INTERNATIONAL WORKING GROUP ON THE STANDARDISATION OF GENOME AMPLIFICATION TECHNIQUES (SoGAT) FOR CLINICAL DIAGNOSTICS

SoGAT Clinical Diagnostics I, 24-25 June 2008

National Institute for Biological Standards and Control (NIBSC), UK

Report of the meeting prepared by J Fryer (NIBSC) and C Morris (NIBSC)

The SoGAT working group for the safety testing of blood, tissues and organs for blood-borne pathogens has played an important role in the standardisation of nucleic acid amplification techniques (NAT) for blood safety. Regular meetings of this group have taken place since 1995, and have provided a valuable and unique forum for the development of WHO International Standards and other reference reagents for the standardisation of NAT for blood-borne pathogens. At the 20th SoGAT meeting, held on 12th -13th June 2007 in Warsaw, Poland, it was agreed that the knowledge and experience of SoGAT could be applied to other areas of public health, such as the standardisation of NAT assays used in the diagnosis of other clinical pathogens. It was therefore announced that the SoGAT blood safety meeting would be moved to a 2-year cycle, and that a new working group focusing on the standardisation of clinical diagnostics would be established. The first meeting of the SoGAT Clinical Diagnostics working group was held at NIBSC on 24th -25th June 2008 and was chaired by Dr Philip Minor (Deputy Director, NIBSC). Presentations from the meeting are available at the following link: www.nibsc.ac.uk/spotlight/sogat.aspx

Session 1: Introduction to Clinical Diagnostics

Clinical NAT: why we need run controls
Dr Bill Carman (WoSSVC, UK) described the implementation of NAT-based assays into the routine diagnostic service at the West of Scotland Specialist Virology Centre in Glasgow. Since the year 2000, the replacement of traditional cell culture and direct immunofluorescence diagnostic tests with PCR (and more recently real-time PCR) has resulted in an improved detection rate for many clinical pathogens. The resulting increased demand for molecular assays (and increased cost) was overcome through the development of a specimen-based approach to streamline testing by multiplexing real-time PCR assays according to clinical indication. The centre currently performs a total of 9 multiplexed real-time PCR tests on clinical samples including; vesicle fluids, genital specimens, gastroenteritis stool samples, eye swabs, blood samples from transplant patients, and CSF samples; reduced from 21 single and duplex assays performed in 2006. Dr Carman also highlighted the difficulty in sourcing material for day-to-day control of PCR assays and the need for standardised run controls. Dr Carman acknowledged that while virological NAT testing is advancing rapidly, there is also a need to pre-empt the widespread implementation of molecular assays for bacterial, fungal and parasitic targets, and help standardise these assays.

Clinical microbiology and infection control: molecular technology and quality control in microbiology laboratories
Professor Brian Duerden (Department of Health, UK) highlighted the importance of quality control (QC) in microbiology laboratories. Traditionally in the UK, external
quality assurance (EQA) has been provided by proficiency programmes such as those run by UK NEQAS. Meanwhile, internal quality control (IQC) has been the responsibility of individual laboratories. Professor Duerden questioned whether this approach was sufficient considering the changes in laboratory practice, including the implementation of molecular methods, automation, and the use of commercial kits and reagents. Professor Duerden also supported the need for obligatory (CPA/UKAS) accreditation and the use of standardised (and generally externally-sourced) quality control material in every run to monitor inter-assay performance.

Session 2: Standardisation and Aims of SoGAT

Aims of SoGAT and the role of NIBSC
**Dr Philip Minor (NIBSC, UK)** provided a background to the aims of SoGAT and the role of NIBSC in the standardisation of NAT assays for blood-borne viruses. SoGAT was initially established in 1995 to harmonise the NAT testing of blood and blood products for blood-borne pathogens associated with ‘window period’ donations. Proficiency studies performed in the early 1990’s demonstrated variability in the performance of these assays between manufacturers of blood products and industry regulators, which could lead to a delay in the release of a product. The approach to standardise assays was through the use of common reference materials which would improve comparability of results. The principal aim of the SoGAT group is to plan and coordinate collaborative studies for the development, evaluation and provision of reference materials for these targets, such as international standards and working reagents. Meetings of this group are currently held once a year, and involve representatives from official medicines control laboratories (OMCLs), plasma manufacturers, manufacturers of diagnostic kits, regulatory authorities, and academic and diagnostic laboratories. Over a number of years the group has facilitated the development of international standards and reference preparations for blood-borne viruses which have resulted in an overall improvement in the performance of NAT-based assays developed for these targets.

