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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 07-2014 to 07-2015

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 candidate material (PEI code 129096/12) was finally adopted in Oct. 2014 by the ECBS as the “1st WHO International Standard for HCV core antigen” with an assigned potency of 3200 International Units per mL (IU/mL) when reconstituted in 0.5 mL of distilled water.

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

The proposal for the development of a WHO International Standard (IS) for Hepatitis D Virus (HDV) RNA was endorsed by the WHO ECBS in October 2009. The candidate material, an HDV genotype 1 strain, was obtained from a clinical plasma specimen. Its usefulness for a standard preparation was shown by a feasibility study. The material was diluted in negative human plasma, aliquoted in 0.5 mL preparations, and lyophilized. Fifteen laboratories from nine countries participated in an international collaborative study to evaluate the candidate preparation alongside the corresponding liquid frozen bulk material and a liquid frozen neat HDV RNA positive plasma specimen with their routine HDV NAT. The results of the study indicate the suitability of the candidate material as the proposed international reference material for HDV RNA. In October 2013 the ECBS established the material (PEI code 7657/12) as the “1st WHO International Standard for HDV RNA for NAT based assays” with an assigned potency of 5.75×10^5 International Units per mL (IU/mL) when reconstituted in 0.5 mL of nuclease-free water. Two follow-up studies, national and international, were performed to address the commutability of the reference material. The study results also demonstrated that various differences exist with regard to the detection efficiency of different tests. For further harmonization, the tests need to be standardized using reference materials calibrated against the WHO International Standard. In addition, the WHO IS was included in a panel to be evaluated in external quality assessment schemes (EQAS) organized by QCMD (Quality Control of Molecular Diagnostics). In these studies the commutability of the WHO material will be further investigated in routine testing settings.

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, has been lyophilized between August 2013 and August 2014. A collaborative study was initiated in November 2014, the aim was to evaluate the panel against the "1st World Health Organization (WHO) International Standard (IS) for HEV RNA" (code number 6329/10). Also included in the study was a candidate Biological Reference Preparation (BRP) for HEV RNA prepared from an HEV 3f virus strain on behalf of the European Directorate for the Quality of Medicines and HealthCare (EDQM). The samples for evaluation were distributed to 24 laboratories from 14 different countries. The samples were assayed on three separate days and the data were collated and analysed at the PEI. Data were returned by 23 of the participating laboratories. In total 32 sets of data were returned; 17 from quantitative assays and 15 from qualitative assays. The assays used consisted of a mixture of in-house developed and commercially available assays. The results showed that all samples were detected consistently by the majority of participants. It is proposed that the panel, consisting of 11 members, be established as the "1st 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; the study report will be reviewed by the ECBS in October 2015. In the case of the candidate BRP, a unitage of 40,850 IU/ml (4.6112 log₁₀ IU/mL) has been assigned to the material after calibration against the WHO IS. Real-time stability studies have indicated that the panel of HEV samples and the candidate BRP are very stable under normal conditions of storage, i.e., at 20°C or below, and are therefore suitable for long term use. Ongoing real time stability studies of the panel members and the BRP are in progress.

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

The 11 candidate strains which were finally included in the collaborative study are as follows:

Aerobic spore forming bacteria: *Bacillus cereus* spores PEI-B-P-07-S, *Bacillus thuringiensis* spores PEI-B-P-57-S.

Gramnegative species: *Enterobacter cloacae* PEI-B-P-43, *Morganella morganii* PEI-B-P-74, *Proteus mirabilis* PEI-B-P-55, *Pseudomonas fluorescens* PEI-B-P-77, *Salmonella cholerae-suis* PEI-B-P-78, *Serratia marcescens* PEI-B-P-56.

Coagulase positive *Staphylococcus*: *Staphylococcus aureus* PEI-B-P-63.

Streptococcus species: *Streptococcus dysgalactiae* PEI-B-P-71, *Streptococcus bovis* PEI-B-P-61.

