Institute. The positive panel members were diluted to approximately  $10^6$  IU/ml with the negative plasma that had tested negative for parvovirus B19 DNA and anti-B19 antibodies and a range of other blood borne virus markers. Panels have been distributed to 35 laboratories for evaluation and testing in parallel with the 2<sup>nd</sup> International Standard for parvovirus B19 DNA (99/802). Participating laboratories were asked to confirm that their assays targeted conserved regions of the parvovirus B19 genome, prior to their enrolment into the study. Preliminary data were presented concerning the statistical analysis of the study performed by Alan Heath (NIBSC). The majority of laboratories returned quantitative data by using real-time PCR assays mainly in-house developed assays, although some data were returned from commercially available tests or tests under development. There was a very good agreement by all laboratories for the determination of the absolute estimates in IU/ml for 99/802 and the genotype 1 panel member. In the case of the genotype 2 and 3a panel members the results were a little more variable reflected in a slightly wider range of reported values. There were very few outlying results, and the preliminary potency estimates agreed well with the target concentrations. The panel has been prepared as liquid-frozen and on-going stability studies are in progress to determine the real-time stability of the panel members. Additional stability studies include the analysis of two liquid/frozen plasma preparations of parvovirus B19 which were formulated approximately 10 years ago and submitted as candidate materials when the 1<sup>st</sup> International Standard (99/800) was evaluated in a collaborative study. Once the report is finalized it will be distributed to the participants for comment and the results of the study will be presented at the SoGAT meeting in Brussels in May 2009.

The group agreed to submit the proposal for establishment of the 1<sup>st</sup> International Reference Panel for parvovirus B19 DNA at the next meeting of ECBS in October 2009.

## 6. BACTERIA

### 6.1. *Bacterial antisera reference preparations for serotyping*

Dr Fuchs gave a presentation about the WHO/IHR<sup>2</sup> laboratory strengthening programme for national microbiology reference laboratories. This presentation was held in the parasite session because of the reorganization of the program. Dr Fuchs outlined that the main focus is on the support of the establishment of external quality assessment programmes for the diagnosis of communicable diseases (enteric bacterial pathogens, meningeal bacterial pathogens, plague, and malaria and tuberculosis microscopy) in Africa and the Eastern Mediterranean Region. These programmes allow the identification of major diagnostic failures. First study results demonstrated that the quality of microscopy is acceptable, whereas the quality of culture and biochemical identification is poor and the performance of serotyping (esp. *Haemophilus. influenzae*) and antibiogram (meningeal germs for beta-lactamins) is insufficient. There is an urgent need for the establishment of bacterial antisera reference materials preferentially used for serotyping. The use of comparable commercially available reagents is hampered due to financial reasons. Dr Fuchs explained the possibilities of how to fill the gap of bacterial antisera suitable and available for diagnostics in these regions. WHO should facilitate the emergence of a cost-effective supply center devoted first to the neglected bacterial diseases (e. g. academic or institutional and/or in partnership with private).

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<sup>2</sup> IHR – International Health Regulations

During the discussion the group agreed that there is an urgent need for the establishment of such reference sera. The CCs are willing to support in characterization of candidate materials, in particular NIBSC and PEI.