Standardisation: calibration of international standards, reference preparations and working standards

**Dr Micha Nübling (Paul-Ehrlich Institute (PEI), Germany)** described the types and intended use of different reference preparations that have been developed with the involvement of the SoGAT working group. The World Health Organisation (WHO) establishes biological reference preparations used in the prevention, treatment or diagnosis of human diseases. WHO International Standards are reference preparations with a defined biological activity, expressed in International Units (IU). They are used to calibrate secondary references used in routine assays in terms of the IU, thereby providing a uniform reporting system. Candidate WHO International Standards are assigned an arbitrary potency usually based on a mean titre estimate determined in an international collaborative study involving laboratories performing a range of assays. The report from this study and additional stability assessments are presented to the WHO Expert Committee on Biological Standardisation (ECBS) for formal establishment of the International Standard. Secondary standards, such as working reagents are used to monitor the performance of routine assays and are often calibrated in IU by direct comparison with the WHO International Standard. Dr Nübling described the preparation and calibration of secondary references, in particular, the HCV RNA Biological Reference Preparation produced for the
European Directorate for the Quality of Medicines (EDQM) for the batch release testing of manufacturing human plasma pools.

**Experience with standardisation of blood virology NAT**

Mrs Clare Morris (NIBSC, UK) described in further detail, the development and evaluation of working reagents and WHO International Standards for blood-borne viruses, and in particular, those for HIV-1 RNA. The need for standardisation of early NAT-based assays for HIV-1 was highlighted following the collaborative study evaluation of the first HIV-1 DNA proficiency panel at NIBSC in 1992. Results from this study showed that 54% of participants reported incorrect results. Subsequently, a working reagent was developed at NIBSC which would enable routine monitoring of both intra and inter-assay performance. Similar working reagents were also developed by other laboratories and organisations and expressed in different unitages. Therefore, an internationally-accepted higher order reference was needed against which these different working references could be calibrated. The collaborative studies to establish the first WHO International Standard for HIV-1 RNA in 1999, as well as its replacement in 2005, were summarised.

**Session 3: Standardisation for Clinical Diagnostics Part 1**

**WHO collaborative study to establish WHO International Standards for human papillomavirus (HPV) type 16 DNA and HPV type 18 DNA nucleic acid amplification technology (NAT)-based assays**

Dr Dianna Wilkinson (NIBSC, UK) described the development the first WHO International Standards for human papillomavirus (HPV) types 16 and 18 DNA for NAT-based assays. The rationale for the development of these new International Standards followed the introduction of new vaccines for these viruses and the need to calibrate HPV NAT assays used in vaccine testing and epidemiological studies. Candidate standards comprised two freeze-dried, and one liquid, plasmid DNA preparations of the complete genome of HPV 16 and 18 DNA, because of the inability to culture HPV in vitro. Data was presented on the freeze-drying and stability assessment of these preparations. The candidate standards were evaluated in an international collaborative study involving 21 laboratories performing range of both commercial and in-house assays targeting different regions of the HPV genome. Data returned for the freeze-dried candidates showed a two-log spread in viral loads around the theoretical mean of 1×10⁷ HPV genome copies/mL, with a lower sensitivity for qualitative assays compared with those for quantitative assays. The agreement between laboratories was markedly improved when the potency of the study materials was expressed relative to the candidate standard. On the basis of these results, proposals will be put to the ECBS in October 2008 for establishment of these WHO International Standards for HPV 16 and 18 DNA with assigned potencies of 5×10⁶ and 1×10⁷ IU/mL, respectively. In the discussion following this presentation Dr Wilkinson described plans to develop further HPV DNA standards for genotypes 31, 33, 45, 52 and 58.

