Collaborating Centres ANNUAL REPORT

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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	07-2013 to 07-2014		

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 Hepatitis B e-antigen (HBeAg) is a diagnostic marker for HBV infectivity. It is first detectable in the early phase of HBV infection after the appearance of Hepatitis B surface antigen (HBsAg). The titers of both HBV antigens, HBsAg and HBeAg rise rapidly during viral replication in acute infection. A proposal to establish an international standard for HBeAg was presented at the 3rd WHO Collaborating Centres Meeting in 2011 and was subsequently endorsed by the Expert Committee on Bio-logical Standardization (ECBS) in October 2011. Such an HBeAg standard would help to assess the sensitivity and control the quality of HBeAg test kits both by manufacturers and regulators.
A WHO collaborative study was initiated in 2012 to determine the suitability of the candidate material as a standard for the detection of HBeAg in diagnostic assays. Two materials were compared: (1) the candidate HBeAg International Standard preparation (current Paul-Ehrlich-Institut (PEI) HBeAg standard, lyophilized) and (2) the current PEI HBeAg Standard ("HBe-Referenzantigen 82"). Nineteen laboratories from 12 countries worldwide participated and tested the materials using 14 different commercially available HBeAg assays. The dilution range of the candidate HBeAg material was within the dynamic measuring range of all assays. The endpoint titres ranged from 1:237 to 1:3904. As the PEI HBeAg standard 82 (sample 2, 100 PEI U/mL) has been used worldwide since 1982, the antigen content of the candidate material was expressed relative to the PEI standard. The overall potency of the can-didate material was 95 U/mL relative to the PEI standard. Thus, the difference from the source material is approximately 5 U/mL. This difference lies within the normal intra-assay variation of HBeAg assays and is therefore considered negligible.
To prove the commutability of the candidate HBeAg standard, additional material was analysed in the collaborative study. Three HBeAg positive samples from Hepatitis B virus (HBV) infectious carriers (a high positive HBeAg serum sample, a positive HBeAg serum sample and a low positive HBeAg plasma sample) were ana-lysed in the collaborative study and, in addition to that, 51 clinical samples from HBV seroconversion and longitudinal panels were analysed at the Paul-Ehrlich-Institute using five commercially available HBeAg assays. The investigations performed on these samples demonstrated the commutability of the candidate material.
An accelerated stability study on the candidate HBeAg material revealed a stable recovery at temperatures up to 45°C for one week which may suggest long-term stability. A real time long-term stability study is still ongoing and now includes stability data for one year. These data further support the suggested long-term stability.
The standard was adopted by the ECBS as the 1st WHO International Standard for Hepatitis B Virus e Antigen (HBeAg) in October 2013.

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 Scheiblauer
The 1st WHO International Standard for anti-hepatitis B virus e antigen (anti-HBe) was adopted by the ECBS in October 2013. Following acceptance of the proposal for the anti-HBe Standard in 2011, the standard was developed by a WHO collaborative study carried out in 2012 including 21 laboratories from 12 different countries with 16 different anti-HBe test kits. Commutability of the standard was demon-strated by an additional study with a series of panels which received a favourable response from the ECBS. The potency of the anti-HBe Standard was determined to be 120 international units per mL (120 IU/mL).
The presence of anti-HBe is considered to be a clinical sign of recovery from hepatitis B virus (HBV) infection. Testing of anti-HBe antibodies is therefore useful for monitoring the course of an HBV infection, e.g. for successful therapy against HBV. In addition, anti-HBe without detectable HBeAg during HBV infection may indicate the presence of precore stop codon mutants.
The anti-HBe Standard thus offers the possibility to standardize anti-HBe kits for control of analytical sensitivity and for better comparability of anti-HBe results between laboratories.