## **6.2. Blood-borne bacteria panel**

Dr Montag presented a project for the development of panel of blood-borne bacteria which could serve as an international reference panel. The transfusion of blood components containing high counts of bacteria and bacterial toxins may lead to immediate septic shock, multiorgan failure and, not infrequently, to the death of the recipient. The mortality rates for platelet related sepsis range from 1 in 20,000 to 1 in 100,000 donor exposures, the contamination rate of platelet concentrates reaches from 0.16% up to 0.6% at the end of their shelf life. Currently, no international standards exist for investigation of blood component-related bacterial contamination. During the meeting of the Subgroup Bacteria Working Party Transfusion-Transmitted Infectious Diseases (WP-TTID) of the International Society of Blood Transfusion in Macao, 2008, it was decided to organize an international collaborative study to evaluate different blood-borne bacterial strains. Bacterial strains have been selected and characterized regarding their ability to multiply in platelet concentrates up to  $10^8$  to  $10^9$  colony forming units (CFU)/ml. This property of the bacterial strains has been confirmed in platelets from at least 100 different donors in order to exclude antimicrobial effects of donor's host defence. A novel procedure has been developed to manufacture these bacterial strains as deep frozen suspensions consisting of living microbial cells only. They are stable over at least one year and can be shipped on dry ice. After thawing, the bacteria are ready to use and can be used immediately for spiking of blood components (10 CFU per bag corresponding to 0.03 CFU/ml). The following strains were included in the study: *Staphylococcus epidermidis* (PEI-B-06-08), *Streptococcus pyogenes* (PEI-B-20-06), *Escherichia coli* (PEI-B-19-06) and *Klebsiella pneumoniae* (PEI-B-08-10). The deep frozen samples were blinded and sent on dry ice. Thirteen laboratories from 10 different countries participated in the study. According to the study protocol the following experiments were to be performed: i) identification of the bacterial species in the sample; ii) estimation of the bacterial count (CFU/ml) and iii) investigation of the ability of the strains to grow in platelet concentrates after spiking with both 0.3 and 0.03 CFU/ml. The outcome of the study demonstrated that the bacterial strains investigated are suitable materials for use as a reference panel. The panel is intended for the development, validation and comparison of methods for both bacteria screening and pathogen reduction.

In the discussion it was confirmed that there is a need to ensure the bacterial safety of blood components. Available reference materials used for test validation can contribute to the safety of blood components. The participants supported the PEI proposal to be submitted to ECBS in October 2009.

## **7. HERPESVIRUSES / POLYOMAVIRUSES**

### **7.1. Anti-CMV**

Dr Minor summarized the past activities for establishing a WHO anti- cytomegalovirus (CMV) immunoglobulin standard (presented on behalf of Dr Ferguson). The preparation developed 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). No

further progress has been made since the last CCs meeting in January 2007. This topic is again on the agenda of the 2<sup>nd</sup> CCs meeting for re-discussion.

Dr Gross presented the PEI activities in the control of anti-CMV immunoglobulin preparations. CMV is widespread in the population, but infections are rarely problematic in immunocompetent people. Anti-CMV immunoglobulin is used for treatment of the following clinical syndromes: CMV inclusion disease of the newborn, acute acquired CMV infection and CMV infection in immunocompromised patients (e.g. transplant recipients). The PEI performs batch release for several anti-CMV immunoglobulin preparations. Currently only two products from one manufacturer are approved in Germany. Batches release testing is performed on 8-12 lots per year. For CMV immunoglobulins no specific Pharmeuropa Monograph or generally accepted protocols for determination of anti-CMV antibody titres in immunoglobulins are available. Furthermore, because there is currently no international standard for CMV immunoglobulin products available, the recommended dosages remain empirical. Because the 1<sup>st</sup> PEI Reference Preparation for CMV Immunoglobulin (98 U/ml, manufactured in 1988) was nearly exhausted, the 2<sup>nd</sup> PEI Reference Preparation for CMV Immunoglobulin (potency of 110 U/ml, manufactured in 1996) was established during the validation of the serological test. This reference preparation is widely used and will be distributed to other laboratories upon request. The number of requests varies between 8 to 12 vials per year. Taking into account the number of tests for batch release and the number of shipped vials per year, the current reference preparation is sufficient for several years. From the PEI view there is no urgent need for the establishment of a new reference preparation used for the control of immunoglobulin preparations.

Nevertheless, the CC participants see a need for such standards, e. g. for clinical studies and for diagnostic purposes in the transplantation field, as well as for blood products transfused to CMV-susceptible individuals (e.g. neonates, pregnant woman, immunosuppressed patients), for allergenic cord blood stem cells, and for different tissue preparations. For diagnostic purposes it was suggested that separate standards for IgG and IgM should be developed since current available material is mostly IgG. An immunoglobulin preparation can be a suitable candidate material for IgG BRP. Such material is already available. Possible source materials for IgM can potentially be obtained from industry. WHO will contact the European Diagnostic Manufacturers Association to investigate the manufacturer priorities. CBER will discuss with CDRH and the Advanced Medical Technology Association about the availability of such materials. PEI will provide the outcome of the discussion to the IVD Technical Group at the European Commission for potential inclusion of this marker in the new Common Technical Specifications of the IVD Directive. .