**Development of NAT working reagents for clinical diagnostics**

Dr Anna Gottlieb (NIBSC, UK) described an ongoing collaboration between the UK Clinical Virology Network, NIBSC and the UK Health Protection Agency (HPA) to develop working reagents for clinical diagnostic NAT assays. These working reagents are designed to act as low-positive run controls (detected at approximately cycle 30 in
a real-time PCR assay), and used routinely to monitor both the intra and inter-laboratory performance of clinical diagnostic NAT assays. At present, targets comprise clinically-relevant viruses, however, the aim is to develop further controls for bacterial targets. The run controls comprise whole virus in a universal buffer to be representative of different samples types and provide a quality control for the entire diagnostic assay. Candidate run controls are evaluated by participating UK clinical laboratories performing a wide range of mainly in-house assays, and data in the form of cycle threshold (Ct) values is returned to NIBSC for analysis. Data returned thus far on the performance of eight candidate run controls shows considerable intra and inter-laboratory variation in Ct value determined by each assay. Priorities for the future include the development of multiplexed reagents and the development of a data reporting software to enable laboratories to monitor and compare their performance with other participating laboratories in real-time.

NAAT quality control for Chlamydia trachomatis (and Neisseria gonorrhoea)

Mr Joe Vincini (HPA, UK) described the development of NAT quality controls for C. trachomatis (and N. gonorrhoea) at the HPA Quality Control Reagents Unit. A summary was presented on the evaluation of four commercial NAT-based systems used in the diagnosis of C. trachomatis, and has recently been published on the HPA-MiDAS website (www.hpa-midas.org.uk). The HPA QC control for C. trachomatis is formulated using lenticules, which are dried discs of biologically active organisms in a water soluble matrix. These are designed to be diluted in the appropriate swab transport solution at each laboratory and processed as swab samples. Results of the evaluation of four dilutions of C. trachomatis lenticules by external laboratories using a range of commercial and in-house assays, showed some variation in detection of the lower dilutions. CE Marking of these reagents is pending completion of stability assessment, although data thus far demonstrates the material to be stable, highlighting the suitability of the lenticule format for an IQC reagent. Meanwhile, further development and stability assessment of the N. gonorrhoea component is underway.

Open discussion of the aims of the SoGAT Clinical Diagnostics meeting. There followed a brief discussion on the remit and aims of the SoGAT Clinical Diagnostics Working Group. Dr Philip Minor viewed the principle remit of the group to be to coordinate the development of primary international reference reagents for the clinical NAT assays. This would underpin the development and use of secondary references such as working reagents and run controls to monitor routine assay performance. It was generally agreed that such international standards were especially important where quantification was critical for diagnosis, for example, in the case of human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV). Another important role would be to exchange information on the performance of different NAT assays and QC reagents, and potentially assign standardised units to these reagents. The group could facilitate this by organising studies to compare working reagents produced by different organisations, or by including these reagents in the collaborative studies to develop international standards. In the absence or relevance of WHO International Standards for other clinical targets, the group could explore the possibility of developing other primary standards against which secondary references could be calibrated. Dr Jutta Preiksaitis (Provincial Laboratory for Public Health, Canada) suggested the need for a web-based reporting system for comparing the performance of NAT assays and QC reagents, such as has been provided by the Canadian government, which could be linked to similar systems worldwide. However, further
regulation may be required to encourage laboratories to monitor and return data on laboratory performance.

Session 4: Standardisation for Clinical Diagnostics Part 2

**Practical aspects of standardisation for a global controls manufacturer**

Dr Frank Opdam (AcroMetrix Europe B.V., The Netherlands) described some of the practical aspects faced by manufacturers of QC reagents and the difficulties caused by the lack of standardisation of diagnostic NAT assays. The principles of metrology, including the importance of standardisation, calibration and traceability were highlighted. At present, the majority of diagnostic NAT assays (excluding those for blood-borne viruses), are controlled using different QC reagents prepared either in-house or by external organisations. They are often based on a specific assay system and have individually defined units. Manufacturers of independent QC reagents must comply with international regulations for medical devices and where values are assigned, these must comply with ISO 17511. In the absence of higher order references against which each reagent can be calibrated, values are assigned using the manufacturers own calibrators, however, results generated through these controls are not comparable. Dr Opdam described the development of the OptiQual® MRSA positive control, in compliance with ISO17511, and the procedure for value assignment.

