Proposals for WHO International Standards for CMV and EBV for NAT-based assays

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SoGAT – Clinical Diagnostics, 24th/25th June 2008
Overview of presentation

- Background to CMV, EBV and diagnostic NAT-based assays for these viruses
- Need for standardisation
- Role of an International Standard
- Discuss specific requirements for International Standards for NAT-based assays for each virus
- Describe steps to development and establishment
- Open discussion
Background

- Both ubiquitous herpesviruses, establish latency following primary infection
- Reactivation can lead to severe disease
- Importance of viral load measurements:
  - Diagnosis of established disease
  - Identify patients at risk, initiation of pre-emptive therapy
  - Monitor response to therapy
  - Predict likelihood of recurrence of disease
  - Monitor development of resistance to antivirals
CMV diagnostics

- NAT-based assays
- Majority quantitative (range of calibration standards comprising plasmid DNA or cell-cultured virus)
- In-house methods:
  - Based on real-time PCR
  - Range of clinical samples
    - Immunocompromised; blood, plasma, serum, PBMC, PBL, CSF, BAL (lung transplants)
    - Congenital; blood, serum, urine, saliva, CSF, DBS
  - Platforms; PRISM, LC, Rotor-Gene
  - Gene targets; UL54 (pol), UL55 (gB), UL122 (IE), UL123 (MIE), UL83 (pp65), UL32 (pp150), HXFL4, US17
  - Primer/probe sequences
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Name</th>
<th>Technology</th>
<th>Viral target</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche</td>
<td>COBAS Amplicor CMV Monitor</td>
<td>PCR</td>
<td>UL54 (pol)</td>
<td>Plasma, PBL/ whole blood</td>
</tr>
<tr>
<td>Digene (Murex)</td>
<td>Hybrid Capture CMV DNA assay</td>
<td>Signal amplified hybridisation</td>
<td>17% CMV genome</td>
<td>Leukocytes, Whole blood</td>
</tr>
<tr>
<td>bioMérieux</td>
<td>NucliSens CMV pp67</td>
<td>NASBA</td>
<td>pp67 mRNA</td>
<td>Whole blood</td>
</tr>
<tr>
<td>Argene</td>
<td>CMV R-gene Kit</td>
<td>Real-time PCR</td>
<td>UL83 (pp65)</td>
<td>Plasma, whole blood</td>
</tr>
<tr>
<td>LightUp</td>
<td>ReSSQ CMV assay</td>
<td>Real-time PCR</td>
<td>Not specified</td>
<td>Plasma, serum</td>
</tr>
<tr>
<td>QIAGEN</td>
<td>artus CMV LC/TM/RG PCR Kit</td>
<td>Real-time PCR</td>
<td>UL122 (IE)</td>
<td>EDTA plasma</td>
</tr>
<tr>
<td>Nanogen (Amplimedical)</td>
<td>Q-CMV</td>
<td>Real-time PCR</td>
<td>UL123 (MIE)</td>
<td>Not specified</td>
</tr>
<tr>
<td>Cepheid</td>
<td>Affigene CMV trendr</td>
<td>Real-time PCR</td>
<td>Not specified</td>
<td>Plasma, whole blood</td>
</tr>
<tr>
<td>Cepheid</td>
<td>Smart CMV</td>
<td>Real-time PCR</td>
<td>Not specified</td>
<td>Plasma</td>
</tr>
<tr>
<td>Roche</td>
<td>LightCycler CMV Quant Kit</td>
<td>Real-time PCR</td>
<td>UL54 (pol)</td>
<td>Plasma</td>
</tr>
</tbody>
</table>
EBV diagnostics

- NAT-based assays
- Majority quantitative (range of calibration standards comprising plasmid DNA, EBV-positive cells; Namalwa, JY, Raji)
- In-house methods:
  - Based on real-time PCR
  - Range of clinical samples
    - Immunocompromised; blood, PBMC, plasma, serum
    - Other EBV-associated disease; biopsies, urine, stool, CSF
  - Platforms; PRISM, LC, Rotor-Gene
  - Gene targets; BALF-5 (pol), EBNA-1, BNRF p143, BamH1-W, BamH1-K, BXLF-1, BZLF-1, EBER1, LMP-1/2, p23 (VCA)
  - Primer/probe sequences
  - Reporting units; copies/ml, copies/µg DNA, copies/10^n cells
## EBV diagnostics: commercial ASR

