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**Name of the University, Hospital, Research Institute, Academy or Ministry**

Paul-Ehrlich-Institute (PEI)

**Name of the Division, Department, Unit, Section or Area**

Division of Haematology and Transfusion Medicine

**City** Langen **Reference Number** DEU-117

**Title** WHO Collaborating Centre for Quality Assurance of Blood Products and in vitro Diagnostic Devices

**Report Year** 07-2016 to 07-2017

**1. Please briefly describe the progress made in the implementation of your agreed workplan as WHO collaborating centre during the past 12 months (or the reporting period listed above). Please report on how each workplan activity was implemented, if any outputs have been delivered, if any results have been achieved and if any difficulties have been encountered during this time. If an activity has previously been completed, has not started yet, or been placed on hold, please indicate this.**

**Activity 1**

Title: Development of an International Standard for Hepatitis B Virus e Antigen (HBeAg).

Description: It is proposed to prepare an international HBeAg standard to evaluate HBeAg assay analytical sensitivity. It may also serve for calibration of secondary standards by manufacturers of diagnostic kits, for quality control by competent authorities and by users. The participants of the collaborative and commutability study include reference laboratories for viral hepatitis, public health laboratories, competent authorities and IVD manufacturers developing HBV assays that are located worldwide.

20 laboratories contribute to establish an overview of assay sensitivity over a range of countries covering Europe (France, Germany, Netherlands, United Kingdom, Russia), America (Canada, USA, Brazil), and Asia (Thailand, China, Korea, Japan, Russia). Thirteen different assays covering different assay formats (ELISA, ChLIA, RIA and ECL) are used.

Annette Reissinger, Sigrid Nick

The standard was adopted by the Expert Committee on Biological Standardization (ECBS) as the "1st WHO International Standard for Hepatitis B Virus e Antigen (HBeAg)" in October 2013.

Stability studies are ongoing.

**Activity 2**

Title: Development of a WHO International Standard for Antibodies to Hepatitis B Virus e Antigen (anti-HBe-IgG)

Description: The Paul-Ehrlich-Institut (PEI) anti-HBe IgG-material has been used since 1982 for calibration of the anti-HBe kits and many manufacturers have referred the sensitivity to PEI units. There is continuous demand for this anti-HBe standard. Human plasma positive for anti-HBe-IgG in high titres, has been characterized to establish the international standard. The value assignment in IU will be performed in an international collaborative study involving reference laboratories, test kit manufacturers and competent authorities from worldwide.

The anti-HBe-IgG international standard will be used for determination of the analytical sensitivity of anti-HBe assays. It may also serve for calibration of secondary standards by manufacturers for their test kits, for quality control by competent authorities and by users.

Olivia Knauer, Heiner Scheiblaue

The “1st WHO International Standard for Anti-Hepatitis B Virus e Antigen (anti-HBe)” was adopted by the ECBS in October 2013.

Stability studies are ongoing.

### **Activity 3**

Title: Development of a WHO International Standard for Hepatitis C virus (HCV) Core Antigen

Description: A HCV core antigen standard would be especially useful for estimating the sensitivity of HCV core antigen assays and of HCV antigen/antibody (Ag/Ab) combination assays. It may also serve for calibration of reference materials by manufacturers of diagnostic kits.

First qualitative and quantitative HCV core antigen assays intended to be used for blood screening and / or for HCV patient monitoring have already been introduced into the market. In addition, also HCV Ag/Ab combination assays have become available now. For comparable antigen detecting assays, such as HBsAg (Hepatitis B Virus surface antigen) or HIV-1 p24 (Human Immunodeficiency Virus capsid antigen) tests, the availability of internationally recognized standard preparations has been of great value and the correlation between analytical and clinical sensitivity is well established. Such preparations have proven essential for manufacturers for standardizing their devices. Regulatory bodies benefit from such materials for the assessment of the sensitivities or failures of devices. Even users take advantage of international reference preparations for selecting high quality devices. The benefit for HCV infected patients will be the availability of improved HCV core assays for monitoring the virus load and thus optimized anti-viral therapy.

Sigrid Nick, Heiner Scheiblaue

The “1st WHO International Standard for Hepatitis C virus (HCV) Core Antigen” (HCV Ag, PEI code 129096/12) was adopted by the ECBS in October 2014.

Stability studies are ongoing.

### **Activity 4**

Title: Development of a WHO International Standard for Hepatitis D Virus RNA

Description: For the preparation of the standard material several HDV RNA-positive plasma samples representing the most predominant clade HDV-1 were provided by the Institute of Hepatology, Ankara University, Turkey in 2010. A feasibility study was performed involving several laboratories to characterize the materials and to find out the suitable candidate material for the standard preparation. The lyophilized standard preparation will be evaluated in a worldwide collaborative study.

The HDV RNA International Standard will be used by clinical diagnostic laboratories, IVD manufacturers and NCLs for the development and calibration of NAT assays, for the calibration of secondary references and working standards, and for the evaluation of standardized preparations used in quality control and quality assurance.

Michael Chudy

In October 2013, the ECBS established the “1st WHO International Standard for HDV RNA for NAT based assays”. Further follow-up investigations will be performed to underpin the commutability of the WHO material, e. g cooperation with EQA (external quality assessment) studies organized by the Quality Control for Molecular Diagnostics (QCMD) organization. Based on the availability of an international standard the Hepatitis D International Network is now going to discuss the algorithm for diagnosis and treatment of chronic HDV infection.

### **Activity 5**

Title: Development of a WHO International Genotype Panel for Hepatitis E Virus (HEV) RNA for Nucleic Acid Amplification Technique (NAT)-based assays

Description: The need for standardization of NAT assays for HEV RNA was initially demonstrated in the first ever international proficiency testing study coordinated by the Paul-Ehrlich-Institut. In a follow-up study, the 1st International Standard (IS) for HEV was established by the Expert Committee on Biological Standardization (ECBS) in October 2011.