**Controls for Chlamydia trachomatis and Neisseria gonorrhoea**

Dr Mark Manak (SeraCare Life Sciences, Inc., USA) described the development of the ACCURUN 341 controls for *C. trachomatis* and *N. gonorrhoea*. The aim was to develop a single non-infectious whole process multi-analyte run control suitable for three commercial assays currently used in the detection of *C. trachomatis* and *N. gonorrhoea*. In the absence of a higher order reference for these targets, stocks comprising well-characterised strains for each organism, were quantified by several methods, including; in-house TaqMan PCR, EM count, spectrophotometry. The concentration required to challenge the sensitivity of each assay system (C$_{50}$ and C$_{95}$ levels), was determined by analysing several dilutions of both stocks on each platform, and converting these dilutions to a theoretical particle count. The results of this evaluation showed that there was a wide variation in the sensitivity of each assay, preventing the use of a single control in all systems. Therefore, SeraCare proposed to produce three variations of this control, each at 5× C$_{50}$ value of each platform.

**ZeptoMetrix Corporation: Standardisation of full process nucleic acid testing controls**

Dr Peter Trabold (ZeptoMetrix Corporation, USA) described the development of Zeptometrix NATtrol™ reagents, which are a range are non-infectious whole organism full-process controls for routine NAT assays. Culturable targets are grown in-house and purified by sucrose gradient, meanwhile, non-culturable targets are sourced from human plasma. Inactivation of the target is achieved using a proprietary cross-linking agent and confirmed using a cell culture-based assay. The concentration of stock material is determined using different real-time PCR platforms (amongst other direct nucleic acid quantification methods) and calibrated against WHO International Standards and commercial standards where available. Where these are not available, in-house calibrators have been developed. Viral controls are additionally evaluated using a bacteriophage functional assay and by particle
counting, using an instrument developed by Virus Detection Systems Corporation. Data using this instrument is limited at present, however, it is hoped that further information can be presented at future meetings.

Session 5: Assessment of Laboratory Performance and Calibration of EQA panels

**EQA schemes for CMV, HPV and mycobacteria**

Dr Vivienne James (HPA, UK) described the results of recent UK NEQAS molecular proficiency programmes for HCMV, HPV and mycobacteria. The HCMV quantification programme comprised four specimen pairs of HCMV-seronegative human plasma spiked with cell culture grown AD169 strain at concentrations of between 5 and 7 log_{10} copies/mL. Samples were distributed to approximately 50-70 participants using a range of commercial and in-house assays. The range of viral loads returned for each sample varied between 2 and 5.5 log_{10} copies/mL, indicating significant variation in inter-laboratory performance. Meanwhile, intra-laboratory variation was demonstrated for paired samples. Results of the qualitative EQA programme for mycobacteria demonstrated increased detection of *M. tuberculosis* over other strains despite the use of a number of different direct and post-culture detection methods. For the pilot HPV EQA programme, four cervical specimens comprising different HPV genotypes were distributed in PreservCyt™ medium. Data was returned from 46 participants performing a range of genotyping and non-genotyping methods. Results showed that while 97% of participants accurately detected HPV, genotyping results were variable.

**QCMD molecular EQA past, present, and future**

Unfortunately at short notice Dr Paul Wallace (QCMD, UK) was unable to attend the meeting to give his presentation on the past, present and future of QCMD’s molecular EQA programmes.

Session 6: Standardisation of CMV and EBV viral loads

**Viral dynamics and the importance of viral load measurements in the management and prevention of CMV disease**

Professor Vincent Emery (Royal Free and University College Medical School, UK) described the importance of viral load measurements in the management and prevention of CMV disease. Research on HCMV replication dynamics in transplant recipients performed in the 1990’s showed that peak viral load during active infection was a major risk factor for the development of HCMV disease. Based on these investigations viral load thresholds were widely applied for the initiation of antiviral therapy in transplant patients. At the Centre for Virology, Royal Free Hospital, HCMV viral load is monitored in whole blood from transplant recipients using an in house real-time PCR assay. The viral load threshold for the initiation of pre-emptive antiviral therapy is set at 3000 genome copies/mL, and therapy is stopped upon two consecutive negative PCR results. Other laboratories prefer to monitor HCMV viral load in plasma rather than whole blood, and although the dynamics of HCMV replication is different in each sample type, both provide prognostic information for the management of CMV disease. In addition to peak viral loads, recent research suggests that baseline viral loads of patients on antiviral therapy can be used to predict the response to therapy and duration required. Lower levels of persistent replication
are also important in the pathogenesis of certain CMV diseases. Antiviral therapy with ganciclovir (GCV) continues to be the mainstay for treatment of HCMV infection, and clinical trials of the antiviral agent maribavir and a HCMV vaccine are ongoing.

