Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

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

2nd IS for HIV-2 RNA (2018)

1st IS for Adenovirus DNA (2018)

1st IRR for MERS CoV (2018)

6th IS for HCV RNA (2019 - genetic variability and batch size)

1st 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 Syncitial Virus

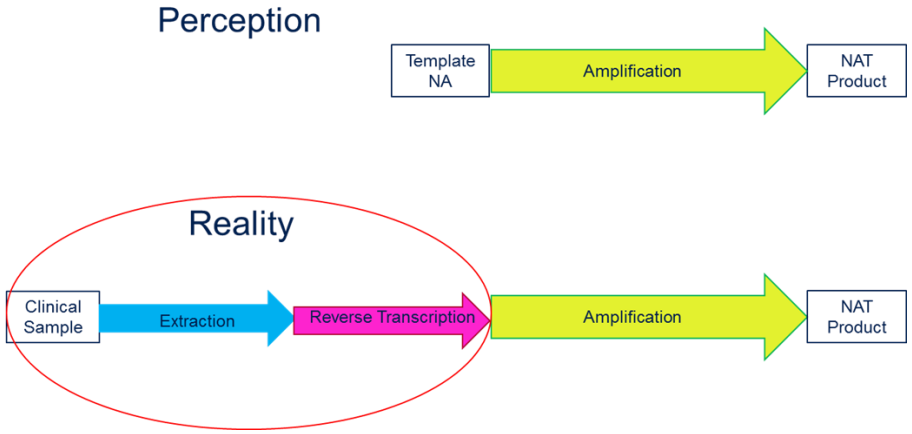
Crimea Congo Haemorhagic Fever
Rift Valley Fever

Commutability

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

Issues to consider:

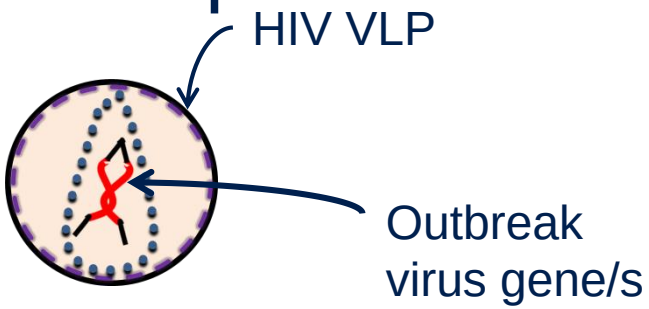
1) Does the reference standard “control” the whole process?



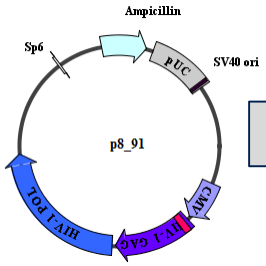
2) Is the assay fit for purpose?
   a. How is it calibrated?
   b. Is the diagnostic amplification fit for purpose?

(Hayden et al JCM 2019)

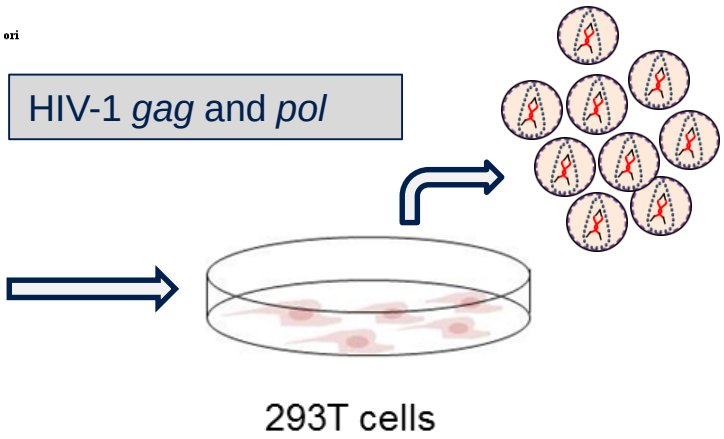
Chimaeric HIV-outbreak virus RNA particles

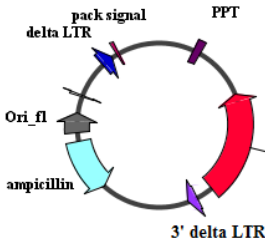


Production



HIV-1 *gag* and *pol*





Outbreak RNA virus genes

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

Mattiuzzo et al., PLoS One, 2015

Do recombinant lentiviruses reflect Disease X?