The IS has been prepared from a genotype 3a HEV strain, obtained from a Japanese blood donor. HEV can be classified into at least four main genotypes. Genotypes 1 and 2 can be found in humans, whilst genotypes 3 and 4 are found in both humans as well as a range of animal species, particularly pigs and wild boar where the sequences between the animal and human strains, circulating in the same geographic region, are closely related with evidence for zoonotic infection.

The geographical distribution of HEV genotypes is complex. Genotype 1 consists of strains circulating in Africa and Asia (Egypt, Algeria, Morocco, Sudan, and Chad; India, Pakistan, Nepal, Bangladesh and China). Genotype 2 is found in Mexico and in some African countries (Nigeria, Namibia, Chad, and Sudan). Genotype 3 is widely distributed, mainly being reported in the USA, Europe and Japan. Genotype 4 is restricted to India and East Asia. However, genotype 1 viruses, and more recently genotype 4 viruses, are found in patients in Europe, North America and elsewhere after travelling to endemic areas and represent imported cases. Epidemics and sporadic cases of hepatitis E occur in areas of endemicity (genotypes 1, 2 and 4); more isolated clinical cases occur among a sizeable group of mostly asymptomatic seropositive residents in developed countries (genotype 3). It is now well recognised that genotype 3 HEV can cause chronic infection in transplant patients with monitoring of viral loads in response to antiviral therapy.

From sequence analysis of different HEV strains, at the nucleotide level, there is in the order of 74% nucleotide identity between genotypes. In the case of genotype 3 for example, there are at least 10 sub-genotypes which vary by up to 15% nucleotide identity. In order to ensure appropriate coverage of NAT assays for HEV, the availability of a genotype panel would be a valuable tool.

A panel of different HEV genotypes and important sub-genotypes will be evaluated by a group of laboratories including reference laboratories for viral hepatitis, public health laboratories, blood banks/plasma fractionation organizations, control laboratories, research laboratories and organizations developing vaccines and IVD manufacturers developing HEV NAT assays. The panel samples will be evaluated alongside the WHO IS. Such a panel will be valuable to ensure adequate detection of HEV from both human and zoonotic sources.

Sally Baylis

At the annual meeting of the WHO ECBS in October 2011, the proposal was made by the PEI to develop an international reference panel for hepatitis E virus genotypes. The ECBS endorsed the proposal. A panel of HEV-positive samples, comprising eleven different members including genotype 1a (2 strains), 1e, 2a, 3b, 3c, 3e, 3f, 4c, 4g as well as a human isolate related to rabbit HEV was lyophilized, evaluated in an international collaborative study and established by the WHO ECBS in October 2015 as the "1st WHO International Reference Panel (IRP) for all HEV genotypes for NAT-based assays" (code number 8578/13) with no unitage being assigned to the individual panel members.

At the same time, a candidate Biological Reference Preparation (BRP) for HEV RNA was prepared from an HEV 3f virus strain on behalf of the European Directorate for the Quality of Medicines and HealthCare (EDQM). The candidate BRP was assigned a unitage of 40 850 IU/ml (4.6112 log<sub>10</sub> IU/mL) after calibration against the WHO IS. The BRP was adopted by the European Pharmacopoeia Commission in February 2016.

Stability studies are ongoing.

#### **Activity 6**

Title: Extension of the 1st WHO Transfusion-Relevant Bacterial Strain Repository

Description: In 2010, the ECBS approved the proposal to establish the first WHO Repository for Transfusion-Relevant Bacteria Reference Strains. The repository consists of four bacteria strains which were included in the international collaborative study (i.e. *Staphylococcus epidermidis* (PEI-B-P-06), *Klebsiella pneumoniae* (PEI-B-P-08), *Streptococcus pyogenes* (PEI-B-P-20), and *Escherichia coli* (PEI-B-P-19).

(Störmer, M. et al. International Validation Study on Blood Transfusion Bacteria Standards Relevant to Transfusion Medicine-ISBT Working Party Transfusion-Transmitted Infectious Diseases (WP-TTID), Subgroup on Bacteria. *Vox Sang* 10-2011) (ISBT = International Society of Blood Transfusion).

The panel members are bacterial strains selected for their ability to replicate in blood platelet concentrates (PCs) under routine storage conditions used in transfusion medicine. They are prepared using a specially developed procedure which guarantees defined bacterial suspensions (deep frozen, ready to use, stable, shippable, defined in count of living cells). The panel is designed to allow objective validation of methods for bacterial screening as well as technologies for pathogen reduction in PCs under "real life" conditions, i.e. inoculating the PCs with a very low bacteria count (0.03 to 0.3 CFU/ml) followed by growth in the matrix.

An enlargement of the repository was requested and agreed by ECBS (2010).

The list of 21 bacterial candidate strains was discussed during the annual meetings of WP-TTID, Subgroup on Bacteria San Diego 2011 and Cancun 2012). As an outcome a list of 11 selected bacterial strains was proposed which includes spore forming bacteria (*Bacillus* spp. spores) as well as coagulase-negative *Staphylococcus* (CNS), *Klebsiella*, *Pseudomonas*, *Streptococcus*, *Salmonella* and *Serratia* species. These strains had been produced by PEI. After stability testing and sequencing of bacterial 16srRNA the 11 candidate strains as well as the four approved reference strains will be included in the planned enlargement study and distributed to the participating laboratories for testing in platelet concentrates (under real life conditions) regarding their ability to proliferate in platelet concentrates after low spiking (< 1 CFU/ml). The study will be divided in 2 phases and will be performed in different regions of the world.

The Transfusion-Relevant Bacterial Strain Panel will be available to blood banks and manufacturers of approaches for improvement of bacterial safety of blood worldwide. Furthermore it will allow regulatory agencies to decide on those approaches in an objective and standardized manner.

Eva Spindler-Raffel

Platelet Transfusion Relevant Bacteria Reference Strains are a feasible tool for validation and assessment of various microbiological methods for improving blood safety. In the second collaborative study, ten candidate strains demonstrated growth independently of donor effects under “real life” conditions and were included in the WHO International Reference Repository of Platelet Transfusion.

They were adopted by ECBS to be included into the “1st WHO Repository of Platelet Transfusion-Relevant Bacterial Reference Strains” in 2015.