**Development of an international standard for EBV and CMV viral load assessment**

Dr Jutta Preiksaitis (Provincial Laboratory for Public Health, Canada) presented the results of a joint study between the American and Canadian Societies of Transplantation examining the inter-laboratory variability in HCMV and EBV viral load measurements. HCMV samples comprised purified nucleocapsids of cell culture-grown HCMV Merlin strain in human plasma, as well as clinical plasma samples (including UL54 or UL97 GCV resistant mutants). Prior to dispatch, nucleocapsid samples were quantified by electron microscopy (EM) and a mean viral load result determined from seven reference laboratories ('expected result'). Viral loads returned by 33 laboratories performing a range of qualitative and quantitative NAT assays, demonstrated significant variation in both detection and quantification of HCMV, particularly for samples with a low viral load. Only 57% of viral loads were within 0.5 log_{10} of the ‘expected result’, with more variability observed for in-house compared with commercial assays. EBV samples comprised varying concentrations of EBV-containing Namalwa cells and EBV-negative Molt-3 cells diluted in human plasma, as well as clinical samples. Viral loads returned by 28 laboratories using a range of qualitative and quantitative assays varied significantly, independent of the sample or assay system examined. Only 47% of quantified results were within 0.5 log10 of the expected viral load. Overall, the study highlighted the need for an internationally-accepted reference to help standardise NAT-based assays for these viruses.

**Standardisation of EBV viral load measurement: results of a questionnaire**

Professor Barbara Gärtner (Saarland University Hospital, Germany) presented the preliminary results of a questionnaire arising from the EBV Viral Load Standardisation Workshop held at the Third European Congress of Virology on 4th September 2007. The aim is to determine a standardised approach to EBV viral load testing (from sampling to interpretation of PCR result) through several rounds of targeted questions and analysis of responses using the Delphi method. Questionnaires have been distributed to workshop attendees and clinical laboratories in eight countries. Responses thus far highlighted differences in current testing strategies, including: specimen type (plasma or whole blood), source of control/calibration material (plasmids, EBV cell lines, or virus particles), and PCR method. However, there was a consensus that assay controls should be extracted, and an acceptance for results to be defined in IU/ml when an international standard is available.

**Proposals for WHO International Standards for HCMV and EBV for NAT-based assays**

Dr Jacqueline Fryer (NIBSC, UK) described proposals for the development of the first WHO International Standards for HCMV and EBV for NAT-based assays. A summary of the range of in-house and commercial NAT-based diagnostic assays available for the detection and quantification of each virus was presented. The lack of standardised reference reagents with which to calibrate these assays makes it difficult to compare results and develop uniform treatment strategies, highlighting the need for
internationally-accepted references for these viruses. It was put forward that proposed international standards for HCMV and EBV would comprise infectious whole virus formulated in a universal matrix, prepared from well-characterised laboratory or clinical strains, and freeze-dried to maintain stability. The concentration would be in the order of $10^6$ genome equivalents/mL [subsequently amended to $10^7$ genome equivalents/mL], to cover the quantification range of current assays. A plan for generating a consensus on the above proposals, sourcing virus, filling and evaluating candidate standards in an international collaborative study was put forward. Proposals would be presented to WHO at the ECBS meeting in October 2008 for approval to develop these references at WHO International Standards.

**Open discussion on the standardisation of HCMV and EBV viral loads.** A general discussion followed on the proposed international standards for these viruses with consideration of virus source and strain, formulation and concentration.

**Virus source and strain:** It was agreed that since it would be difficult to source sufficient clinical material for each virus, laboratory-cultured strains similar to circulating clinical isolates and containing all potential PCR gene targets would be used. Cell cultured whole virus preparations of strains Merlin or AD169 were considered to be suitable for HCMV candidate standards, although it was noted that the AD169 genome lacks specific sequences within the unique long region. Meanwhile, the laboratory-cultured B95-8 strain was considered to a suitable EBV candidate standard. In addition, Namalwa and Raji cells, containing 2 and 50-60 copies of the EBV genome per cell respectively, were considered to be representative of the EBV genome. Dr Philip Minor noted that certain lineages of B95-8 cells have been reported to be contaminated with a Squirrel Monkey Retrovirus (SMRV).