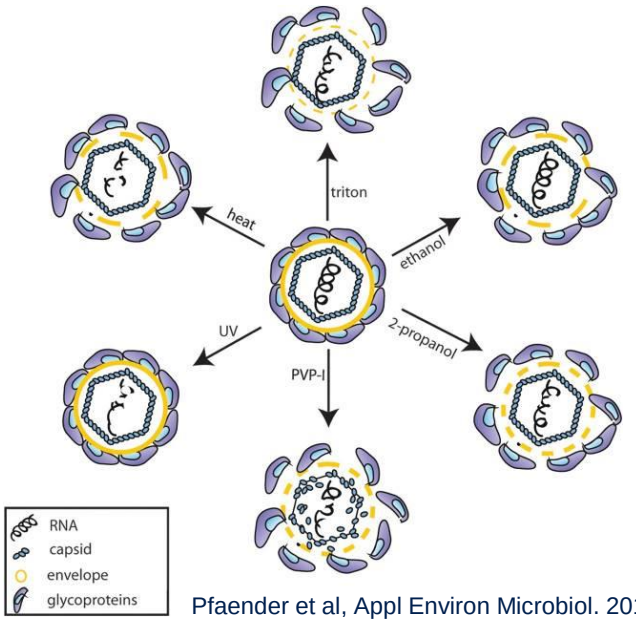
Maintaining the Standard

6th IS for HCV RNA - Outline of Collab Study

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

* evaluated in quantitative assays only

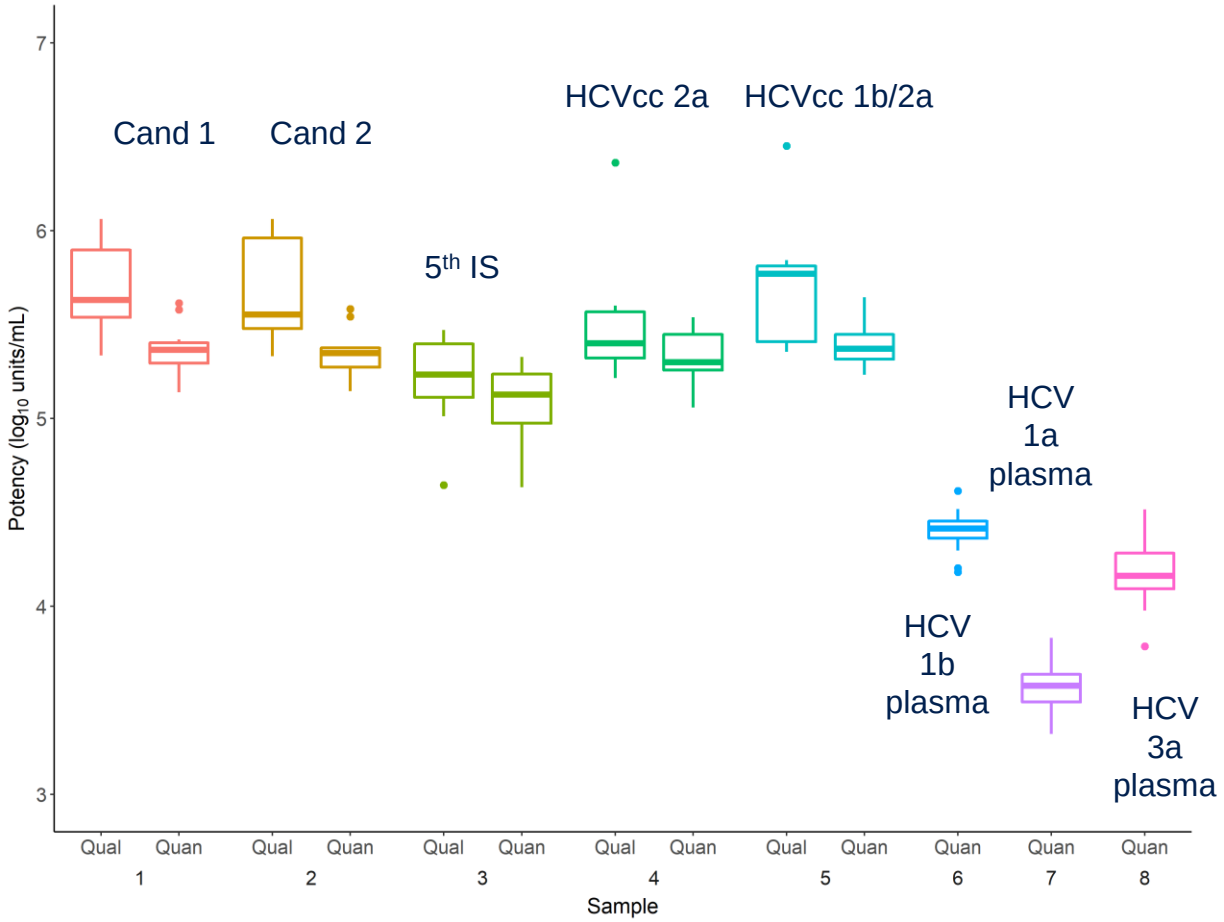
HCVcc kindly provided by Dr Wakita, NIID, Japan. Virus inactivated by UV irradiation and purified by sucrose density gradient.