## **7.2. CMV DNA**

The rationale for the establishment of an IS for human CMV DNA was explained by Dr Fryer. This topic was already discussed in the SoGAT-Clinical Diagnostic group which was established to coordinate the development of reference materials for NAT-based assays for clinically relevant pathogens in 2008. The project for the establishment of an IS for CMV DNA was endorsed by the ECBS in 2008. Candidate material is the strain Merlin propagated in cell culture. Five thousand vials will be filled with a virus concentration of about 1.00E+07 copies/ml in May/June 2009. The collaborative study will evaluate this material in parallel with strain AD169 and purified DNA from the Merlin strain. The report will be submitted to ECBS in 2010.

### **7.3. EBV DNA**

The establishment of an IS for Epstein-Barr virus (EBV) DNA is to be seen in the same line as for CMV DNA. This NIBSC project, presented by Dr Fryer, was also endorsed by the ECBS in 2008. As candidate material serves the strain B95-8 which was propagated in cell-culture. Five thousand vials will be filled with a virus concentration of about  $1.00E+07$  copies/ml in May/June 2009. The collaborative study will be designed to evaluate the candidate material alongside with three liquid preparations, B95-8 bulk material, EBV-positive Namalwa cells, and EBV-positive Raji cells. About 30 participants coming from clinical and research laboratories as well as from manufactures will be invited to represent a broad range of NAT assays. The report will be submitted to ECBS in 2010.

### **7.4. JC virus DNA and BK virus DNA**

NIBSC is interested in the development of reference materials for further pathogens which play a role in clinical diagnostics. Dr Fryer presented two further projects of reference materials for polyoma viruses. JC and BK viruses are ubiquitous, which can cause persistent infection. JC virus is responsible for progressive multifocal leucoencephalopathy, a severe demyelinating disease of the central nervous system. It affects individuals with impaired cell-mediated immunity (e. g. AIDS patients and transplant recipients). BK virus can cause polyoma-associated nephropathy in kidney transplant recipients. The diagnosis of these diseases relies on NAT, esp. on viral load measurements. Most assays are in-house developed and needed standardization. Several EQA studies demonstrate significant degree of variation in both detection and quantification of JC and BK viruses. Source materials for candidate IS preparations could be either whole virus or viral episomes.

The CCs participants agreed that a general discussion is needed before the proposals are submitted to the ECBS. The priorities for global reference materials in clinical diagnostics should be further discussed. . Additional information and input from other interested parties would be helpful, e. g. outcome of the SoGAT-Clinical Diagnostic meeting September 2009, Istanbul.

## **8. TSE BLOOD REFERENCE PREPARATIONS**

Dr Minor pointed out that there are three main questions should be addressed regarding transmissible spongiform encephalopathies (TSE) blood screening tests: i) how good is the test at detecting the PrPsc marker in terms of sensitivity and specificity?; ii) how relevant is the marker, i.e. at predicting whether the unit is infectious or the donor is infected?; and iii) what is the consequence for the donor/patient, i.e. does the marker predict eventual disease? The calculation of the assay sensitivity based on assumptions of the infectivity in blood of approximately 1-10 IU/ml and of the infectivity in brain which is  $10^6$  - $10^8$  IU/g then a 10% homogenate contains 100,000 to 10,000,000 infectious units per ml. An assay must be able to detect a  $10^{-4}$  or  $10^{-7}$  dilution of a 10% brain homogenate. To cover these unresolved questions Dr Minor presented study results of the Amorfix test (Canadian manufacturer) performance on a panel of plasma samples spiked CJD uninfected and CJD infected brain materials, blood samples from uninfected sheep and scrapie infected sheep materials. There was an extensive overlap of results from samples from clinically affected and clinically uninfected animals. Additionally, the test was affected by the nature of the samples, e. g. anticoagulant (ETA or citrate), freeze-thawing. The overall outcome of the study was that clinically affected and clinically unaffected animals could be distinguished as groups, but not as individuals; but that a better differentiation between affected and unaffected individuals is needed. The specificity was determined in about 1,000

plasma samples from UK blood donors (0.5% repeat-reactive (RR) samples). In a study done in France 6 RR in 10,000 donors were observed, but were mostly false-positive.