The repository now comprises 14 bacterial strains all with individual growth curve kinetics varying from slow to fast growth.

Overview of all strains held and distributed by the PEI:

Type of standard - PEI Code number - Bacteria strain

1st WHO International Repository for Platelet-Transfusion-Relevant Bacterial Reference Strains - 8483/13

Staphylococcus epidermidis PEI-B-P-06

Streptococcus pyogenes PEI-B-P-20

Escherichia coli PEI-B-P-19

Klebsiella pneumoniae PEI-B-P-08

Enlargement of WHO International Repository for Platelet Transfusion- Relevant Bacterial Reference Strains

11162/16 Bacillus cereus PEI-B-P-57 (spore suspension)

11163/16 Bacillus thuringiensis PEI-B-P-07 (spore suspension)

11164/16 Enterobacter cloacae PEI-B-P-43

11165/16 Morganella morganii PEI-B-P-91

11166/16 Proteus mirabilis PEI-B-P-55

11167/16 Pseudomonas fluorescens PEI-B-P-77

11168/16 Serratia marcescens PEI-B-P-56

11169/16 Staphylococcus aureus PEI-B-P-63

11170/16 Streptococcus bovis PEI-B-P-61 (reclassified Streptococcus gallolyticus)

11171/16 Streptococcus dysgalactiae PEI-B-P-71.

### **Activity 7**

Title: Development of a WHO International Standard for Mycoplasma Nucleic Acid Amplification Techniques (NATs)

Description: Mycoplasma nucleic acid amplification techniques (NATs) play an increasing role both in the testing of biologicals for contaminants and in the diagnosis of patients for bacterial infections.

The lack of standardization of Mycoplasma NATs impedes comparative assessment of the performance of different NAT systems, regulatory assessment of NAT systems and reporting of NAT test results in a “common language”.

Nucleic Acid Amplification Techniques (NATs) have been introduced as potential alternative methods in European Pharmacopoeia for the detection of Mycoplasma as part of the 2.6.7 monograph. Similar approaches have been chosen in the US where Mycoplasma NATs may be used for contamination testing of biological, after proper validation.

Micha Nübling (as former PEI staff member)

The candidate material was adopted by the ECBS as the “1st WHO International Standard for mycoplasma DNA for NAT assays, designed for generic mycoplasma detection”.

Stability studies are ongoing.

### **Activity 8**

Title: Participation in the Blood Regulators Network (BRN)

Description: The BRN is a working group of seven leading regulatory authorities in the field of blood products with the task of reacting rapidly to emerging risks, assessing new technologies, and providing advice to WHO. Professor Seitz had been the first chairperson of the BRN (2006 to 2008). Current topics are e.g. support for implementation of Resolution WHA63.12 and the BRN document "Assessment Criteria for National Blood Regulatory Systems", assessment of new developments such as pathogen inactivation technology, discussion of opportunities for international convergence of regulations.

Rainer Seitz (2006-2016), Margarethe Heiden (2006-2015)  
Anneliese Hilger (as of 2015), Dorothea Stahl (as of 2016)

The BRN reported to the ECBS during the annual ECBS meeting at the WHO headquarters in Geneva and met for a closed meeting in October 2016. Furthermore, regular BRN telephone conferences were held in 2016.

In March 2014, the BRN had provided the Position Paper on Collection and Use of Convalescent Plasma or Serum as an Element in Middle East Respiratory Syndrome Coronavirus Response. During the serious outbreak of Ebola in West Africa, the WHO secretariat had coordinated the organization of clinical trials aiming at the scientific evaluation of the so far experimental therapy option of convalescent whole blood (CWB) or plasma (CP). To this end, WHO convened an ad hoc working group including members of the BRN, which operated by regular telephone conferences. This ad hoc working group elaborated the document Interim Guidance for National Health Authorities and Blood Transfusion Services on the Use of Convalescent Whole Blood or Plasma Collected from Patients Recovered from Ebola Virus Disease for Transfusion, as an Empirical Treatment during Outbreaks. The document was updated in 2015 and 2016.

The Ebola crisis dramatically increased the awareness for the importance of functioning national blood systems as part of the response to emergencies. In 2012, the BRN had provided the document Assessment Criteria for Evaluation of Blood Regulatory Systems. Another important milestone was the addition of blood and blood components to the WHO Model List of Essential Medicines. The BRN initiated its work on the development of a guidance document on the management of blood and blood components as essential medicines, which should also be an element of the implementation of national blood regulatory systems. The actual items of discussion as well as the minutes of BRN meetings and teleconferences are confidential. However, general information about the BRN and documents produced by the BRN for publication are available on the BRN website <http://www.who.int/bloodproducts/brn/en/>.

### **Activity 9**

Title: Support for the project "Improving Access to Safe Blood Products through Local Production and Technology Transfer of Technology in Blood Establishments"

Description: The project was started with the workshop "Improving Access to Safe Blood Products in Low- and Middle- Income Countries (LMIC): A Framework to improve Public Health" at WHO Headquarters, Geneva, 14 -15 June 2012. Professor Rainer Seitz and Dr Micha Nübling contributed presentations from PEI. The initiative is an important element in the implementation of Resolution WHA63.12, which has been supported also by the WHO BRN, e.g. by elaborating the document "Assessment Criteria for National Blood Regulatory Systems". PEI experts will contribute to drafting further guidance documents, and activities towards their implementation. Dissemination by relevant WHO departments, WHO Regional Offices; Government support for local manufacture of medical products.

Rainer Seitz, Sabine Wegehaupt

PEI was not involved in any activities during the reporting period.

### **Activity 10**

Title: Contribution to the Development of a WHO Technical Document on the Residual Risk in Blood Components

Description: The PEI has profound experience due to involvement in several national and international regulatory committees, including EMA (European Medicines Agency) working parties and expert groups of the European Pharmacopoeia, and scientific societies, such as ISTH, ISBT. Experts of the PEI, as desired and appropriate, will be ready to actively contribute to the elaboration and/or updating of guidance documents, such as the guidance on blood products in Technical Report Series, no. 840, and no. 932 (revision of the Recommendations for the Preparation, Characterization and Establishment of International and other Biological Reference Standards). Currently, a WHO Guideline on the residual risk in blood components is being drafted which may facilitate decision-making in regard to testing strategies for blood borne pathogens, taking the regional epidemiological background of the donor population into consideration.