To characterize candidate strains for enlargement of the WHO Repository which should be able to grow in PCs from very low initial concentrations, hence mimicking contamination with few bacteria, a collaborative study was started in 2013 with test laboratories in different regions of the world. The deep frozen bacteria (the four strains of "1st WHO International Reference Repository of Platelet Transfusion Relevant Bacterial Strains" plus 11 candidate strains) were shipped to the participating laboratories. At the request of ECBS, the four Repository Strains were again included in the enlargement study as a reference.

The study protocol includes the enumeration of inoculum, low spiking of test bacteria directly in PC bags (inoculum concentration 10 to 25 cfu per PC bag). Growth kinetics will be documented by sampling and enumeration after storage day 2, 4 and 7.

New lots of the four WHO Repository strains (*Staphylococcus epidermidis*, PEI-B-P-06, *Streptococcus pyogenes*, PEI-B-P-20, *Klebsiella pneumoniae* PEI-B-P-08 and *Escherichia coli* PEI-B-P-19) as well as of the candidate strains were produced. Stability testing and identification was performed. The 15 strains were shipped on dry ice to the study partners, 14 collaborating laboratories (Austria 1, Canada 1, England 1, Germany 3, Japan 1, Mexico 1, Pakistan 1, South Africa 1, The Netherlands 1 and USA 3). The tests in the collaborating laboratories were finished in June 2014.

With the exception of the *Morganella morganii* strain, all bacterial strains showed moderate to excellent growth at day 7 after inoculation. The individual growth curves showed variation from slow to fast growth. *Bacillus cereus*, *Bacillus thuringiensis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Staphylococcus aureus* and *Streptococcus dysgalactiae* showed a growth of significantly more than 2 log₁₀ CFU/mL up to 8 log₁₀ CFU/mL by day 2 of storage. For *Enterobacter cloacae*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *Streptococcus bovis* and *Streptococcus pyogenes* this growth level was reached at day 4. Growth for *Salmonella choleraesuis* was lower than for the other strains and showed a high variability among the results of the different participants. In addition, the study provided information regarding the growth behaviour and kinetics of different bacterial species in PCs.

Those nine strains that demonstrated donor independent growth properties under "real life" conditions and show stability during the storage time will be recommended for inclusion in the WHO International Reference Repository of Platelet Transfusion Relevant Bacterial Reference Strains. As the tested *Morganella morganii* PEI-B-P-74 strain showed no growth, a second strain was tested in eight laboratories in accordance with the study protocol. The new strain showed growth in all tested PCs. The statistical evaluation shows very consistent data. The growth potential as well as the match of inoculum is comparable to the already existing WHO bacteria repository. It is recommended to add this strain to the bacteria extension list.

Stability testing of all investigated strains was performed routinely at PEI during the study period and beyond until April 2015.

The results of the collaborative study were presented to the TTID WP in several meetings (i.e. ISBT Congress Seoul, June 2014, extraordinary meeting TTID WP subgroup on bacteria Philadelphia, October 2014 and ISBT Regional Congress London June, 2015).

Literature

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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 (currently WHO)

In October 2010, the ECBS endorsed the project proposal to establish a WHO International Standard (WHO IS) for Mollicutes (mycoplasma) NAT. During the years that followed, a feasibility study with worldwide representation was performed which led to the identification of Mycoplasma fermentans as the most promising mycoplasma species for the use as the candidate WHO IS. In a comparability study, the candidate preparation containing Mycoplasma fermentans was characterized and International Units were proposed close to NAT detectable units. The whole project and the proposal were presented to ECBS in October 2013. The candidate material was adopted by the ECBS as the “1st WHO IS for mycoplasma DNA for NAT assays, designed for generic mycoplasma detection”.

The WHO IS standard project for mycoplasma NATs was summarized in a scientific publication which was accepted by the Journal “Applied and Environmental Microbiology” for its September 2015 issue.

As follow up of this project the WHO IS material was included in a panel of the organization QCMD (Quality Control of Molecular Diagnostics) to be evaluated in external quality assessment schemes (EQAS). In these studies the commutability of the material was further investigated in routine testing settings and no concerns of potential non-commutability were raised. Furthermore, the study showed quite variable sensitivities of the mycoplasma NAT assays used by the participants, which were mainly composed of in-house developed tests.