Pfaender et al, Appl Environ Microbiol. 2015

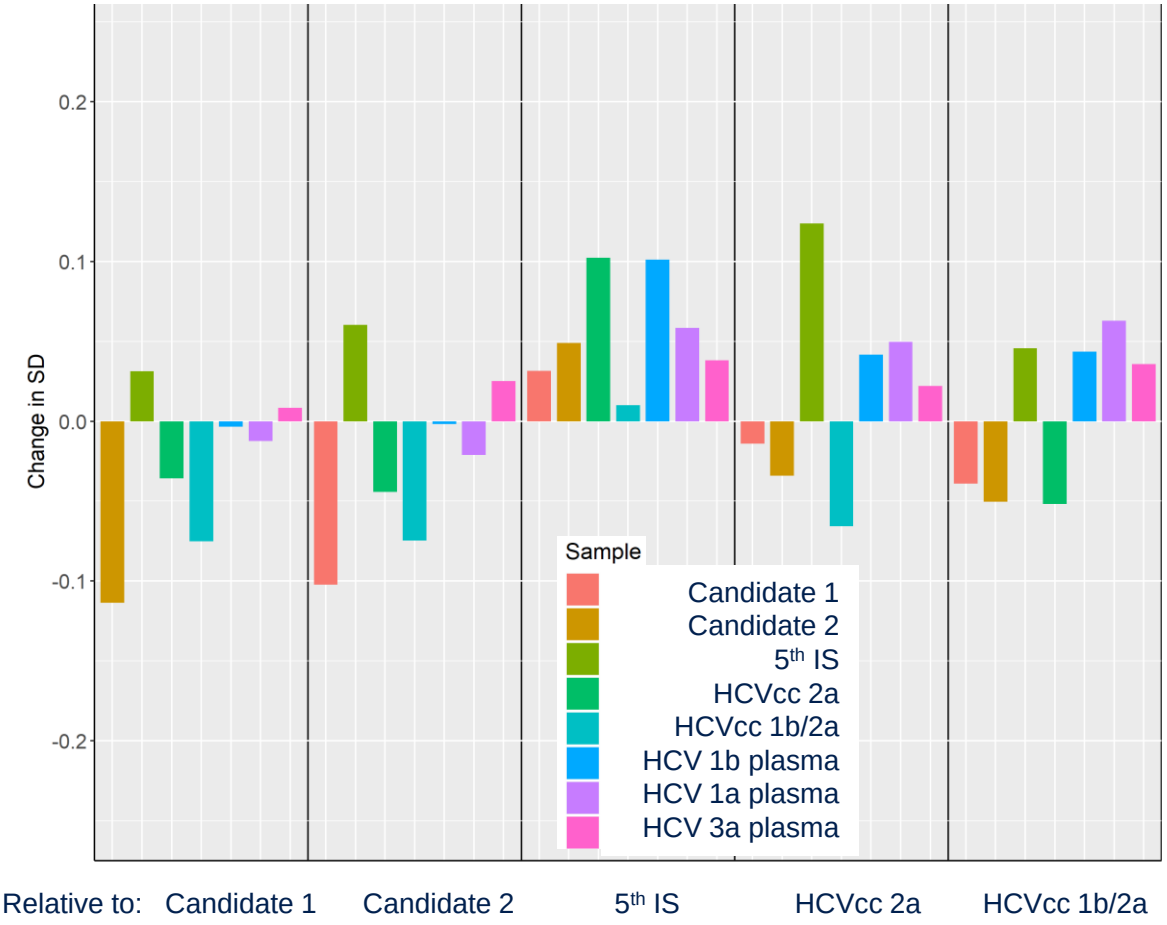
Overall potency estimates (Qual vs Quant)