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 Scheiblauer
An International Standard for Hepatitis C Virus Core Antigen (Ag) was proposed by PEI and endorsed by the ECBS in 2009. After intensive search and characterization of a suitable material in 2011 and 2012 and further discussions with the WHO Collaborating Centres in April 2013, a collaborative study was carried out in 2013/14.
The candidate standard is a lyophilized plasma preparation obtained from a donor infected with HCV genotype 1a. Twelve laboratories from nine countries worldwide participated in the collaborative study to evaluate and characterize the candidate material (sample A) using the assays in routine use in their laboratory alongside with the corresponding liquid frozen bulk material (sample B) and four liquid frozen neat and diluted HCV core antigen positive plasma specimen (samples C to F). Six assays were used including two quantitative and one qualitative HCV Ag assays as well as three qualitative HCV Ag/Ab combination assays. The assays´ analytical sensitivities were determined to assess the potency. The results showed considerable differences in analytical sensitivity between the various assays yielding an endpoint titre range from 1:2 for the least sensitive assay to 1:3200 for the most sensitive assay. The same differences in sensitivity between the assays as with the candidate standard A were reflected by samples C to F. Intra-laboratory and inter-laboratory variability for sample A and the bulk material sample B were in an expected range for immunoassays, and it can therefore be assumed that the candidate material was still homogenous after the lyophilization step.
To prove commutability of the candidate standard, a complementary study was performed with a selected number of representative assays using low, medium and high HCV core antigen positive clinical samples from early HCV infection (seroconversion). This additional study demonstrated that seroconversion sensitivity is correlated to analytical sensitivity as determined with the candidate standard sample A.
Altogether, the study results show that there was good comparability of sensitivity for HCV core antigen of the candidate standard sample A between the various assays and laboratories. The potency of the candidate standard material is however dependent on the sensitivity of the test kits. It was therefore decided to determine the units based on highest sensitivity.
Accelerated and on-going real-time stability studies of the proposed 1st WHO International Standard for HCV core antigen indicate that the preparation is stable and suitable for long-term use when stored as recommended.
It is proposed that the candidate material (PEI code 129096/12) is established as the 1st WHO International Standard for HCV core antigen for use with HCV core antigen assays 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 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 5.75×105 International Units per mL (IU/mL) when reconstituted in 0.5 mL of nuclease-free water. Follow-up national and international studies are ongoing to also address commutability aspects of the reference material.

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. Since that time, HEV-positive plasma sam-ples have been evaluated from blood/plasma donors including genotype 3 strains which are clinically important in chronic HEV infections in Europe. Additional strains available from earlier studies include 3b (Japanese derived), and 4c (from Japan) and these will be supplemented by a further genotype 4g sample from Japan. Genotype 1 plasma samples have been obtained from India and Africa which will be included in the panel. The plasma samples have been lyophilized, with ~1000 vials prepared for each sample. In addition, high titre stool samples are available and include a further genotype 1 virus strain from India and a genotype 3 sample from an immuno deficient French patient where the HEV strain is highly related to rabbit HEV as well as genotype 2. Formulation of the additional samples has been investigated, and lyophilization is pending prior to launch of a collaborative study in Q4, 2014.

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 pneumonia (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 random study was started, Q1, 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 Rele-vant Bacterial Strains plus 11 candidate strains) were shipped to the participating laboratories. At the request of ECBS, the Repository strains were included again in the enlargement study as reference strains.
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) were produced. Stability testing and identification was performed. The shipment of the 11 candidates as well as the four WHO Repository strains started (Q4, 2012, Q1 and Q2 2013). Start of the study (Q1, 2013) with 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 labs were finished in June 2014. For the time being, the results are being statistically evaluated and reviewed for reporting.
Literature
1_Palavecino EL, Yomtovian RA, Jacobs MR. Bacterial contamination of platelets. Transfus Apher Sci. 2010; 42:71-82.
2_Montag T, Strategies of bacteria screening in cellular blood components. Clin Chem Lab Med. 2008; 46:926-32.
3_ Störmer M, Arroyo A, Brachert J, Carrero H, Devine D, Epstein JS, Gabriel C, Gelber C, Goodrich R, Hanschmann KM, Heath DG, Jacobs MR, Keil S, de Korte D, Lambrecht B, Lee CK, Marcelis J, Marschner S, McDonald C, McGuane S, McKee M, Müller TH, Muthivhi T, Pettersson A, Radziwon P, Ramirez-Arcos S, Reesink HW, Rojo J, Rood I, Schmidt M, Schneider CK, Seifried E, Sicker U, Wendel S, Wood EM, Yomtovian RA, Montag T. Establishment of the first international repository for trans-fusion-relevant bacteria reference strains: ISBT Working Party on Transfusion-Transmitted Infectious Diseases (WP-TTID), Subgroup on Bacteria. Vox Sang. 2012; 102:22-31.