Dr Epstein explained the reasons to consider the establishing of WHO TSE Blood Biological Reference Materials. In this regard the maximum duration of infectivity in blood of persons with pre-clinical/sub-clinical vCJD as well as the prevalence of pre-clinical/sub-clinical vCJD in various countries is not known. Consequently, the long-term risk of transfusion-transmitted vCJD for donors exposed to BSE agents in various countries is not known. Antemortem testing to detect either TSE agent in blood or other evidence of pre-clinical/sub-clinical TSE would help to reduce risk of transfusion-transmitted vCJD (and, possibly, other forms of CJD) without wasteful deferral of otherwise suitable donors. TSE Blood Biological Reference Preparations could be used as for the validation of candidate blood-based TSE diagnostic and screening tests by test kit manufacturers and regulators, for the validation of devices purported to remove TSE agents from blood components, and for studies of the properties of blood-borne TSE infectivity. Possible candidate materials for the TSE BRPs could be non-blood-derived spiking material (already existing WHO reference materials of sporadic CJD and variant CJD human brain suspensions and control uninfected suspension). Alternatively, TSE-infected tissue rich in blood cells (e.g., spleen) could serve as blood surrogate. NIBSC has already prepared suspensions of spleens from CJD cases as possible UK CJD reference materials. If available, TSE-infected blood and/or blood components (animal or human origin) are the favored option for the preparation of BRPs, but their available is very limited. There are some possible reasons why WHO TSE BRPs are currently being not developed: i) reduced resources (funding) available to address public health issues related to TSEs; ii) probable failures in early efforts to validate several candidate blood-based TSE tests (limited current demand for reference materials); and iii) projected market for human TSE tests (blood-based or tissue-based) is probably limited. CBER solicits the support for resuming WHO activity to establish TSE BRPs.

Regulatory developments for vCJD screening tests in Europe were outlined by Dr Nübling. Currently vCJD tests may be self-certified in Europe. This means that the manufacturer may approve (CE mark) a vCJD test for the European market without intervention of a third party (notified body). However, since these devices would potentially be used analogous to virus blood screening devices, there are currently attempts to classify these tests "high-risk" IVDs (like e.g. anti-HIV-1/2 assays). In anticipation of this classification by the European Commission there is already the exercise to define product-specific minimal requirements for product quality and product evaluation (CTS). An update of this effort was presented. A challenge for potential practical use of the assay(s) in pipeline for blood screening is the lack of a confirmatory assay, leaving the blood centre with reactive test results without final resolution. This again poses ethical issues about how to deal with vCJD reactive blood donations and corresponding donors.

Although there will be no funding by WHO, possibilities should be identified as to how WHO can support the project. The participants of the meeting suggested that the discussion about the development of suitable TSE blood reference preparations (vCJD panel) should be continued in future WHO meetings and manufacturers should be encouraged to develop appropriate diagnostic tests. It was agreed that future reference materials should cover all kind of diagnostic tests. The way to identify suitable human blood samples as potential candidate material was discussed. Materials from sporadic CJD cases could be an option, but the specimens have usually a low infectivity. Repeat reactive samples (in at least two or three different tests) from the blood donor study could be an appropriate material for the panel. A further discussion should focus on the barriers to secure infectious human plasma. The WHO CCs will work on a protocol which should be developed for appropriate collecting and storage of blood specimens containing potentially the vCJD agent both from infected humans as well as animals.

## **9. RETROVIRUSES AND HUMAN HERPESVIRUS TYPE 8**

### **9.1. *HIV epidemiology***

An overview about the actual global data of HIV epidemiology was presented by Dr Osmanov. In 2007 about 33 million people were living with HIV worldwide. In the same year there were 2.7 million newly HIV-infected persons and a total of 2 million AIDS deaths. More than 96% of the new infections are in low and middle income countries. Molecular epidemiology of HIV-1 is characterized by increasingly complex patterns with a clear shift from pure subtypes to recombinant strains. Relevance of genetic subtypes and circulating recombinant forms (CRFs) of HIV-1 for vaccine protection needs to be critically assessed by appropriately designed vaccine efficacy trials. The need for the development of appropriate reference reagents was emphasized to ensure highly effective diagnostics for the prevention of the infection and for the control of the disease.