**Formulation:** It was proposed to prepare candidate standards in a universal matrix, for further dilution in the specific matrix relevant to the working reference or assay system being calibrated. In the case of HCMV, some concern was expressed for the presence of heterogenous virus populations produced in cell culture, and it was suggested that purified full-length DNA should be considered as an alternative to HCMV culture supernatant. It was noted that the complete genome of the Merlin strain of HCMV had been cloned into a bacterial artificial chromosome (BAC), and that this could represent a source of purified viral DNA. Alternatively, HCMV DNA material prepared by Dr Marcia Holden at the National Institute of Standards and Technology in the US could be considered. The inclusion of both culture supernatant whole virus and purified DNA in the collaborative study would enable the suitability of each formulation to be evaluated. In addition, sucrose gradient-purified nucleocapsids from cell culture were suggested as a clean alternative to culture supernatant virus.

**Concentration:** It was agreed that the concentration of proposed international standards for these viruses should be at or above the upper end of the quantification range for HCMV and EBV NAT assays, in order to be suitable for the calibration of secondary references. Accepting that final concentrations of the WHO International Standards would be expressed in IU, the concentration of candidate standards for HCMV was proposed to be in the order of $10^6$ genome equivalents/mL. For EBV, the concentration of candidate standards was proposed to be in the order of $10^8$ genome equivalents/mL, as higher viral loads than $10^6$ genome equivalents/mL are sometimes seen in patients. While it would not be possible to prepare a candidate of Namalwa cells at this concentration, it would be possible to include a preparation of these cells at $10^6$ genome copies/mL for comparison in the collaborative study.
[NB: a subsequent consensus was reached to prepare candidate standards for both viruses at approximately $10^7$ genome equivalents/mL.]

Session 7: Standards for Bacteria and Parasites

**Standardisation of meningococcal epidemiology – diagnostics**

Dr Steven Gray (HPA North West Laboratory, UK) summarised the service provided by the Meningococcal Reference Unit at the Manchester Royal Infirmary, which includes a series of PCR-based assays for the confirmation of meningococcal disease. Dr Gray described an EQA programme to evaluate meningococcal strain characterisation and DNA detection in European meningococcal reference laboratories, organised in collaboration with UK NEQAS, and funded by the European Invasive Bacterial Infections Surveillance Network. Panels (distributed between 2005 and 2007) comprised culture and non-culture (simulated septicaemia) samples, representative of the main disease-causing lineages in Europe as well as some unusual strains. Overall, phenotypic characterisation results (presented relative to the consensus result) were good, where laboratories had access to reagents, with those for culture samples better than those for non-culture samples. PCR-based detection (PorA and MLST) was generally successful, with a practical limit of detection observed of $10^3$ organisms/mL. Other future work includes the development of either DNA or whole organism PCR controls for different meningococcal serogroups, although the formulation and concentration of these controls remains to be determined.

**Malaria and toxoplasma standardisation of NAT assays**

Mr David Padley (NIBSC, UK) described the development of the first WHO International Standard for *Plasmodium falciparum* DNA for the standardisation of NAT-based assays used in blood screening, diagnosis and clinical management, and anti-malarial vaccine studies. *P. falciparum* accounts for the majority of fatal cases of malaria. Four samples of *P. falciparum* comprising; freeze-dried whole blood from an exchange transfusion patient (AA), a liquid preparation of cultured *P. Falciparum* (BB), and two liquid preparations of patient whole blood (CC and DD), were evaluated in an international collaborative study involving 14 laboratories from 10 countries. Data on freeze-drying and stability assessment was presented. Based on the results of the collaborative study, the freeze-dried material AA, was established as the first WHO International Standard for *P. falciparum*, with an assigned potency of $1\times10^9$ IU/mL. Sample DD is proposed as a working reagent for use in routine NAT assays. Mr Padley also described a proposal to develop an international standard for *Toxoplasma gondii* for NAT-based assays. Proposed candidates for this standard would comprise the RH (genotype 1) strain cultured in HeLa cells and in mice, at a concentration of approx. $1\times10^7$ parasites/mL.