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
In October 2010, the ECBS had endorsed the project proposal to establish a WHO International Standard (WHO IS) for Mollicutes (mycoplasma) NAT. During the following years, 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”.
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 will be further investigated in routine testing settings though there are no concerns of potential non-commutability so far.

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 WHO Blood Regulators Network (BRN), established following a recommendation of the 11th International Conference on Drug Regulatory Authorities (ICDRA, Madrid, Spain, 16–19 February 2004), has been operating since 2006.
The BRN reported to the ECBS during the annual ECBS meeting at the WHO head-quarters in Geneva and met for a closed meeting on 24 October 2013. Furthermore, BRN telephone conferences were held on 16 October, 11 December 2013, and on 24 January, 7 March, 14 March, and 1 May 2014.
A major focus of the BRN in the past few years was the preparation of Assessment Criteria for Evaluation of Blood Regulatory Systems. The document is available on the WHO BRN website. The project is expected to be an important contribution to the implementation of the WHA Resolution on Availability, Safety and Quality of Blood Products (WHA 63.12).
The BRN was involved in discussions with WHO experts concerning the emergence of Middle East Respiratory Syndrome Coronavirus (MERSCoV). In July 2009 the BRN had provided the document Position Paper on Collection and Use of Convales-cent Plasma or Serum as an Element in Pandemic Influenza Planning. This document was taken as the basis for drafting recommendations accordingly adapted for MERSCoV outbreaks. The resulting document Position Paper on Collection and Use of Convalescent Plasma or Serum as an Element in Middle East Respiratory Syndrome Coronavirus Response is also available on the BRN website.
In the last ten years, there was an upcoming public discussion on the life-long ban for men who have sex with men as blood donors. Internationally, there is an increasing pressure upon regulatory bodies using more or less scientific argumentations against this permanent deferral. To give an insight as to how decisions in the field of blood safety should be made, a report on A shared regulatory perspective on deferral from blood donation of men who have sex with men (MSM) was elaborated by the BRN members and published in Vox Sanguinis DOI: 10.1111/vox.12166).
The actual items of discussion as well as the minutes of BRN meetings and telecon-ferences are confidential. However, general information about the BRN and docu-ments 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
Dr Sigrid Nick and Dr Micha Nübling attended the “WHO Workshop on Blood Testing and Risk Assessment as Part of Good Manufacturing Practices in Blood Establishments” which took place in Jakarta, Indonesia on 9-13 June 2014. During this workshop, the different sessions were organized by the two PEI employees, together with Dr Ana Padilla from WHO. The workshop identified the need in countries like Indonesia for regulation of in vitro diagnostic devices (IVD) covering blood screening devices.
Dr Sabine Wegehaupt participated in the “WHO Workshop on Enforcement and Im-plementation of Good Manufacturing Practices (GMP) for Blood Establishments” held in Jakarta, Indonesia, 24-27 June 2014. She provided training on “Practical Aspects - How to perform an inspection in Blood Establishments” and “Good Manufacturing Practices - Standard Operating Procedures, Premises & Equipment”. In addition, she held a workshop in the style of a mock-inspection at a blood establishment.
All activities were financed by WHO.

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
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 compo-nents. This residual risk depends on the epidemiology of infections and testing strategy. Calculations based on different scenarios were introduced. This approach will be explained in the planned WHO Technical Document on the Residual Risk in Blood Components. The project is still ongoing.

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 SoGAT meeting (Standardization of Gene Amplification Techniques, see 2.6.2) in Graz, Austria, in May 2014 a round table discussion was held dealing with key elements which should be covered by the WHO Technical Document on the Preparation and Calibration of Secondary Reference Preparations (IVD standards). A follow-up consultation with NIBSC colleagues in September 2014 is planned to proceed with the draft of the WHO document.