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, Margarethe Heiden

The BRN reported to the ECBS during the annual ECBS meeting at the WHO headquarters in Geneva and met for a closed meeting on Thursday, 16 October 2014. Furthermore, BRN telephone conferences were held on 12 August and 9 September 2014, and on 20 February and 26 May 2015.

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. When the serious outbreak of Ebola in West Africa became evident, the BRN elaborated a similar Position Paper on Collection and Use of Convalescent Plasma or Serum as an Element in Filovirus Outbreak Response in August 2014, which was posted on the BRN website. The WHO secretariat co-ordinated the organization of clinical trials aiming at 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 which operated by regular telephone conferences. Members of the BRN were included in and commented on drafts produced by this working group. In September 2014, the 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 was published, which was updated in April 2015.

The Ebola crisis dramatically increased the awareness for the importance of functioning national blood systems as part of the response to emergencies. The BRN had provided in 2012 the document Assessment Criteria for Evaluation of Blood Regulatory Systems. The document is available on the WHO BRN website. This document could be used to provide assistance in the implementation of national blood systems in line with the 2010 WHA Resolution on Availability, Safety and Quality of Blood Products (WHA 63.12). The BRN initiated its work on the development of a guidance document on the implementation of national blood regulatory systems.

The BRN discussed the proposal of desmopressin as candidate for the “WHO Model List of Essential Medicines”. Although desmopressin is not a blood product, and several BRN are not responsible for its regulation, the BRN endorsed the proposal since it considered desmopressin to be a useful alternative in the treatment of mild and moderate haemophilia. Further topics addressed by the BRN included risk-based decision making, potential hazards of transfusion, and national decision making on the MSM (men who have sex with men) deferral.

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 web site <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.

Uwe Unkelbach, Rainer Seitz, Sabine Wegehaupt, Micha Nübling (currently WHO)

The next activity of this project is scheduled for August 2015.

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 (currently WHO)

In the “WHO Workshop on Blood Testing and Risk Assessment as Part of Good Manufacturing Practices in Blood Establishments” (Jakarta, Indonesia, 9-13 June 2014) a session covered the estimation of residual risk for viremic blood components. This residual risk depends on the epidemiology of infections and testing strategy. Calculations based on different scenarios were introduced. This approach was followed up in a WHO -organized meeting in Geneva end of June 2015 where a small working group of experts, including Dr Sigrid Nick (PEI) and Dr Micha Nübling (ex-PEI, currently WHO) discussed a “WHO Guideline on the Residual Risk in Blood Components”. This technical document will provide guidance to the calculation of the residual risk of virus infections in blood components, including recovered plasma potentially being used for further manufacturing. Screening test features (diagnostic sensitivity in early infection) and donor epidemiology need to be known as parameters. Selective evaluation and assessment of new screening tests reflecting regional needs will also be covered in this document.

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

At the last SoGAT meeting (Standardization of Gene Amplification Techniques, see 2.6.2) in London, UK, in June 2015 the draft WHO guidance document on the Preparation and Calibration of Secondary Reference Preparations (IVD standards) was discussed. The participants confirmed the importance of this document. The draft document will be revised by including all relevant comments from the meeting discussion and sent out for a further review to the SoGAT people as well to regulators, authorities, and relevant associations of IVD manufacturers by the end of August 2015. The document will be updated and presented to the ECBS meeting in October 2015 for comments. The guidance document will be finalized in accordance with the outcome of the ECBS meeting.

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 (currently WHO), Sally Baylis

Discussions between the WHO Collaborating Centres (WHO CCs) on addressing commutability issues during development of WHO reference materials took place at different occasions (e.g SoGAT, see 2.6.2). There is an agreement between the WHO CCs that commutability needs to be addressed either in the collaborative study, in separate studies prior to establishment of a new reference material, or after establishment. Inclusion into proficiency testing programmes may provide further insight into commutability.

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.

Uwe Unkelbach, Sabine Heinz-Stempel, Sigrid Nick, Micha Nübling (currently WHO)

There were no activities between July 2014 and June 2015.