Micha Nübling (as former PEI staff member)

The document was finalized in 2016 and adopted by ECBS as WHO Guideline on Estimation of Residual Risk of HIV, HBV or HCV Infections via Cellular Blood Components and Plasma (published 2017; WHO TRS 1004, Annex 4; 166-196).

#### **Activity 11**

Title: Contribution to the Development of a WHO Technical Document on the Preparation and Calibration of Secondary Reference Preparations (IVD standards)

Description: The PEI has profound experience in the development of WHO International Standards for the in vitro diagnostic (IVD) area. An important task is the assurance of the continuity of the International Units for secondary standards. Currently, different approaches are obviously followed by different parties for the establishment and calibration of secondary standard preparations. A document is proposed which covers the steps and issues to be considered on the establishment of secondary standards.

Michael Chudy

The document was finalized in 2016 and adopted by ECBS as WHO manual for the preparation of secondary reference materials for in vitro diagnostic assays designed for infectious disease nucleic acid or antigen detection: calibration to WHO International Standards (published 2017; WHO TRS 1004, Annex 6; 389-455).

#### **Activity 12**

Title: Contribution to the Development of a WHO Technical Document on Commutability

Description: The PEI has profound experience in the development of WHO International Standards for the IVD area. These materials are used for standardization of different diagnostic assays. An important prerequisite for the proper use of these standards is their feature to be representative for routine clinical specimens, e.g. in regard to the analyte tested for or the test matrix (e.g. human plasma or serum). The representation of clinical specimens by a reference material is called commutability. If a reference material is non-commutable to clinical specimens, a bias may be introduced between different assays. Currently not all commutability aspects are fully addressed by the collaborative studies organized for the establishment of WHO International Standards.

Micha Nübling (as former PEI staff member), Sally Baylis

It is common understanding between the WHO CCs that commutability needs to be addressed by either the collaborative study, separate studies, or by inclusion into proficiency testing programmes after establishment. Due to differences between WHO reference materials (some even originate from clinical specimens), and numbers of different assays to be included into collaborative studies further details of how exactly to address commutability have not been defined, for good reasons.

#### **Activity 13**

Title: Assuring safety and availability of blood products in developing countries: Good Manufacturing Practices (GMP) & Educational Tools for blood/plasma collection establishments

Description: This is an initiative to establish and implement GMP in blood establishments by the organization of meetings and training courses in several regions of the world, with presentations by experienced inspectors (including PEI), and attendance of pharmaceutical inspectors, heads of blood national programs and delegates from regulatory authorities in the respective nations/regions.

National/regional benefit: Optimal use of and benefit from donated blood plasma. Locally available essential blood plasma derived medicinal products. Incorporation of developing countries in the international transfusion community and associated industries.

Support to the quality control of blood safety related IVDs both serology and NAT.

Sabine Heinz-Stempel, Sigrid Nick

PEI was not involved in any activities during the reporting period.

**2. Please briefly describe your collaboration with WHO in regards to the activities of the WHO collaborating centre during the past 12 months (e.g. means of communication, frequency of contact, visits to or from WHO). Please feel free to mention any difficulties encountered (if any) and to provide suggestions for increased or improved communication (if applicable).**

2.1 67th Meeting of the Expert Committee on Biological Standardization (ECBS), Geneva, Switzerland, 17-21 October 2016

The president of the PEI, Professor Cichutek, was designated chairman of the ECBS.

Dr Heidi Meyer provided an update on the activities of the PEI WHO Collaborating Centres (CC) and discussed current scientific issues. Concerning the WHO CC for Quality Assurance of Blood Products and in vitro Diagnostic Devices, PD Dr Dorothea Stahl was introduced as new head, following Professor Rainer Seitz. Dr Meyer replaced Dr Michael Pfeleiderer as head of the WHO CC for the Standardization and Evaluation of Vaccines.

PEI participated in collaborating studies for measurement standards; submitted a proposal for new anti-Chikungunya virus reference reagent, and contributed to the development of the residual risk of blood components guideline and the guidance on secondary reference preparations for IVD, and also on vaccine related guidelines (clinical evaluation, maternal immunization, Ebola, Flu, core working group member Y. Sun).

Furthermore, PEI provided ongoing support for the WHO prequalification programme to support prequalification of IVDs and vaccines.

During the public health emergency on Zika virus (ZIKV) infections, PEI developed a candidate IS for ZIKV for NAT-based assays. By the end of July 2016 WHO agreed to an immediate distribution for inclusion in different EQA (external quality assessment) programmes. The candidate IS was proposed for adoption as WHO IS by ECBS 2016.

PEI researchers investigated virus reduction methods on ZIKV, and effective inactivation / removal of ZIKV e.g. pasteurization (albumin, 58°C), solvent/detergent treatment, and retentive virus filtration.

Dr Meyer also pointed out the various good collaborations of PEI within the BRN, the Collaborating Centres, and with non-ECBS countries, e.g. Ghana and Liberia, for the establishment of bilateral interactions to strengthen the Blood Regulatory Systems in Ghana and Liberia (coordinated by Jens Reinhardt).

In the light of the recent Ebola crisis and as a consequence of the G7 summit in June 2015, the German Ministry of Health (MoH) agreed to fund two PEI projects in the context of the German MoH's Global Health Program to facilitate access to medical countermeasures (MCM) in low and middle income countries, focusing on i) availability, safety and quality of blood and blood products, providing support in establishing regulatory structures and their adaptation to crisis situations in the partner countries (lead: PD Dr Dorothea Stahl); and ii) to support regulation and control of clinical trials, providing support and regulatory training on clinical trial approval for vaccines and biomedicines (lead: Dr Christoph Conrad).

At the end of this ECBS meeting, the committee adopted the "1st WHO International Standard for Zika Virus for Nucleic Acid Amplification Technique (NAT)-Based Assays".