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, Sally Baylis
Discussions between the WHO Collaborating Centres on addressing commutability issues during development of WHO reference materials took place at different occa-sions (e.g SoGAT, see 2.6.2). The final outcome will be summarized in a Technical Document on Commutability (work in progress). PEI is supporting WHO in drafting the guidance document.

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
Sabine Heinz-Stempel and Micha Nübling participated in the “WHO Workshop on Blood Regulatory Systems”, held in Johannesburg, South Africa from 9-11 Septem-ber 2013. Ms Heinz-Stempel provided training on the “Principles of Good Manufacturing Practice in the Production of Blood and Blood Components” as well as the ” Principles of Good Manufacturing Practice - Impact on Blood Safety and Plasma Fractionation Programs”. This activity was financed by WHO.
Dr Nübling gave presentations on the “Evaluation and Quality Assurance of Tests Kits for Transfusion Transmitted Infections” and on “Impact of Epidemiology and Laboratory Testing Strategies on Blood Safety”. This activity was financed by PEI.

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 64th Meeting of the Expert Committee on Biological Standardization (ECBS), Geneva, Switzerland, 21-25 October 2013
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. The need for global harmonization of regulation was mentioned against the background of increasing manufacturing activities of biopharmaceuticals or APIs (active pharmaceutical ingredients) in third countries. Current activities of the PEI in the field of standardization of testing methods (e.g. replacement tests for animal testing) and of more basic research with

a potential of future application (e.g. vaccine platform based on recombinant measles virus) were highlighted. Standardization efforts in the field of allergies might become cooperation projects with WHO. Advanced therapy medicinal products (ATMPs) or biomarkers covered by companion diagnostics are new fields where input and request of stakeholders should be sought to understand their needs before standardization efforts are started.
At the end of this ECBS meeting several reference preparations developed by the PEI were adopted by the Committee, including the 1st WHO ISs for HDV RNA, mycoplasma DNA, HBeAg and anti-HBe (see respective activities). The main topics at the 64th ECBS included follow-up information on the so-called ACHILLES project, a WHO initiative to assure safety and availability of blood products in developing countries with different activities organized by WHO in Indonesia as pilot country (see activities 9 and 13).
The “WHO Workshop on Blood Regulatory Systems held in South Africa” in September 2013 with the participation of both blood establishment representatives and regulators from 11 sub-Saharan countries was also reviewed. This workshop elaborated recommendations for further WHO and national activities.
Another important activity was the “WHO Consultation on Commutability of WHO Biological Reference Preparations for in vitro Detection of Infectious Markers” held in April 2013 with 50 participants. There was a general consensus on the various approaches to address this issue.
Progress had been made with blood and blood components now being listed as essential medicines. This listing will facilitate more appropriate support and infrastructure in blood systems in low and middle-income countries.

2.2 New project endorsed at 64th ECBS Meeting, Geneva, Switzerland, 21-25 October 2013
2.2.1 Development of an International Standard for IgG Antibodies to Human Cyto-megalovirus (Anti-HCMV-IgG)
Nina Wissel, Dr Heiner Scheiblauer
Human Cytomegalovirus (HCMV) is spread worldwide with a prevalence between 40% and 100%. The virus causes relatively mild and asymptomatic infections in im-mune-competent persons, but can cause congenital disease and severe complica-tions in those with immunodeficiency, e.g. immunological immaturity, acquired immunodeficiency or immunosuppression. In addition, avoidance of blood products from primarily seropositive HCMV donors is especially helpful to avoid transfusion transmitted HCMV. 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 relatively weak positive and not defined in international units.
Therefore a project was initiated to establish the 1st International Standard for anti-HCMV IgG. The project was presented at the WHO Collaborating Centres Meeting in April 2013, subsequently proposed to WHO and endorsed by the ECBS in October 2013.
The project activities in 2013 focused on a feasibility study to select and characterize human plasma specimens adequate for a candidate standard preparation. The material finally selected is high positive for anti -HCMV-IgG with high avidity and is anti-HCMV-IgM negative to avoid interference with IgG. The selected material was filled in 1900 ampoules à 1.0 mL and was lyophilized. A collaborative study was started in late 2013 including 15 laboratories comprising reference laboratories, manufacturers and users, from eight countries worldwide with 10 different anti-HCMV assays of various test formats and principles. In addition, to demonstrate commutability to the candidate material, a reference panel with eight anti-HCMV positive plasma samples has been included: eight anti-HCMV-IgG positive samples with low or high IgG avidity and anti-HCVM-IgM positive (low to high) or anti-CMV-IgM negative. Moreover, 50 human plasma samples negative for anti-HCMV-IgG and IgM were included with a range of potential cross-reactivities to other herpes viruses and to check specificity. The collaborative study is on-going and the data available are currently evaluated. If possible, 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. The standard may also serve the calibration of the manufacturer´s diagnostic kits, for quality control by competent authorities and by users.