### **9.2. *HIV molecular epidemiology. Update of the 1st International Reference Panel for HIV-1 RNA Genotypes***

Dr Hewlett summarized the historical timeline and key milestones in HIV-1 molecular epidemiology. CRFs were detected in the latter half of the 1990's and unique recombinant forms could be identified preferentially in the African continent. There was an increase in new recombinants in the last couple of years. In 2007 14 new CRFs were assigned. Some of the unusual subtypes and CRFs are important pandemic strains. Several publications describe an impact of HIV genetic diversity on diagnosis. It is not known whether current NAT kits can detect all subtypes and CRFs with equal sensitivity. CBER has developed a national HIV-1 RNA subtype panel currently in use for lot release (subtypes A-G, groups O, N, virus isolates spiked in negative plasma). A further panel for CRF02\_AG and CRF01\_AE has been characterized and will be formulated in the future. Dr Hewlett presented an outline of an international project in close cooperation with the National Institute of Health (NIH) that is being planned in the US to study HIV-1 diversity and to develop a repository panel with HIV variants for future reference panel development.

It was already agreed in the 1<sup>st</sup> CCs meeting in January 2007 that CBER and NIBSC would collaborate in identifying useful subtypes and CRFs for the extension of the 1<sup>st</sup> Reference Panel. Dr Holmes presented an update of this project. Currently the following well characterized strains and CRFs are available as possible candidate materials: subtypes A, C, G, H, J, K, group O virus, CRF01\_AE, CRF02\_AG, CRF07\_BC, CRF11\_A01GJ, CRF13\_A01GJU, and CRF14\_BG. Viruses are being grown in PBMC cultures. Stocks of clarified culture supernatant are being stored as 1ml aliquots under liquid nitrogen vapour. Following previous discussions at the 1<sup>st</sup> WHO CC meeting, viruses will be heat inactivated according to the CBER procedure (60°C / 60 minutes). Freeze-drying will be undertaken at NIBSC. Due to logistic reasons it is proposed to limit the panel size to 12 members (corresponding to 4 freeze-dry runs). An international collaborative study will be organized to evaluate the panel.

Further to discussion of the above proposal, CBER can provide further materials (rare CRFs) for updating of the HIV-1 RNA genotype panel. The group agreed on the proposed activities of this HIV project, with Dr Hewlett and Dr Holmes as the coordinators. Proposal should be submitted to ECBS in 2010 for endorsement.

### **9.3. *HIV-2 RNA International Standard***

Ms Morris gave an update about the development of the 1<sup>st</sup> IS for HIV-2 RNA, a project which was endorsed by the ECBS in 2006. In consultation with CBER, two subtype A strains, ROD and CAM 2, were identified. Both strains are well characterized including full length sequencing and grow well in the CEM cell line. Viral titre was determined to be  $>10^8$  copies/ml by in-house real-time LTR based assay. Viral stocks were heat inactivated following the CBER protocol at 60°C for 60 minutes. Efficacy of the inactivation procedure was controlled by p24 measurement after cultivation for 3 weeks. No p24 antigen could be detected. About 2500 vials of each virus were freeze-dried. Both strains were evaluated in a collaborative study by 33 laboratories. The data will be analysed by NIBSC. The report of the study and the proposal for establishment of the 1<sup>st</sup> IS for HIV-2 RNA will be submitted to ECBS in October 2009.

### **9.4. *Anti-HTLV I/II antibody reference panel***

The proposal for an anti-human T-cell lymphotropic virus (HTLV)-1/2 reference panel was already presented by Dr Cowan from CBER at the CCs meeting in January 2007. No progress has been made to date. This proposal was discussed again at the 2<sup>nd</sup> CCs meeting in Langen. CBER and NIBSC see a need for the development of a reference panel. NIBSC can provide candidate materials (HTLV I: two positive blood donor samples; HTLV II: positive pooled plasma) which could further characterized by different enzyme-immuno assays in a feasibility study (CBER, NIBSC, PEI and WHO Collaborating Centre for Quality Control of Serology in Blood Banks from Sao Paulo, Brazil). WHO will hold a conference call with the three CCs to discuss the study outcome and to coordinate the preparation of the proposal and work plan for the development of the Anti-HTLV-1/2 Reference Panel for submission to ECBS.

### **9.5. *Human herpes virus type 8 standard preparations***

Dr Hewlett informed the group about the situation with human herpes virus type 8 (HHV-8) transmissions through transfusion and by organ transplants. A better understanding in this area is needed because of the conflicting reports in the literature. At present there is no strong desire from the CCs to develop international reference materials. Nevertheless, CBER is active in this area and is trying to acquire candidate materials representing the different major subtypes. Argentina has shown interest in development of WHO BRPs and can also provide antibody-positive materials. CBER will contact specialists from Argentina for discussion regarding possible HHV-8 panel development. Also the development of large scale assays is needed for broader prevalence studies.