ECBS also endorsed the project to develop an antibody standard (IgG/IgM) to Chikungunya virus as 1st WHO International Standard.

Moreover, the committee endorsed both the extension of the “1st WHO Repository of Platelet Transfusion-Relevant Bacterial Reference Strains” by 10 strains and the project to establish the “1st WHO Repository of RBC Relevant Bacteria Strains”.

Dr Jens Reinhardt (PEI) shared the rapporteur ship for the blood/IVD track with Dr Clare Morris (NIBSC).

## 2.2 New Projects endorsed at ECBS Meetings

### 2.2.1 Development of an International Standard for IgG Antibodies to Human Cytomegalovirus (Anti-HCMV-IgG), endorsed by ECBS in 2013

Description: Human Cytomegalovirus (HCMV) is spread worldwide with prevalence between 40% and 100%. The virus causes relatively mild and asymptomatic infections in immune competent persons, but can cause congenital disease and severe complications in those with immunodeficiency, e.g. immunological immaturity; acquired immunodeficiency or immunosuppression. Diagnostics of HCMV specific IgG and IgM antibodies and IgG avidity play a major role in prenatal care, blood transfusions and especially transplantation medicine. Current anti-HCMV assays differ considerably in their sensitivity: However, no international reference material is available at the moment. PEI anti-HCMV-IgG reference material has been available since 1982. It is frequently requested by manufacturers for calibration of anti-CMV test kits. But this material is of limited supply and is weakly positive and not defined in international units. The proposed standard may be used for the calibration of the manufacturer’s diagnostic kits, for quality control by competent authorities, and for the calibration of secondary standards by users.

Nina Wissel, Heiner Scheiblaue

The project to establish the “1st WHO International Standard for Anti-HCMV IgG” was endorsed by the ECBS in October 2013.

The aim is to develop a CMV IgG antibody (anti-CMV-IgG) standard for diagnostic purposes to improve the comparability of the divergent outputs of the current anti-CMV IgG assays. A collaborative study was conducted between 2014 and 2016 to characterize the candidate standard, assess its performance, and determine whether it is suitable for the intended purpose, i.e. calibration of anti-CMV IgG diagnostic tests. The study was designed to include a broad representation of assay methods, laboratory types and countries. In addition, 10 additional study samples were used covering the relevant infection stages relevant to CMV serology, to ensure that the results with the candidate standard can be transferred to other anti-CMV IgG samples.

The candidate material resulted in a mean end point titre of 46.4, which was used as the overall potency. Overall, the study results showed that the candidate standard was effective for the majority of the assay methods to normalize the results of the anti-CMV IgG test kits and thus to improve the comparability of the results. The standard is suitable for test calibration, comparisons of analytical sensitivity and quality control. The study report was submitted to the ECBS in June 2017. The candidate material is proposed to ECBS as the “1st WHO International Standard for Anti-CMV IgG” with an assigned unitage of 46.4 International Units per vial.

### 2.2.2 Development of a WHO International Anti-Hepatitis E Panel

Description: The diagnosis of HEV requires a variety of tests including the detection of IgM and IgG antibodies. Analysis of IgM antibodies is particularly useful for the confirmation of acute infection. Anti-HEV IgG may also be detectable during acute infection. Anti-HEV IgG is also a marker of past HEV infection and seroprevalence and indication of previous exposure of different populations to hepatitis E virus. Anti-HEV IgG assays vary in their performance and different studies have given widely different estimates of the seroprevalence of anti-HEV. Despite the establishment of an interim WHO International Reference Reagent (IRR, NIBSC code number 95/584) for antibodies to HEV in 1997, there are still wide discrepancies in the performance of anti-HEV assays. It was noted by the ECBS, at the time of establishment of the IRR that assays for anti-HEV were at an early stage of development and acknowledged that full assessment of new

antibody assays requires panels of sera. Since the IRR was established, no internationally agreed unitage has been assigned to the preparation. In the original collaborative study, seven laboratories evaluated a range of mainly in-house developed assays and two commercial assays available at the time.

Fifteen years later, diagnostic sensitivities and specificities as well as inter-assay agreement have been shown to vary widely for different kits for the detection of both anti-HEV IgM and IgG. Anti-HEV IgM is the major serological marker of acute or recent HEV infection; however, the main problem with detection of this marker is specificity; this is further compounded by a lack of sensitivity. Significant underestimations of seroprevalence of HEV (IgG) have been the result of lack of sensitivity of available assays. These assay issues, compounded by the failure of many clinical diagnostic laboratories to test for HEV infection as an alternative diagnosis of acute hepatitis, have impacted on the surveillance of incident hepatitis E and understanding the extent of asymptomatic infection.

Sally Baylis

The proposal to prepare the anti-HEV panel was endorsed by the ECBS in October 2015. Plasma has been obtained from recovered patients and donors with further units being screened.

### 2.2.3 Proposal for extension of the “1st WHO Repository of Platelet Transfusion-Relevant Bacterial Reference Strains” by Red Blood Cell Transfusion-Relevant Bacterial Strains

Description: Bacterial reference strains are a suitable tool for objective validation and assessment of various microbiological methods for blood safety and development of new techniques. As a first milestone, four platelet transfusion-relevant bacterial strains were established in cooperation with the ISBT WP TTID (International Society of Blood Transfusion Working Party Transfusion-Transmitted Infectious Diseases) Bacterial Subgroup in 2010 as the “1st WHO Repository of Platelet Transfusion-Relevant Bacterial Reference Strains”. This panel was extended by 10 strains which had been previously evaluated in a second collaborative study and adopted by ECBS in 2015 (see Activity 6).

In line with the strategy to establish relevant bacterial reference strains for all blood components and advanced therapy medicinal products (ATMPs), the next step will be to establish a bacteria panel for red blood cells (RBC). Statistically, the prevalence of bacterial contamination in RBC is 1 in 30 000 with septic reactions of 1 in 500 000 and projected fatality rates of approximately 1 in 10 million. Four fatalities were reported between 1997 and 2012 caused by transfusion of bacterially contaminated RBC in Germany. Further transfusion incidents were reported in the last years caused by *Staphylococcus aureus*, *Serratia marcescens*, *Yersinia enterocolitica*, *Pseudomonas fluorescens*, *Pseudomonas koreensis*, *Klebsiella pneumoniae*.