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 65th Meeting of the Expert Committee on Biological Standardization (ECBS), Geneva, Switzerland, 13-17 October 2014

At the annual meeting of the ECBS, the president of the PEI, Professor Cichutek, described the recent activities of the WHO Collaborating Centre and the current work plan. In particular, he highlighted the development of a hepatitis C core antigen standard which was established later by the committee (see Activity 3). Another standardization project at the PEI is a genotype panel for hepatitis E virus RNA for nucleic acid amplification technique (NAT)-based assays. Two existing reference preparations, HDV-RNA and mycoplasma DNA, are covered by follow-up studies. Another project is the enlargement of the first WHO International Reference Repository for platelet transfusion relevant bacterial strains by 11 further strains (see also resp. activities).

He also reviewed the recent activities of the PEI WHO CC for the Standardization and Evaluation of Vaccines, including participation in the activities of the WHO Strategic Advisory Group of Experts (SAGE), supporting the Developing Country Vaccine Regulators' Network (DCVRN) and the provision of training and technical assistance in a range of relevant areas.

The PEI was further involved in activities like (a) development of vaccines and treatments for Ebola; (b) epidemiological study of narcolepsy; (c) methods, particularly novel methods, for product testing; (d) illegal and falsified medicines; and (e) research activities.

At the end of this ECBS meeting, the HCV core antigen reference preparation was established as first WHO International Standard for this marker.

2.2 New project endorsed at 64th ECBS Meeting, Geneva, Switzerland, 13-17 October 2014

2.2.1 Development of an International Standard for IgG Antibodies to Human Cytomegalovirus (Anti-HCMV-IgG)

Nina Wissel, Dr Heiner Scheiblaue

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 and there is no international reference material available at the moment. There has been a PEI anti-HCMV-IgG reference material since 1982, which is frequently demanded by manufacturers for calibration of their anti-CMV test kits. But this material is of limited supply and is weak positive and not defined in international units. The proposed standard may serve for the calibration of the manufacturer's diagnostic kits, for quality control by competent authorities, and by users. The project to establish the "1st International Standard for anti-HCMV IgG" was endorsed by the ECBS in October 2013.

The candidate standard material is human plasma, highly positive for anti-HCMV-IgG with high avidity and is anti-HCMV-IgM negative to avoid interference with IgG. After lyophilization in 2013, 1900 ampoules were obtained. A collaborative study initiated in 2013 included (I) the candidate material, (II) the PEI anti-HCMV-IgG reference material, (III) a commutability panel with eight anti-HCMV positive samples with various IgG avidity and various anti-HCMV-IgM titer, as well as (IV) 50 human plasma samples negative for anti-HCMV-IgG and IgM to check specificity. The collaborative study included 15 laboratories from seven countries worldwide comprising reference laboratories, manufacturers and users. Overall, 20 different anti-HCMV-IgG/-IgM assays of various test formats and principles, including one neutralization assay (US FDA) were used. By the end of June 2015, data were returned by 13 laboratories. The evaluation of study results and statistical analysis is ongoing in 2015. A unitage will be assigned to the candidate material in order to evaluate the analytical sensitivity of anti-HCMV IgG assays and to improve comparability of results between laboratories. Submission of the report of the collaborative study to ECBS is planned in 2016.

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

Karin Weißer

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. 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.

Dr Weisser assessed 95 INN requests of biological substances from July 2014 to June 2015. She attended two consultations of the INN expert group (59th and 60th consultation in October 2014 and April 2015) where all comments were discussed and decisions on the selection of INNs were taken.

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

WHO normative guidance on post-market surveillance of in vitro diagnostic devices

Heiner Scheiblaue

WHO has developed guidance on post-market surveillance for in vitro diagnostic devices (IVD), with specific emphasis on applicability in resource-limited settings

(http://www.who.int/diagnostics_laboratory/postmarket/en/). The target audience for this document is: National regulatory authorities and national reference laboratories, end-users, procurers, implementing partners and manufacturers. Post-market surveillance aims to ensure that IVDs continue to meet the same quality, safety and performance requirements as when they were initially placed on the market.