## **10. FLAVI-, ARTHROPOD-BORNE, HEMORRHAGIC FEVER VIRUSES**

### ***10.1. WNV reference panel***

Dr Rios explained the current situation on WNV screening by NAT in the US. These tests were developed to detect WNV RNA as the most promising tool for the identification of infectious blood donations. Standardization is an open issue for all kind of WNV assays. CBER has already prepared standard reagents which comprise of both a human isolate and stocks of the flamingo isolate NY99 (using CDC viral seed). The correlation of plaque forming unit (PFU) to copy number was determined to be 1 to 500 using intermediate dilutions. Heat treatment of the virus results in loss of infectivity as determined by PFU and 2 to 3 log<sub>10</sub> reduction of copy number as determined by TaqMan NAT. The formulated panel composed of 14 coded members (1000, 500, 100, 50, 10, 5 and 0 viral copies/ml, one from each isolate) and were distributed to 7 independent laboratories, and tested with 12 different assays. There was a great variability of results with low copy number panel members. The used isolates were produced and exist in large quantities. The stability of the liquid materials can be an issue at 4 °C but it is stable for up to 6 year at -20 °C and -80 °C, and stability at lyophilized stage is yet to be determined. The proposal by CBER is to extend the characterization of the materials by a collaborative study to develop a WHO BRP for WNV RNA.

A study on WNV and blood safety in Germany was presented by Dr Nübling. This study had been initiated to determine the prevalence and incidence for WNV in a healthy blood donor population in order to be able to estimate potential risks for WNV transmission via blood transfusion. A complex test algorithm was established and a confirmation assay (WNV Western Blot) developed. In summary, only very few (<0.1%) of blood donors were confirmed anti-WNV-positive, and no WNV-RNA positive donors were identified in this study. Some plasma pools from the US used for the manufacturing of plasma derivatives tested WNV-RNA positive, in contrast to pools from Europe and Asia. However, virus inactivation procedures are predicted to be effective against low viral load contamination in manufacturing pools.

At present NIBSC and PEI did not consider a priority in developing WHO BRP for WNV since the incidence of this infection is very low in Europe. However for further discussion of the above proposal, the PEI experts pointed out that the European Centre for Disease Control will hold a WNV meeting in May 2009. The outcome of this meeting will determine the global need of BRP for WNV RNA. WHO will initiate a discussion among the experts of the CCs. It was also suggested that the incidence among blood donors should be looked in high endemic areas during the outbreak of an epidemic. Also the sequence of viral strains during the 2008 outbreak in certain European countries should be studied for mutation since there has been possible link between virulence and mutations in WNV genome sequences in the US epidemics.

### ***10.2. DENV and the development biological reference panels***

Dr Drager from WHO presented an overview of the global epidemic situation of Dengue fever caused by Dengue virus (DENV). Dengue fever is the most rapidly spreading mosquito-borne viral disease in the world. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries. Nearly 75% of current global dengue burden is in the two WHO regions of South-East Asia and Western Pacific. Dr Drager explained the Asia Pacific Dengue Strategic Plan for both regions (2008-2015) which has been prepared in consultation with member states and development aid partners to reverse the rising

trend of epidemic dengue by enhancing their preparedness to detect, characterize and contain outbreaks rapidly and to stop the spread to new areas. The WHO representative pointed out that the interpretation of results of DENV diagnostic tests is sometime difficult due to different reasons. Differential diagnosis is made more difficult by cross-reactivity with other non-dengue flavivirus infections. Additionally, there is no regulation of diagnostics in many countries and no standardization of the tests exists. The Special programme for Research and Training in Tropical Diseases (TDR) developed a serum panel for the evaluation of commercial anti-DENV IgM tests and rapid tests. Furthermore, a short overview of the current efforts in the development of vaccines was presented.

Dr Rios highlighted the fact that DENV is a blood borne pathogen and the risk of transmission via blood transfusion has already been documented in two cases. The availability of a DENV BRP would facilitate the development and the quality control of new blood screening tests. Such assays tests would significantly improve the safety of the blood supply worldwide. CBER proposes the development of a BRP for DENV including the 4 serotypes for NAT assays. CBER has access to viral isolates acquired from infected humans through collaborators at CDC. The candidate materials include the 4 DENV serotypes and human isolates will be tested in an international collaborative study involving several laboratories who wish to participate.

