SoGAT — Progress in the development of reference standards for NAT detection and measurement of infectious disease

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Standardisation of Genome Amplification Techniques (SoGAT) - AIMS

• Lead the development of WHO Reference Reagents and International Standards (ISs) suitable for NAT and serological infectious disease assays (for screening of blood donations, plasma pool testing and diagnostics)
• Provide guidance on the preparation of external control materials calibrated against the WHO ISs to be included in each run to ensure the reliability of the results
• Understand the relationship between clinical samples and the WHO ISs
• Promote standardisation of NAT and serological assays through inter-laboratory comparison studies or collaborations with EQA providers
• Provide a forum for the exchange of information to develop standards to support new technologies
• Provide a forum to react quickly to the standardisation needs of emerging or re-emerging pathogens
Activities of SoGAT

Forum for scientific and clinical experts, EQA providers and standards producers

• To prioritise public health/clinical need for standards
• To review and assess the impact of new technologies
• To provide early technical expert review of data from collaborative studies for primary standards
• To review issues pertaining to existing standards
• To disseminate outputs to the wider scientific community
Topics from SoGAT 2019

5 Sessions:
1) Review of new projects submitted to WHO
   – New standards for establishment
   – New projects for endorsement
2) Commutability of reference materials
   – Do standards reflect clinical isolates sufficiently?
3) Nucleic acid extraction methods and Genetic Variability
   – The challenge of different clinical materials and pathogens
4) ddPCR and NGS – can this help with replacing standards?
5) Standardising Point of Care/Point of Impact Tests
   – Supporting the introduction of new clinical technologies
New Standards for NAT at ECBS

Established standards
2\textsuperscript{nd} IS for HIV-2 RNA (2018)
1\textsuperscript{st} IS for Adenovirus DNA (2018)
1\textsuperscript{st} IRR for MERS CoV (2018)
6\textsuperscript{th} IS for HCV RNA (2019 - genetic variability and batch size)
1\textsuperscript{st} IS’s for HPV DNA types 6,11, 31, 33, 45, 52, 58 (2019)

Ongoing projects

- West Nile Virus
- Plasmodium vivax
- Babesia microti
- HIV-1 CRF extension panel
- HIV-1 DNA (neonatal and “cure”)
- Trypanosoma cruzi
- Leishmania spp
- Herpes Simplex Virus 1 & 2
- Varicella Zoster Virus
- Enterovirus (non polio)
- Influenza types A and B
- Respiratory Syncititial Virus
- Crimea Congo Haemorhagic Fever
- Rift Valley Fever
Commutability

Do WHO International Standards and Reference Materials perform in assays like patients samples?

Issues to consider:

1) Does the reference standard “control” the whole process?
   - Perception

2) Is the assay fit for purpose?
   - a. How is it calibrated?
   - b. Is the diagnostic amplification fit for purpose?

(Hayden et al JCM 2019)
Chimaeric HIV-outbreak virus RNA particles

Advantages:
- Safe: non-replicative HIV VLP, non-infectious (lack of Env), no expression of outbreak virus genes (no promoter and added stop codons)
- Easy and fast production
- HIV-1 ΔU3 LTR allows for genome quantification

Do recombinant lentiviruses reflect Disease X?

Mattiuzzo et al., PLoS One, 2015
### Maintaining the Standard 6th IS for HCV RNA - Outline of Collab Study

<table>
<thead>
<tr>
<th>Study sample</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Candidate 1 (individual HCV gt. 1a plasma donation, lyophilized)</td>
</tr>
<tr>
<td>Sample 2</td>
<td>Candidate 2 (individual HCV gt. 1a plasma donation, lyophilized)</td>
</tr>
<tr>
<td>Sample 3</td>
<td>5th IS, (individual HCV gt. 1a plasma donation, lyophilized)</td>
</tr>
<tr>
<td>Sample 4</td>
<td>Inactivated HCVcc (gt. 2a) in plasma</td>
</tr>
<tr>
<td>Sample 5</td>
<td>Inactivated HCVcc (gt. 1b/2a chimaera) in plasma</td>
</tr>
<tr>
<td>Sample 6 *</td>
<td>Individual HCV gt. 1b plasma donation</td>
</tr>
<tr>
<td>Sample 7 *</td>
<td>Individual HCV gt. 1a plasma donation</td>
</tr>
<tr>
<td>Sample 8 *</td>
<td>Individual HCV gt. 3a plasma donation</td>
</tr>
</tbody>
</table>

* evaluated in quantitative assays only

HCVcc kindly provided by Dr Wakita, NIID, Japan. Virus inactivated by UV irradiation and purified by sucrose density gradient.