Marcel Prax

The proposal to extend the repository was endorsed by the ECBS in October 2015.

In January 2017, Dr Prax, who is the head of laboratory in the section “Microbiological Safety”, assumed the coordination of the study.

The majority of bacteria isolated from platelet components, which are stored at room temperature, are unable to grow or even survive in RBC under mandatory cold storage conditions from 1°C to 6°C. Therefore, most of the strains of the platelet repository are not suitable as bacterial reference strains for RBC and specific candidates have to be selected. Between November 2015 and September 2016, PEI collected transfusion-relevant candidate strains, most of them involved in transfusion incidents worldwide. Cultivation, identification and pretesting in RBC concentrates were performed to select for appropriate strains for the collaborative study. Within the pre-study, a total of 32 strains were analysed and inoculated in RBC concentrates for 49 days at 4°C without agitation and the number of colony forming units was determined over time. Six of them demonstrated growth independently of donor effects.

The candidate strains which are finally included in the collaborative study are as follows:

*Listeria monocytogenes* PEI-A-199, *Serratia marcescens* PEI-B-P-56, *Serratia liquefaciens* PEI-A-184, *Pseudomonas fluorescens* PEI-B-P-77, *Yersinia enterocolitica* PEI-A-105, *Yersinia enterocolitica* PEI-A-176, *Bacillus cereus* PEI-B-P-57.

They mainly comprise meso- and psychrophilic bacteria with a permissive growth temperature below 25°C. The individual growth curve kinetics revealed a growth of significantly more than 2 log<sub>10</sub> CFU/mL up to 7

log<sub>10</sub> CFU/mL by day 14 of storage starting from an initial inoculum of approximately 0.03 CFU/mL. After the identification of promising candidates, stability studies have been performed and are still ongoing. So far, the candidate strains are very stable under storage conditions of -80°C. The results of the pre-study were presented and discussed at the TTID WP meeting (ISBT Copenhagen), June 2017. Due to the fact that growth ability may vary among the bacteria and species and is also dependent on a number of other factors, it is crucial to validate the candidate strains in an international collaborative study (cooperation with the ISBT WP TTID Bacterial Subgroup) which will start in the second half of 2017.

#### 2.2.4 Development of a WHO International Standard for Zika Virus RNA Nucleic Acid Amplification Techniques (NATs)

Description: On the 1st of February, 2016, Dr Margaret Chan, the former director general of the WHO, declared a Public Health Emergency of International Concern (PHEIC) due to the increase in neurological disorders and neonatal malformations seen in the Americas, strongly suspected to be linked to the Zika virus (ZIKV) outbreak. This declaration came after a meeting of the International Health Regulations (IHR, 2005) Emergency Committee. The WHO asked the PEI to develop a candidate international standard (IS) for ZIKV for nucleic acid amplification technique (NAT)-based assays. Such an IS or reference material will be used in diagnostic testing to ensure accurate diagnosis of ZIKV infection in the ongoing outbreak and beyond.

Sally Baylis

A candidate IS was developed using an inactivated, lyophilized ZIKV preparation formulated in a stabilizing, neutral solution and intended for dilution using a range of different types of sample matrix. The virus strain used for the preparation of the candidate IS originated from a ZIKV infected patient from French Polynesia, closely related to ZIKV strains currently circulating in the Asia-Pacific region and central and South America. Further strains from the Asian ZIKV lineage were included in an international collaborative study as well as two preparations derived from African ZIKV isolates and the candidate international standard. The samples consisted of a mixture of inactivated ZIKV reference materials as well as clinical materials (urine or plasma) from ZIKV infected patients. In addition, a panel of in vitro transcribed RNAs covering partial ZIKV genome sequences was included in the study. The candidate standard was established by the WHO ECBS in October 2016 as the 1st IS for ZIKV RNA with an assigned unitage of 50 000 000 International Units per mL.

#### 2.2.5 Development of the 1st WHO International Standard for Chikungunya virus RNA for Nucleic Acid Amplification Technique (NAT)-based assays

Description

Chikungunya virus (CHIKV) is an Alphavirus (family Togaviridae) transmitted by Aedes mosquitos that causes a fever-rash-arthralgia syndrome in humans, known as chikungunya fever. During the last decade CHIKV has expanded its geographic range and caused major outbreaks in Africa, Asia, Europe, the Indian Ocean, the Caribbean and the Americas. There are three major, geographically distinct CHIKV lineages: West African, East/Central/South African (ECSA), and Asian. Co-circulation of CHIKV with Dengue virus (DENV) and Zika virus (ZIKV) has been reported for certain regions, which complicates the clinical management of patients. Nucleic acid amplification technique (NAT)-based assays are considered the most sensitive detection method for laboratory diagnosis and blood screening of acute CHIKV infections. Worldwide, a range of commercial and in-house developed methods are used. However, due to the lack of a reference material NAT assays are not standardized. Hence, test results show high variability and are difficult to compare. A reference material of higher order is needed for assay validation and the calibration of secondary references. Potential users are IVD manufacturers, clinical laboratories, blood transfusion services and regulatory authorities.

Julia Kreß

At the WHO ECBS Meeting in October 2010, a proposal for the establishment of an International Standard (IS) for CHIKV RNA for NAT-based assays presented by the Center for Biologics Evaluation and Research (CBER) of the U.S. Food and Drug Administration (FDA) was endorsed by WHO ECBS. In the following years, CBER established a well characterized (national) CHIKV RNA reference reagent (Añez G et al., Genome Announcement, 2014; Añez G et al., Vox Sang., 2015). At the WHO CC Meeting in July 2015, a decision was

made to use the same vial stock for the development of the candidate IS. In March 2016, the project was transferred from CBER to PEI.