2.3 International Nonproprietary Names (INN) of blood products and monoclo-nal 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 selection and publication of INNs fall within the responsibility of the WHO unit Quality Assurance and Safety of Medicines (QSM), Department of Essential Medicines and Health Products in the Health Systems and Innovation (HIS) cluster at WHO headquarters. 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 94 INN requests of biological substances from July 2013 to June 2014. She attended one consultation of the INN expert group (58th consultation in April 2014) where all comments were discussed and decisions on the selection of INNs were taken. In addition, she attended one separate discussion meeting on Biological Qualifiers on 7 April 2014.

2.4 Cooperation with WHO in the area of WHO´s Prequalification Programme for in vitro Diagnostic Devices (IVD) and Procurement of IVDs
PEI IVD Laboratory continued to participate in the WHO Programme for the Prequali-fication 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 the following:
• A product dossier review of the Advanced Quality Rapid Anti-HCV Test was performed. These evaluations by PEI-IVD support WHO in their prequalification of diagnostics for access to safe, appropriate and affordable in vitro diag-nostics of good quality in an equitable manner.
• In the context of quality control of prequalified diagnostics, a guideline was elaborated based on the results of the WHO training workshop on post-market surveillance of HIV diagnostics at the Paul-Ehrlich-Institut, 21-23 February 2013. The guideline is currently distributed for revision.

As in previous years, further international activities in the context of quality control concerned batch testing of HIV rapid test devices for other international organiza-tions like Population Services International (PSI, Washington, DC, USA) and Global Fund Quality Assurance of Diagnostic Products.

2.5 Meetings and Workshops at WHO headquarters, Geneva
2.5.1 Expert Review Panel for Diagnostics (EPRD) Meeting on Technologies for HIV Early Infant Diagnosis, Pilot_EoI of 13 February 2014
Dr Micha Nübling was invited to participate in the 1st meeting of the Expert Review Panel for Diagnostics. This panel has been established to review applications of IVD manufacturers to be listed for procurement despite currently missing prequalification. This mechanism shall allow fast access of IVDs to markets where they are needed, based on benefit risk estimation. During the first meeting, different applications for the HIV early diagnosis in infants were reviewed, and recommendations of the panel were prepared. See also 2.4, WHO Prequalification Programme.

2.6 Other (non- WHO) meetings and workshops, related to WHO and PEI CC ac-tivities (chronological order)
2.6.1 IPFA/PEI (International Plasma Fractionation Association) 21st Workshop on Surveillance and Screening of Blood Borne Pathogens, Rome, Italy, 21-22 May 2014
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 Julia Kress, Dr Sally Baylis, Dr Sigrid Nick, Dr Margarethe Heiden, and Dr Heiner Scheiblauer attended the meeting in May 2014.
The well attended 21st anniversary workshop - more than 200 participants mainly from blood banks, academic institutions, regulatory authorities and diagnostic industry - focused on advances in pathogen removal and inactivation, on borderline trans-fusion risks and on donor policies in different regions. Further topics included 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.

2.6.2 SoGAT (Standardization of Gene Amplification Techniques) – 2nd Joint Blood Virology and Clinical Diagnostics Meeting, Graz, Austria 28-29 May 2014
The second joint meeting of the two SoGAT groups (blood virology, clinical diagnos-tics) was attended by Dr Michael Chudy and Dr Micha Nübling. The meeting started with an update session on International Standard development. Another session covered the issue of commutability of reference preparations. For the first time, a separate session on serology related topics was organized with the decision to con-tinue in this way in future SoGAT meetings. Discussions took place on the draft guideline on secondary reference materials, followed by a session on regulatory topics, especially screening requirements.

