Overview of SoGAT BV XXI

SCIENTIFIC WORKING GROUP
ON THE STANDARDIZATION OF GENOME AMPLIFICATION TECHNIQUES
FOR THE SAFETY TESTING OF BLOOD,
TISSUES AND ORGANS
FOR BLOOD BORNE PATHOGENS

Held at the Brussels Marriott Hotel
Brussels, Belgium
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NIBSC

National Institute for Biological Standards and Control
Assuring the quality of biological medicines
SoGAT Blood Virology Aims

• To develop, evaluate and provide reference reagents and International Standards for qualitative and quantitative nucleic acid assays for blood borne pathogens, including viruses, bacteria and parasites

• To organise collaborative studies to evaluate candidate materials, validate methods and establish reproducibility between laboratories

• To exchange information on the technical and scientific aspects of nucleic acid assays, assist in the development of regulatory approaches and exchange views on the technology and its application between professionals in:
  – Control Authorities
  – Academic laboratories
  – Manufacturers of blood products
  – Kit manufacturers
  – Diagnostic laboratories
  – Blood banks
SoGAT Blood Virology Aims

• To investigate and develop a new generation of reagents and standards, e.g. synthetic nucleic acids, non-infectious biological materials

• To develop standards for new assays for emerging and re-emerging pathogens and genetic variability

• To develop standards in support of new technologies, e.g. multiplex nucleic acid assays, micro-arrays

• To develop methods for the correlation of nucleic acid concentration with antigen concentration and infectivity

• To evaluate pathogen reduction methods and to develop standards for monitoring infectivity e.g. for quantitation of pathogen reduction
Session 1: Parvovirus B19 (Chair: Gerold Zerlauth)

- Replacement International Standard for B19:  
  Sally Baylis

- Active B19 virions production in hepatoblastoma a  
  and hepatocarcinoma cell lines: amplification and genomic stability:  
  Anne Op de beeck,

- Current status of B19V NAT screening in the U.S.  
  and the preparation of 4-member genotype panel:  
  Mei-ying Yu (jointly with S Baylis)

- B19 genotype panel:  
  Sally Baylis (jointly with M-y Yu)

- Dual testing with quantitative Parvo B19 DNA assays;  
  genotype recognition and isolates with mismatches:  
  Theo Cuypers, M Koppelman

Summary:


Following a successful collaborative study to evaluate a B19 genotype panel, recommendations made to ECBS to establish 1st reference panel for B19.

High genetic variation can lead to under reporting even in genotype 1, DNA tests could therefore be developed to target two regions of genome to overcome suboptimal detection.

Development of a highly sensitive assay using tissue culture to produce infectious B19 virus with genomic stability.
Session 2: Hepatitis A/C/E (Chair: Micha Nuebling)

- Plans for HAV genotype panel: Rob Anderson
- Yield obtained by mandatory HCV and HIV1-NAT in Germany and break-through transmissions: Micha Nuebling
- Role for HCV antigen detection: a new generation of assays: Richard Tedder
- Hepatitis E in Belgium: an import infection or an emerging viral zoonosis?: Isabel Micalessi
- Proposal to prepare standards and reference panels for hepatitis E virus: Sally Baylis
- Indigenous HEV infection in the UK: a hazard for blood donation?: Samreen Ijaz

Summary:

Sub optimal detection of some HAV genotypes lead to a proposal to produce a genotype panel, request for viruses.

Low levels of infection can be shown as negative by NAT in donor population but mandatory detection levels provide a suitable safety margin.

Use of HCV antigen assay shows good correlation with NAT but not absolute and leads to wonder the relationship between antigen and RNA.

Problems of HEV infections in blood donations, potentially a rise in cases or just due to raised awareness and better diagnosis? In some cases demonstrated to be a zoonosis from pigs. Need NAT standardisation to assess assays.
Session 3: HIV (Chair: Albrecht Groener)

- Update on CBER HIV CRF02_AG and 01_AE NAT panels and international HIV panel development efforts: Indira Hewlett,
- Preparation of reference panels for current and emerging HIV variants: Mark Manak,
- Development of HIV-2 International standard: Harvey Holmes

Summary:

Increase in circulating recombinant forms of HIV has lead to the need for new reference panels, becoming an international collaboration to source new CRF’s for inclusion in the panel.