The lyophilized candidate IS consisted of a cell culture grown, heat inactivated CHIKV isolate (ECSA lineage) diluted in human plasma. An international collaborative study was conducted to assess its suitability for use in NAT assays. Further clinical materials from CHIKV infected patients were included in the study to evaluate commutability between the candidate IS and patient specimens. A total of 25 laboratories from 14 countries agreed to participate in the study. 31 datasets from 24 laboratories have been received. The assays used consisted of a mixture of in-house developed and commercial assays, most of which were qualitative real time PCR assays. Statistical analysis of data was performed to evaluate the potency of the candidate IS with the aim of assigning an internationally agreed unitage.

The evaluation of study results, statistical analysis and the preparation of the report is still ongoing. Submission of the report of the collaborative study to ECBS is planned for 2017.

## 2.2.6 Development of a WHO International anti-Chikungunya virus reference reagent

### Description

The mosquito-borne Chikungunya virus (CHIKV) is a member of the Alphavirus genus in the Togaviridae family. Chikungunya was first identified in Tanzania in the early 1950s. The disease occurs not only in Africa but also in Asia and the Indian subcontinent and, since 2013, has spread to the Americas, particularly central and Southern areas. Small outbreaks have also occurred recently in Europe.

The diagnosis of Chikungunya requires a variety of tests including detection of IgM and IgG antibodies. Co-circulation of Chikungunya virus with Dengue virus (DENV) and Zika virus (ZIKV) frequently occurs and infections caused by these viruses share common signs and symptoms in infected patients. Accurate diagnosis and discrimination of CHIKV from other virus infections is important for patient care. Analysis of IgM antibodies is particularly useful for the confirmation of acute infection. Anti-CHIKV IgG may also be detectable during acute infection. Anti-CHIKV IgG is also a marker of past CHIKV infection and seroprevalence of anti-CHIKV and indication of previous exposure of different populations to Chikungunya virus.

Anti-CHIK assays vary in their performance (for example, Niedrig et al. Clin Microbiol Infect. 2009;15:880 and Prat et al. Emerg Infect Dis. 2014;20:2129).

Sally Baylis

The proposal to establish a reference reagent was endorsed by the WHO ECBS in October 2016. Plasma has been collected from blood donors as well as recovered patients and is undergoing further characterization prior to the launch of an international collaborative study.

## 2.3 Cooperation with WHO in the area of WHO's Prequalification Programme (PQ) for in vitro Diagnostic Devices (IVD) and Procurement of IVDs

PEI IVD Laboratories continued to participate in the WHO Programme for the Prequalification of Diagnostics (see [http://www.who.int/diagnostics\\_laboratory/evaluations/en/](http://www.who.int/diagnostics_laboratory/evaluations/en/)).

### 2.3.1 4th Annual Meeting of WHO Prequalification of in vitro Diagnostics Dossier Assessors and Inspectors, WHO HQ, Geneva, Switzerland, 25-27 April 2017

Dr Heiner Scheiblauer and Dr Michael Chudy participated in the meeting which was held to discuss, improve and harmonize WHO's inspection and dossier assessment activities. Participants were informed and trained on the new documents of the Technical Guidance Series (TGS) and Technical Specification Series (TSS).

### 2.3.2 Participation in training activities of regulatory authorities and manufacturers linked to the PQ programme

#### 2.3.2.1 Joint CFDA/ WHO meeting: Dossier assessment of in vitro diagnostic medical devices (IVD), Swissotel Hotel, Beijing, P.R. China, 12-16 December 2016

Dr Heiner Scheiblauer participated. Within the framework of WHO Prequalification of Diagnostics Programme

(WHO PQDx), a training course was held for Chinese IVD manufacturers to strengthen the capability for production of IVDs, in particular HIV rapid test kits, and to harmonize dossier submissions and the evaluation of IVDs.

2.3.2.2 Joint WHO/ PEI Training on batch testing of HIV rapid tests, panel selection and preparation, Paul-Ehrlich-Institut, Langen, Germany, 19-27 April 2017

The training was hosted by Dr Heiner Scheiblaue, Dr Olivia Knauer, Nina Wissel, and Dr Sigrid Nick, PEI IVD Testing Laboratory. Three trainees from Chinese regulatory authorities, i.e. the National Institute for Food and Drug Control (NIFDC) and the HIV/HCV Reference Laboratory, National Center for AIDS/STD Control and Prevention (NCAIDS), China CDC were trained at the PEI for seven days.

Topics included the batch verification process, selection and characterization of suitable samples, reading of results using an intensity scale, setting of specifications, documentation and work instructions.

2.4 International Nonproprietary Names (INN) of blood products and monoclonal antibodies

Dr Karin Weisser of PEI has been working as Biological Advisor for the International Nonproprietary Names (INN, i.e. generic names) expert group since May 2006. In April 2016, Dr Weisser became a full member of the INN expert group. The programme is responsible for the selection and publication of INN for new pharmaceutical substances upon request by manufacturers. An INN identifies a pharmaceutical substance or active ingredient by a unique name that is globally recognized and is public property.

The ECBS is informed about decisions and developments at the annual meeting by a WHO representative of the group.

INNs are assigned to a range of biologicals including recombinant blood products, monoclonal antibodies and gene therapy medicinal products which fall within the responsibility of the PEI.

In addition to the regular two consultations each year, separate WHO INN meetings on biological products have been held at intervals since 2002 to address general and specific aspects of nomenclature, including discussions on cell therapy products or biosimilars.

In 2017, Dr Weisser assessed 130 INN requests of biological and 138 INN requests of chemical substances from July 2016 to June 2017. She attended one consultation of the INN expert group (64st consultation, April 2017) where new and outstanding applications were discussed and decisions on the selection of INNs were taken. In addition, she attended an ad hoc meeting on biologicals in September 2016 where a subgroup of INN biological experts met to review the current INN approach for monoclonal antibodies, cell therapies and vaccine-like substances.

2.5 WHO Meetings and Workshops (chronological order; HQ = Headquarters, Geneva, Switzerland)

2.5.1 Global Technical Expert Consultation on “Estimating the impact of emerging infections to the blood supply: requirements for risk estimation and decision making support”, WHO HQ, 14-15 June 2017

The objective of the consultation was to define the preconditions, requirements and components for a software tool to estimate the potential impact on the blood supply when confronted with the emergence of a pathogen. The expected outcome is a report defining and outlining potential tools and approaches to protect the blood supply from emerging infectious diseases and to define their essential prerequisites. From PEI, Dr Juliane Dörrbecker, Dr Jens Reinhardt, Dr Verena Klümpers, and Mr Washington Samukange attended the workshop. Dr Jens Reinhardt was the rapporteur.