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Pfaender et al, Appl Environ Microbiol. 2015
Overall potency estimates (Qual vs Quant)

• Mean estimates from quantitative assays lower than qualitative assays.
• Inter-lab variation lower for quantitative assays (twice as many assays).
• Overall mean estimate for 5th IS slightly higher than 2015 CS.
• Inter-lab variation slightly reduced compared to 2015 CS.
Relative potencies – change in SD

- Only small changes in SD when results expressed as relative potencies.
- No differences in harmonization between HCV genotypes.
- For the majority of samples candidates 1 and 2 improved the agreement between laboratories.
Relative potencies – change in SD

- Only small changes in SD when results expressed as relative potencies.
- No differences in harmonization between HCV genotypes.
- For the majority of samples candidates 1 and 2 improved the agreement between laboratories.
- HCVcc does not significantly worsen the agreement between laboratories.
- HCVcc provide the potential to prepare larger batches of IS.
Biological Standardisation allows assay improvement to be quantified

- 20 years of IS for HCV RNA reveals improvement in measurement by commercial and in house assay

Variations in calibration of replacement standards of $0.2 \log_{10}$ detectable by state of the art commercial assays
dPCR in Infectious Disease Diagnostics

Digital PCR is NOT a frontline test for infectious disease diagnosis

HOWEVER to SoGAT dPCR:

- Method with potential for more accurate quantification of replacement standards (maintaining the Unit)
- An opportunity to engage and interact with metrology laboratories (CCQM-NAWG)
- Provided valuable orthogonal data on the quality of reference standards (eg Polyoma viruses JC and BK vs EBV)
Point of Care/Impact Testing
Influenza types A and B, RSV (1st IS’s)
Complexity of Studies increasing – need for Pilot studies

Pilot study samples:

<table>
<thead>
<tr>
<th>Virus</th>
<th>Strain</th>
<th>Virus</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu A</td>
<td>A/Christchurch/1/2003, H1N1</td>
<td>Flu B</td>
<td>B/Jiangsu/10/2003, Flu B Yamagata</td>
</tr>
<tr>
<td>Flu A</td>
<td>A/Wyoming/3/2003, H3N2</td>
<td>Flu B</td>
<td>B/Maryland/15/2016, Flu B Victoria</td>
</tr>
<tr>
<td>Flu A</td>
<td>A/PuertoRico/8/34, H1N1</td>
<td>Flu B</td>
<td>B/Phuket/3073/2013, Flu B Yamagata</td>
</tr>
<tr>
<td>Flu A</td>
<td>A/Brisbane/2/2018, H1N1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flu A</td>
<td>A/Kansas/14/2017, H3N2</td>
<td>RSV A</td>
<td>A2</td>
</tr>
<tr>
<td>Flu A</td>
<td>A/England/195/2009, pdm09H1N1</td>
<td>RSV B</td>
<td>B</td>
</tr>
<tr>
<td>Flu A</td>
<td>Anhui/1/2013, H7N9 (grown and inactivated at Colindale)</td>
<td></td>
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</tbody>
</table>
Conclusions

SoGAT provides the WHO:

1) An expert group of scientists (clinical>technical experts>mfrs) wanting the numbers in NAT assays to be accurate and comparable through time and space

2) A forum to share experience and practical challenges in quantification of NAT assays used in clinical diagnosis

3) A forum to support the development of reference standards
   a. Design of pilot and collaborative studies
   b. Review of data
   c. Formulation of next steps before submission to WHO

ECBS

Overall:
A forum where the WHO and SI measurement systems meet for benefit to the greatest number of patients.