The Guideline was initially developed with a number of countries (Ivory Coast, Burkina Faso, Republic of South Africa, Tanzania, and People's Republic of China) in a WHO workshop at PEI (21-23 February 2013). Following a 2nd meeting at WHO headquarters in Geneva (21-22 October 2014) the guideline was eventually finalized in March 2015.

Post-market surveillance consists of reactive surveillance after an issue has occurred related to the IVD, and proactive surveillance to scan for potential issues related to the IVD. Proactive post-market surveillance activities include lot verification testing (pre-distribution and post-distribution to end-users). The section on

batch testing was drafted by PEI-IVD Testing Laboratory.

Heiner Scheiblauber, Sigrid Nick

PEI IVD Laboratory continued to participate in the WHO Programme for the Prequalification of Diagnostics (see http://www.who.int/diagnostics_laboratory/evaluations/en/).

Activities in the context of the cooperation with the WHO Prequalification Team of the Department of Essential Medicines and Health Products included product dossier reviews and on-site audits of various HIV and HCV IVDs. These evaluations by PEI-IVD support WHO in their prequalification of diagnostics for access to safe, appropriate and affordable in vitro diagnostic devices of good quality in an equitable manner.

Heiner Scheiblauber

2nd Annual Meeting of WHO Prequalification of In Vitro Diagnostic Devices Dossier Assessors and Inspectors

The meeting was held to discuss and improve the WHO inspection and dossier assessment procedures, to further align internationally (Medical device single audit program (MDSAP); International Medical Device Regulators Forum (IMDRF)), and to establish harmonized and standardized principles.

2.5 Meetings and Workshops at WHO headquarters, Geneva

2.5.1 WHO International consultation on regulatory systems strengthening, 13-15 January 2015.

Dr Jens Reinhardt was invited to participate in the "WHO International Consultation on Regulatory Systems Strengthening" which took place at WHO headquarters, Geneva from 13-15 January 2015. The Regulatory Systems Strengthening (RSS) Team of the Department of Essential Medicines and Health Products (EMP) has launched an international consultation on regulatory system strengthening. This consultation started in early October 2014 through a series of online WebEx meeting sessions. The work is organized under the leadership of three main working groups (WG): WG1: Policy, terminology and prequalification, WG2: Methodology and process of National Regulatory Authority (NRA) assessment, and WG3: Functions, indicators and assessment tools. A white paper was available as a basis to discuss policy matters and strategic directions of the WHO programme for strengthening regulatory systems. Furthermore, different multinational as well as regional approaches for the strengthening of the regulatory systems were discussed during the meeting, e.g. the prequalification programme by WHO, as well as possibilities and challenges of their harmonization.

2.5.2 Meeting on WHO Guidelines "Estimation of Residual Risk of Virus Infections in Recovered Plasma /Blood Components", 17-18 June 2015

Dr Sigrid Nick gave an introduction to "State of art CE-marked serological assays: variation of diagnostic window phase lengths for combo, antibody ELISAs and rapid diagnostics".

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) 22nd Workshop on Surveillance and Screening of Blood Borne Pathogens, Prague, Czech Republic, 20-21 May 2015

PEI co-organizes this annual scientific meeting. The primary focus concerns the application of nucleic acid amplification tests (NAT) and other measures to increase blood safety, with standardization of assays being one of the regular topics in this series of meetings. Dr Micha Nübling, Dr Johannes Blümel, Dr Annette Reissinger, and Dr Michael Chudy attended the meeting in May 2015.

The well attended 22nd anniversary workshop – more than 200 participants mainly from blood banks, academic institutions, regulatory authorities and diagnostic industry – focused on risk-based decision making, a special session on HEV, the algorithm to be applied for non-repeatedly reactive test results obtained by NAT testing of small pools or of individual donations. Progress in development of blood screening IVDs was presented in the manufacturers' session. Another session focused on the situation caused by Ebola outbreaks in African countries, and the potential benefit of convalescent plasma for the treatment of patients.