2.5.2 BRN assessment of the regulation of blood and blood products by the Zambia Medicines Regulatory Agency (ZAMRA), Zambia, 28 February- 1 March 2017

The WHO BRN performed an assessment of the regulation of blood and blood products by the Zambia Medicines Regulatory Agency (ZAMRA) upon request from ZAMRA. Dr Micha Nübling (WHO HQ), Dr André Loua (AFRO) and Dr Jens Reinhardt (PEI as BRN member) were in Zambia for two days and assessed the blood regulation system. They also visited the Zambia National Transfusion Service.

2.5.3 Consultation on the WHO International Standard for Anti-Rubella, WHO HQ, 30 June 2017

Sigrid Nick attended the consultation workshop where arguments and considerations for and against continuing the WHO anti-Rubella standard were discussed.

2.6 Other (non- WHO) meetings and workshops, related to WHO and PEI CC activities (chronological order)

2.6.1 IPFA/PEI (International Plasma Fractionation Association), 24th International Workshop on Surveillance and Screening of Blood Borne Pathogens, Zagreb, Croatia, 16-17 May 2017

PEI co-organizes this annual scientific meeting. The primary focus concerns the application of nucleic acid amplification techniques (NAT) and other measures to increase blood safety, with standardization of assays being one of the regular topics in this series of meetings. Dr Sally Baylis, Dr Michael Chudy and Dr Heiner Scheiblaue participated in the meeting in May 2017.

More than 180 participants mainly from blood banks, academic institutions, regulatory authorities and diagnostic industry attended the workshop.

2.6.2 SoGATS/SID (Standardization of Genomic Amplification Techniques and Serology/ Standardisation of Infection Diagnostics), London, the United Kingdom, 26-28 June 2017

Dr Julia Kreß, Dr Marcel Prax and Dr Heiner Scheiblaue participated in the SoGATS/SID 2017 workshop (1.5 days NAT, 1 day serology). The meeting focused on the following topics: Next Generation Sequencing in the clinical setting, current standardization activities of the WHO CCs, bacterial standardization, challenges with molecular assays, emergency preparedness, parasitology diagnostics, standardizing serological tests, avidity. Presentation by Dr J. Kreß: "Development of the 1st WHO IS for Chikungunya Virus (CHIKV) RNA".

Presentation by Dr M. Prax: "Overview: Challenges in the production of bacterial standards".

Presentation by Dr H. Scheiblaue: "WHO 1st International anti-CMV IgG Standard".

Dr Heiner Scheiblaue presented for discussion the results of the collaborative study on the anti-CMV IgG standard.

2.7 Further conferences with CC relevant topics attended by PEI co-workers (chronological order)

International Society of Blood Transfusion (ISBT); Working Party-Transfusion Transmitted Infectious Diseases, Copenhagen, Denmark, 17-21 June 2017.

Presentation by Dr M. Prax: "Update on the ISBT TTID study on establishment of bacterial reference strains for RBC".

Hepatitis C Diagnostic Summit, Centers for Disease Control and Prevention, Atlanta (U.S.A.), 8-9 September 2016.

Presentation by Dr J. Kreß: "Development of WHO International Standards for HCV NATs".

**3. Please briefly describe any interactions or collaborations with other WHO collaborating centres in the context of the implementation of the above activities (if any). If you are part of a network of WHO collaborating centres, please also mention the name of the network, and describe any involvement in the network during the last 12 months.**

3.1 3rd WebEx Meeting of WHO Collaborating Centres to support the development of WHO Biological Reference Preparations for Blood Products and in vitro Diagnostic Devices, 27 April 2017

Participants of PEI: Dr Michael Chudy, Dr Julia Kreß, Dr Sigrid Nick, Dr Jens Reinhardt, Dr Constanze Yü, PD Dr Dorothea Stahl, Dr Winfried Kammer, Dr Manvi Porwal, Dr Marcel Prax, Dr Gabriele Unger.

The meeting was performed to coordinate needs of WHO programmes, to discuss priority projects in order to avoid an overlap of activities and to strengthen the collaboration within the WHO CC Network, but also to agree on proposals to be put forward to ECBS for endorsement or establishment. Thus, the meeting comprised scientific updates on ongoing projects, an update on WHO Guidance Documents, general discussions on important topics and the proposals to ECBS.

Presentation by Dr J. Kreß: "Update on the Development of the 1st WHO IS for Chikungunya Virus RNA for NAT-based Assays"

3.2 Participation in collaborative studies of WHO International Blood Product Standards

Section 7/3, "Batch Release, Blood Products, Logistics" took part in a collaborative study to assess the comparability of new FVIII products with the WHO International Standards (CS540). According to the organizer of the study (NIBSC) the data from all of the labs have been analysed and the report will be written over the summer 2017 with an expected completion by early autumn.

Furthermore, two sections in the Division "Haematology" (Section 7/3 and research group 7/01) have participated in the collaborative study to assign potency to the 2nd International Standard for Factor IXa (CS545). The report was distributed to participants for comments and agreement in May 2017, and discussed during a telephone conference in July 2017.

3.3 Participation in collaborative studies for the establishment or replacement of WHO International Standards for NAT-based assays for blood borne viruses

The "Virus Safety" section at PEI participated in the collaborative study to establish the 1st International Standard for chikungunya virus RNA, organized by the PEI section "Molecular Virology".

Section "Molecular Virology" at PEI participated in the collaborative study to establish the 4th WHO International Standard for HIV-1 for NAT-based assays as well as in the collaborative study to establish the 2nd WHO International Standard for HIV-2 for NAT-based assays. Both studies were organized by NIBSC, UK.

**4. Please briefly describe any type of technical, programmatic, advisory or other support received from WHO during the past 12 months for the implementation of the agreed activities listed above (if any).**

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