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<tr>
<td>Argene</td>
<td>EBV R-gene Kit</td>
<td>Real-time PCR</td>
<td>BXLF-1 (thymidine kinase)</td>
<td>Plasma, whole blood</td>
</tr>
<tr>
<td>Cepheid</td>
<td>affigene EBV trender</td>
<td>Real-time PCR</td>
<td>Not specified</td>
<td>Plasma, whole blood</td>
</tr>
<tr>
<td>LightUp</td>
<td>ReSSQ EBV assay</td>
<td>Real-time PCR</td>
<td>Not specified</td>
<td>Plasma, blood</td>
</tr>
<tr>
<td>Nanogen (Amplimedical)</td>
<td>EBV Q-PCR Alert (Q-EBV)</td>
<td>Real-time PCR</td>
<td>EBNA-1</td>
<td>Not specified</td>
</tr>
<tr>
<td>QIAGEN</td>
<td>artus EBV LC/TM/RG PCR Kit</td>
<td>Real-time PCR</td>
<td>Not specified</td>
<td>Not specified</td>
</tr>
<tr>
<td>Roche</td>
<td>LightCycler EBV Quant Kit</td>
<td>Real-time PCR</td>
<td>LMP-2</td>
<td>whole blood, plasma, CSF, cell culture and other biological samples</td>
</tr>
<tr>
<td>Cepheid</td>
<td>Smart EBV</td>
<td>Real-time PCR</td>
<td>Not specified</td>
<td>Plasma</td>
</tr>
</tbody>
</table>
Requirement for standardisation

- Wide range of NAT technologies used
  - Differ in: extraction method, specimen type, amplification method and instrumentation, gene target, primer/probe, calibration standards, reporting units, etc
- Lack of standardisation between assays makes it difficult to compare performance
- Cannot set uniform threshold levels for pre-emptive therapy as they are assay dependent
- Evidence for variation in the performance of CMV and EBV NAT assays
  - US/Canadian Society of Transplantation study
  - Clinical diagnostic run controls
  - EQA programmes (Barbi et al., 2008)
  - Other studies (Hayden et al., 2008; Michelin et al., 2008; Perandin et al., 2007)
- Highlights need for standardisation of NAT assays for CMV and EBV
Considerations for the development of International Standards for NAT-based assays:

• What is its role

• General requirements for specification
Role of International Standard

- The “Gold Standard” of reference materials
- Defines the International Unit (IU), provides a uniform reporting system
- Provides the basis for calibration of assays and other references
- Allow more accurate comparison of different assays
- Essential for the development of an internationally agreed diagnostic and clinical management approach
General requirements for WHO International standards (for NAT-based assays):

- Biological standard - behaviour should resemble as closely as possible the behaviour of test samples
  - Not necessarily same formulation/matrix as test samples
- Relevant for all NAT assays
  - Evaluated in collaborative study using range of NAT assays
- Assigned internationally agreed unitage based on results of worldwide collaborative study
- Homogeneous (consistent performance)
- Stable (freeze-dried)
Proposal for 1\textsuperscript{st} WHO International Standard for CMV DNA

- **Source material**
  - Whole virus, relevant to all gene targets, can be used to calibrate entire assay
  - CMV-positive clinical sample, difficult to source/replace?
  - Cell cultured virus, purified nucleocapsids?
  - Strain; Merlin, AD169

- **Matrix**
  - Whole blood, plasma (CMV-seronegative)
  - But should be relevant to NAT assays for different specimen types/patient groups
  - Universal buffer (e.g. 10mM Tris, 2% FCS), for further dilution in relevant sample matrix
Proposal for 1\textsuperscript{st} WHO International Standard for CMV DNA

- **Concentration**
  - Above clinically relevant range
  - IU is arbitrary unit but something in order of $10^6$ genomes

- **Filling**
  - Infectious
  - Freeze-dried
Proposal for 1\textsuperscript{st} WHO International Standard for EBV DNA

- **Source material**
  - Whole virus, relevant to all gene targets, can be used to calibrate entire assay
  - EBV-positive clinical sample, difficult to source/replace?
  - EBV-positive B cell line; Namalwa (2 copies/cell), Raji (50 copies/cell)

- **Matrix**
  - Whole blood, plasma (EBV-seronegative)?
  - But should be relevant to NAT assays for different specimen types/patient groups
  - Universal buffer (e.g. 10mM Tris, 2% FCS), for further dilution in relevant sample matrix
Proposal for 1\textsuperscript{st} WHO International Standard for EBV DNA

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- Filling
  - Infectious
  - Freeze-dried
Collaborative study

- To evaluate appropriateness of candidate standards by laboratories worldwide using range of NAT assays
- Collaborative study participants to be recruited to represent
  - Manufacturers of IVD kits
  - Clinical laboratories
  - Research laboratories
- Studies for both materials performed in parallel
  - include clinical samples?
Intended use

- Calibration of commercial IVD kits
- Calibration/validation of in-house clinical diagnostic NAT assays
- Calibration of clinical diagnostic working reagents and secondary references
- Calibration/evaluation of materials distributed through EQA schemes
- Calibration of NAT assays used in research, and those used in development/safety monitoring of potential new antivirals and vaccines
Time-line for establishing International Standards

- Agree specification
- Source virus stocks
- Identify and recruit collaborative study participants
- Proceed with trial then definitive fills
- Launch collaborative study
- Evaluate results, assign unitage, write report
- Submit report to ECBS, Jul 2010
- IS established, Oct 2010
- Held and distributed by NIBSC
Open discussion

For each of proposed CMV and EBV IS:

• Virus source/strain
• Formulation/matrix
• Concentration
• Inclusion of other material in collaborative study