2.6.2 SoGAT (Standardization of Gene Amplification Techniques) – 3rd Joint Blood Virology and Clinical Diagnostics Meeting/ 20th Anniversary SoGAT Workshop - 'Past Successes and Future Challenges', London, UK, 25-26 June 2015

The third joint meeting of the two SoGAT groups (blood virology, clinical diagnostics) was attended by Dr Michael Chudy and Dr Micha Nübling. This meeting was designed more for exchange and discussion on topics related to standardization like real life use of WHO International Standards (IS) beyond calibration of secondary standards (the only WHO IS function described by WHO). Problems with stability of some analytes were also presented and discussed, and fields with gaps of standardization were discussed, with the intention

to define prioritization aspects. Last but not least the draft WHO guideline (prepared by a group around M Chudy, PEI) on calibration of secondary standards was discussed. A proposal to include the standardization of serological assays into the scope of SOGAT was presented.

Dr Sigrid Nick contributed in "Session 4 The birth of Serological International Standards" by giving a talk on HCV core antigen standardization.

2.7 Further conferences with CC relevant topics attended by PEI co-workers (chronological order): IPPC (International Plasma Protein Congress), Rome, Italy, 10-11 March 2015. Dr Micha Nübling gave a presentation on epidemiological data in the Plasma Master File and the EMA approach to assess this information; another presentation of Dr Nübling was on issues around convalescent plasma collected for the treatment of Ebola patients. Dr Anneliese Hilger gave a presentation on recent developments in the field of clotting concentrates. She addressed aspects like new products, availability of factor concentrates, patients' needs and regulatory requirements

14th Planova Worksop (Washington, USA 12-13 July 2014), Dr Johannes Blümel gave a presentation on the regulatory overview with a focus on nanofiltration.

Workshop on viral safety of plasma-derived medicinal products with respect to hepatitis E virus. European Medicines Agency (London, UK, 28-29 October, 2014). Dr Johannes Blümel chaired the workshop. Dr Sally Baylis gave a presentation on testing methods for donor screening and plasma pools.

Blood, Blood Components and Plasma – Quality and Safety (Heidelberg, Germany, 15-16 April 2015). Dr Sally Baylis gave a presentation on hepatitis E virus.

9th Plasma Product Biotechnology Meeting (Cagliari, Sardinia, 11-14 May 2015). Dr Johannes Blümel gave a presentation on "Regulatory Implications of Hepatitis E Virus and Plasma-Derived Medicinal Product's".

IPFA/PEI 22nd International Workshop on "Surveillance and Screening of Borne Pathogens" Prague, Czech Republic, 20-21 May 2015. Dr Blümel gave a presentation on "Regulatory implications of hepatitis E virus." The 20th Annual Meeting of the OMCL (Official Medicines Control Laboratory) of EDQM (European Directorate for the Quality of Medicines & HealthCare) took place in Brussels, Belgium, from 1-5 June 2015. More than 15 participants from PEI participated in this conference. Dr Uwe Unkelbach and M. Wierer chaired the meeting of the first day: the "OCABR (Official Control Authority Batch Release) Blood Session". He also chaired the "Common OCABR Session" on the second day of the conference together with three other colleagues from the OMCL Network.

15th International Symposium on Viral Hepatitis and Liver Disease (Berlin, Germany, 26-28 June, 2015). Dr Sally Baylis gave a presentation on HEV diagnostics at the EASL (European Association for the Study of the Liver) symposium on hepatitis E.

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 Participation in collaborative studies of WHO International Blood Product Standards

In 2014/2015, the PEI section "Batch Release of Blood Products, Logistics", Division "Haematology" participated in the collaborative study to assign value potencies to International Standards for Factor IX (CS519):

1. The 5th International Standard for Factor IX Concentrate as well as a European Pharmacopoeia (EP) Factor XI Biological Reference Preparation (BRP);
2. An International Standard for recombinant Factor IX and an EP recombinant Factor IX BRP;
3. Factor IX antigen to the 4th International Standard for Factor II, VII, IX, X, Plasma.

The study is still ongoing pending the issuing of the report to the participants for comments.

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

In the reporting period the section "Molecular Virology", Division "Virology" at PEI, participated in the following WHO collaborative study:

- Proposed 5th WHO International Standard for Hepatitis C Virus for NAT-based assays (NIBSC, Potters Bar, 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).

n/a