Session 8: Control and Validation of Commercial Assays for Clinical Diagnostics

**Control and validation of Roche Diagnostic tests**

Dr John Saldanha (Roche Molecular Systems, Inc., USA) described how the WHO International Standards and reference preparations for blood-borne viruses have been used to calibrate NAT assays developed by Roche Molecular Systems, and in particular, the COBAS® AmpliPrep/COBAS® TaqMan HCV (CAP/CTM) assay. The analytical sensitivity of the assay for the detection of HCV genotype 1a virus in both
EDTA plasma and serum was determined in IU/mL, by direct comparison against titrations of the WHO International Standard for HCV RNA. Meanwhile, the linear range of quantification and precision of testing was assessed using dilutions of clinical samples and armoured RNA. The genotype inclusivity of the assay to detect and quantify each of the six major genotypes of HCV was assessed using the NIBSC HCV RNA genotype panel. Dr Saldanha highlighted the importance of the International Unit in enabling commercial manufacturers to validate IVD kits and compare assay performance.

**Performance evaluation of standardised QIAGEN sample preparation methods for the artus CMV PCR Kit**

Dr Volker Riemenschneider (QIAGEN GmbH, Germany) described the performance evaluation of two sample preparation methods for the CE Marked artus® CMV PCR kit, assessed in accordance with the European In Vitro Diagnostic (IVD) Directive (98/79/EC). HCMV is classified in the directive as an Annex II, List B agent, which means that the validation of IVDs must be assessed through a Notified Body. The artus® CMV LC PCR kit was validated using the QIAamp® DSP Virus (manual) and EZ1 DSP Virus (automated) kits, which are CE Marked stand-alone sample preparation systems. A number of written standards for the validation of analytical methods were used for guidance. Performance evaluation data for parameters including analytical sensitivity, linear dynamic range, specificity, precision and cross-contamination were presented. In the absence of an internationally-accepted reference standard for HCMV, the limit of detection was determined by titration of an in-house standard and defined in copies/mL. Diagnostic evaluation was performed using clinical EDTA plasma samples and assessed against another commercial assay.

**Session 9: Regulation of Clinical Diagnostics**

**US Regulation of In Vitro Diagnostic Devices (IVDs)**

Dr Francisco Martinez-Murillo (Center for Devices and Radiological Health, FDA, USA) described the regulation and approval process for IVDs in the US, and reviewed the challenges of approving devices in the absence of standardised reference reagents. Regulation of IVDs by the FDA is based on different categories of risk of the device. Class I are low risk devices and mostly exempt from premarket submission. Class II devices are classified as moderate risk and require premarket notification. Meanwhile, Class III devices are the highest risk and require valid scientific evidence of safety and effectiveness for premarket approval. Dr Martinez-Murillo described the key elements required for the submission of a device and the major issues for the review of performance of the IVD. In the absence and lack of traceability to standardised units of measurements, composite references or recognised reference panels from a number of organisations were used in the assessments.

**CE marking and regulation of clinical diagnostics in Europe**

Dr Micha Nübling (PEI, Germany) described the process of CE Marking for the regulation of IVDs in Europe. The IVD Directive (98/79/EC) defines the requirements for placing IVDs onto the common European market. This regulation also applies to run controls used in IVD assays but does apply to WHO International Standards or diagnostic assays developed in-house. The directive classifies IVDs into three
categories based on risk, which require different levels of scrutiny for approval. The majority IVDs are classified as ‘low risk’ and are self-certified. ‘Medium risk’ IVDs, classified in Annex II, List B (includes reagents for HCMV), require an independent Notified Body to assess the quality system and compliance with the IVD Directive. Meanwhile, ‘high risk’ IVDs, classified in Annex II, List A, include reagents for blood-borne viruses involved in blood screening. This class of IVD requires Notified Body assessment and witnessing of batch release testing procedures. For IVD assays, Common Technical Specifications (CTSs) describe the principles and requirements for performance evaluation, including assuring traceability to higher order references such as WHO International Standards if available. Dr Nübling highlighted the importance of the SoGAT working groups and the development of WHO International Standards, to enable the standardisation required for the IVD Directive.

Summary and Closing Remarks

Dr Philip Minor informed the group that a report of the meeting was to be prepared and distributed to all participants. Proposals for WHO International Standards for HCMV and EBV DNA for NAT would be written and distributed to interested parties. The frequency of future meetings of the SoGAT Clinical Diagnostics Working Group was discussed and it was agreed that these should be held at yearly intervals. It was proposed to link future meetings with other international clinical virology and microbiology meetings whilst maintaining association with the SoGAT Blood Virology Working Group. All participants would be notified of the details of the next meeting in due course.