2.7 Further conferences with CC relevant topics attended by PEI co-workers (chronological order):
IPPC (International Plasma Protein Congress), Vienna, Austria, 11-12 March 2014. Professor Rainer Seitz gave a presentation entitled “Wildbad Kreuth Initiative 2013: Outcomes and Next Steps”, Dr Annelie Hilger gave a presentation on the revision of the clinical trial legislation in Europe.
The European Directorate for the Quality of Medicines & Health Care organized a meeting on “Establishment of a Common Test for Procoagulant Activity of Immuno-globulins”, Strasbourg, France, 18-19 March 2014. Priv. Lect. Dr Johannes Dodt chaired the closed session of the meeting. Dr Nannette Gross gave two presenta -tions during the meeting on the status of the dossier evaluation and conclusions from the available data.
European Haemophilia Consortium. World Haemophilia Day Event on: EDQM Hae-mophilia Recommendations Follow Up on the Wildbad Kreuth Symposium 2013, 16 April 2014, hosted by the Paul-Ehrlich-Institut. Presentations by patient representa-tives, physicians and the co-sponsors of the Kreuth Symposium (European Directorate for the Quality of Medicines (EDQM), Ludwig-Maximilians-University Munich and Paul-Ehrlich-Institut). Professor Rainer Seitz gave an overview of the regulation of therapeutic haemophilia products in Europe. The meeting was documented in a video available under https://www.youtube.com/watch?v=XxWAw40W4Ms.
OMCL (Official Medicines Control Laboratory) Annual Meeting of EDQM (European Directorate for the Quality of Medicines & HealthCare), 19-23 May 2014, Interlaken, Switzerland. Various participants from PEI including Dr Susanne Breitner-Ruddock and Dr Uwe Unkelbach.
2014 PDA Virus & TSE Safety Forum, Bethesda, MA, USA, 2-5 June 2014.
PEI participant: Dr Johannes Blümel. Dr Blümel gave an update on European regulatory issues concerning adventitious agents with special emphasis on porcine-derived trypsin, urine-derived medicinal products and hepatitis E virus.
ISTH SSC Meeting, Milwaukee, USA, 23-26 June 2014 Dr Annelie Hilger gave a presentation on “Characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples – outcome of workshop organised by EMA/EDQM November 2013”.
13th International Conference on Thalassemia & Hemoglobinopathies, Abu Dhabi, UAE, 22-23 Oct 2013. PEI participant: Dr Michael Chudy. He gave two presentations on the impact of emerging and re-emerging pathogens on blood safety with special focus on developed and developing countries.
Joint workshop of European Directorate for the Quality of Medicines & Health Care (EDQM) and European Medicines Agency (EMA) “Characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples”, London, United Kingdom, 28-29 November 2013.
Priv. Lect. Dr Johannes Dodt chaired this workshop. Dr Annelie Hilger attended the workshop as chair of the EMA Blood Product Working Party. Both were members of the steering committee.

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
The PEI section “Batch Release of Blood Products, Logistics”, Division “Haematology” participated in several collaborative studies in 2013/2014. The proposed candi-date WHO International Standard (IS) materials were processed at NIBSC, UK in accordance with the WHO guidelines for the production of reference materials.
• BSP 112 Investigate potency discrepancies using Factor VIII concentrates, comparison of different methods: F VIII chromogen and FVIII clotting testing;
• PTS 136 Comparison of Factor IX clotting and FIX chromogen testing methods;
• BSP 119 Establish a new BRP for PKA. The PEI’s results were in good agreement with the other participating laboratories.
PEI was also involved in an extensive collaborative study to investigate the comparability of recombinant and new generation Factor IX products with WHO IS for FIX Concentrate (CS503); the PEI’s results were in good agreement with the other participating laboratories.

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 1st WHO International Standard for Dengue Virus Types 1 to 4 (DENV-1 to -4) RNA for NAT Assays (CBER/FDA, USA).