PEI and NIBSC will support the WHO in characterization and testing of the serum panel. CBER proposal for the development of an International Reference Panel for DENV 1-4 RNA was accepted by the participants. The proposal will be submitted to ECBS in 2010, but CBER will initiate the work in 2009. The WHO Global Alert and Response has an interest in NAT technology as a tool for better differential diagnosis of DENV and for early detection of the disease.

### ***10.3. Chikungunya virus and Japanese encephalitis virus***

Drs Drager and Featherstone, from WHO, gave overviews about the global disease burden caused by Chikungunya virus and Japanese encephalitis virus (JE virus), respectively. Dr Drager informed the participants of the meeting about the geographical distribution of Chikungunya cases in the recent years. Chikungunya infection is characterized by a short duration of the viremia. Therefore, the detection of the viral RNA is limited to a shortened period of less than one week. Differential diagnosis plays an important role in excluding infections caused by other agents. Dr Featherstone pointed out that JE is the leading cause of viral encephalitis in Asia with 30,000-50,000 cases reported annually to WHO. The case fatality is about 20-30% and 10,000-15,000 deaths were estimated per year. Approximately 30-50% of survivors have significant neurological sequelae. JE case definition is based on acute encephalitis syndrome. A reference laboratory evaluation of three commercial diagnostic tests showed good sensitivity but two of them had low specificity due to cross reactivity with dengue virus IgM. However concerns were raised about the sensitivity of these assays when evaluated by "field" laboratories. The WHO Vaccine Preventable Disease Lab Network has established a JE laboratory network in the South East Asian and Western Pacific regions based on the principles of the measles LabNet with often the same labs being used. A JE external quality assurance programme has been established with a comprehensive serum and CSF panel in the process of being compiled. The panel will be used to check the quality of the numerous in-house assays in use and to monitor performance of laboratories in the LabNet.

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

### PROPOSALS TO ECBS FOR ESTABLISHMENT OF WHO BRPs

- HIV-2 RNA (proposed 1<sup>st</sup> International Standard/2009)
- HBV genotype panel: NAT tests (1<sup>st</sup> International Reference Panel/2009)
- B19V genotype panel (proposed 1<sup>st</sup> International Reference Panel/2009)
- HBV genotype panel: HBsAg tests (1<sup>st</sup> International Reference Panel/2010)
- CMV DNA (1<sup>st</sup> International Standard/2010)
- EBV DNA (1<sup>st</sup> International Standard/2010)
- Anti-*T. cruzi* antibody panel (proposed 1<sup>st</sup> International Reference Panel/2010)

### PROPOSALS TO ECBS FOR ENDORSEMENT OF NEW PROJECTS

- HCV genotype panel (proposed 1<sup>st</sup> International Reference Panel/2009)
- HCV core antigen (proposed 1<sup>st</sup> International Standard/2009)
- HDV RNA (proposed 1<sup>st</sup> International Standard/2009)
- HIV-1 genotype panel (proposed 2<sup>nd</sup> International Reference Panel/2010)
- DENV 1-4 RNA for NAT (proposed 1<sup>st</sup> International Reference Panel/2010)
- Anti-Toxoplasma IgM standard (proposed International Standard 2009)
- Toxoplasma gondii (proposed 1<sup>st</sup> International Standard/2009)
- Blood-borne bacteria reference strains (proposed 1<sup>st</sup> International Reference Panel/2009)

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

- HBsAg standard, replacement
- Anti-HCV antibody panel
- Anti-CMV antibody standard (IgG/IgM)
- HEV RNA
- Anti-HTLV-1/2 antibody panel
- other Flavi-, arthropod-borne, hemorrhagic fever viruses (WNV RNA, Anti-DENV, Chikungunya virus and Japanese encephalitis virus)
- Preparations for HHV-8 antibodies and HHV-8 DNA
- BK DNA, JC DNA
- Anti-*T. brucei* antibody BRP
- Anti-*Leishmania* antibody panel
- Anti-*Babesia spec* antibody panel
- Anti-*Plasmodium* species panel
- TSE blood preparations

## ANNEX 1: REFERENCES

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

- 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, Rockville, Maryland, USA (Jan 2007)
- 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>
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