In the US, NIAID formed a study group to ensure screening assays and confirmatory assays can detect CRF's.

A collaborative study has taken place to develop an HIV-2 RNA international standard.
Session 4: Technological development/bacterial infection of blood and products (Chair: Indira Hewlett)

- Experiences with Roche Cobas s 201 in routine minipool screening (HCV, HIV, HBV): Lutz Pichl
- SynTura: A new Ribonuclease resistant viral RNA control material: Ralf Schoenbrunner
- WHO ISBT International Validation Study on Transfusion-relevant Bacterial Strain Panel: Thomas Montag-Lessing
- Development of a real time RT-PCR assay for broad range detection of bacteria in platelet concentrates: Ineke Rood

Summary:

Successful implementation of the new Roche COBAS s201 + MPX system into the German blood banking laboratories.

New approach to controls and calibrators, using synthetic material but remaining true to samples under test.

BacT/Alert systems remains very sensitive with occasion false negative when low titre specimens are encountered. Further work is needed to improve DNA/RNA sensitivity of Gram + bacteria. There is also a high level of background which can lower sensitivity.

A blood bacterial panel has been shown to be valuable for cell therapeutics, gene therapeutics etc (ATMP’s).
Session 5: Proficiency/EQAS/National Standards (Chair: Harvey Holmes)

- The Italian NAT External Quality Assessment Study: the 2008 Programme: Francesca Luciani
- NAT proficiency program in Japan: Saeko Mizusawa
- HIV-1 NAT proficiency study for UK BTS: Clare Morris
- Proficiency Testing of in-house NAT assays used for Blood Screening: Julia Kress
- Calibration Against The WHO Standards Of National Reference Preparations For Detection Of Blood Viruses By NAT: The Italian Experience: Karen Cristiano
- A multi-centre evaluation study of different run and trend control samples: Harry van Drimmelen
- National standards for blood viruses in Taiwan: Yi-Chen Yang

Summary:

Various national and international EQA studies performed throughout the world, in a number of cases the samples are calibrated against the International Standard.

Regulatory requirements differ from country to country, the development and use of national standards and proficiency panels has allowed laboratories to evaluate assays that are on the market in their country and to assess their performance – inter or intra lab.
Session 6: SoGAT-CV – Clinical Diagnostics (Chair: Kurt Roth)

- Standardising NAT for other clinical targets through SoGAT Clinical Diagnostics
- An update on the development of proposed 1st WHO International Standards for human cytomegalovirus and Epstein-Barr virus

Session 7: Hepatitis B (Co-Chairs: Wolfram Gerlich and Nico Lelie)

- Development of HBV genotype reference panels for NAT assays and for HBsAg tests
- Correlation between HBsAg and HBV genome concentrations according to genotype
- Calibration of International HBsAg Units in ng/ml for HBV genotypes A-H and comparison with HBV DNA levels
- Comparison of HBV NAT in small test pools versus large pools with the S201 system
- Establishment of 1st WHO International Standard for anti-HBc
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- **Session 7: Hepatitis B (Co-Chairs: Wolfram Gerlich and Nico Lelie) cont..**

- Screening for HBV/HBc in USA: John Saldanha
- Epitope profiling from HBsAg plasma: Richard Tedder
- HBV genotypes E and A deletions and recombination's: two sides of the same coin? Penelope Garmiri
- Stability of HBV DNA in reference preparations: Corinna Bremer

**Summary:**

Following concern over HBsAg and HBV NAT sensitivity for different genotypes two reference panels have been developed and evaluated, NAT shows good correlation between assays. Further studies to be carried out on HBsAg panel.

Genotypes affected the detection range between when studying HBsAg with lower values seen for genotypes B and D. There was no correlation between HBsAg in plasma and viral load irrespective of genotype.

HBV NAT screening has been introduced in the Netherlands, using the Roche S201 system, detection of HBV in donations increased 10fold due to greater sensitivity. Major benefit, now allows for detection of occult samples as screening for anti HBc not carried out.

The 1st anti-HBc was found suitable for estimation of analytical sensitivity for anti-HBc detection, calibration of anti-HBc test kit sensitivity by manufacturers, to calibrate secondary standards, for quality control procedures, e.g. in batch release testing.
http://www.nibsc.ac.uk/partners/SoGAT