- *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.*



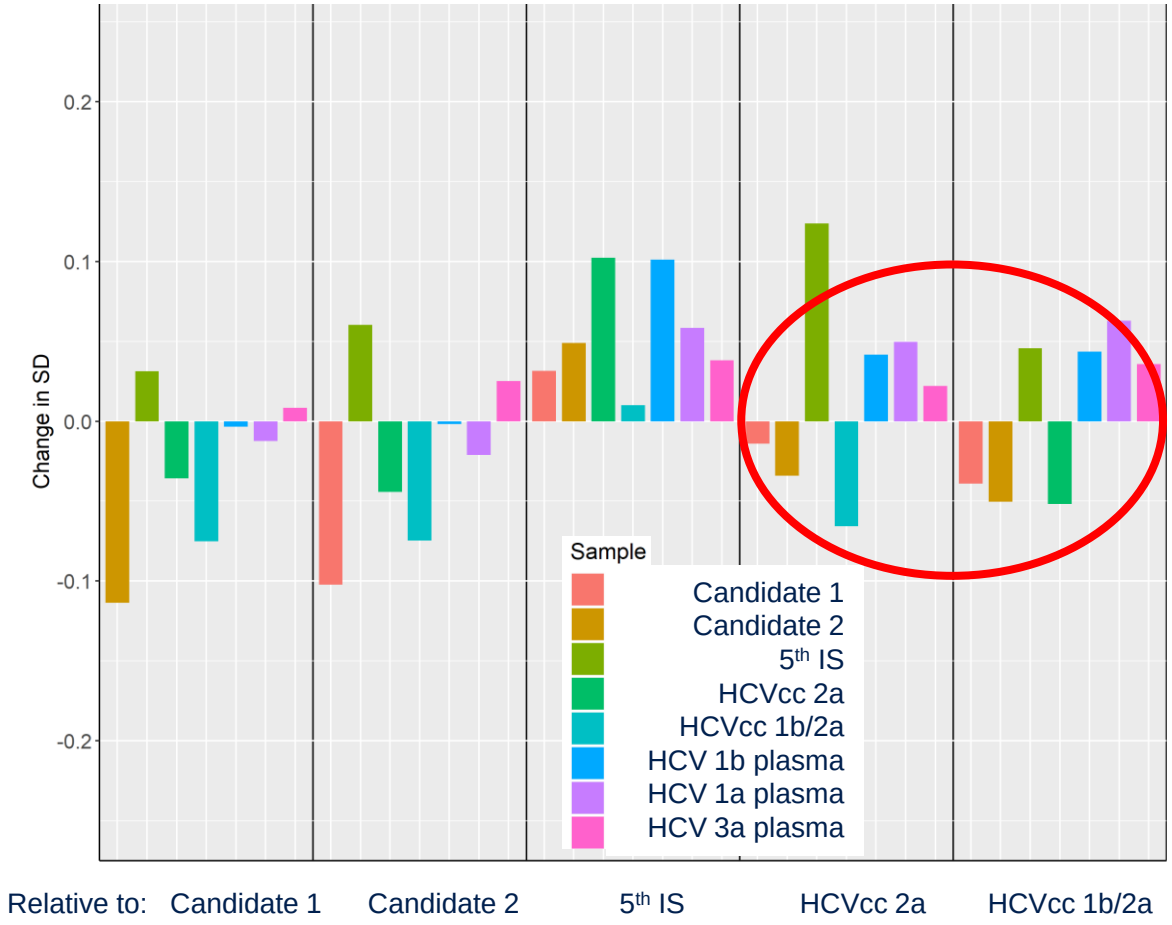
Relative potencies – change in SD

- *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

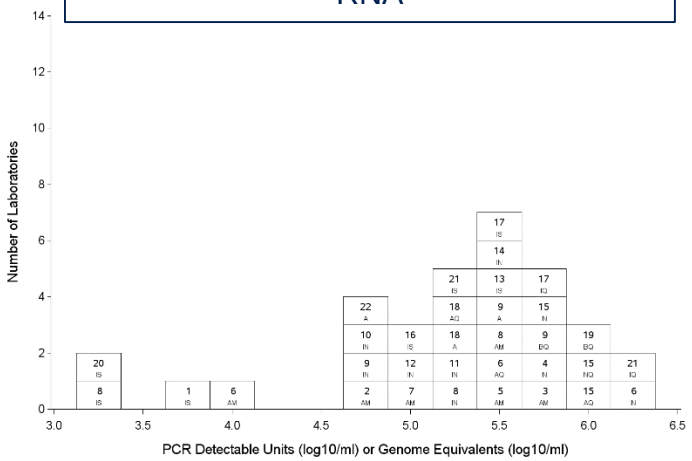
- *Only small changes in SD when results expressed as relative potencies.*
- *No differences in harmonization between HCV genotypes.*
- *For the majority of samples candidates 1 and 2 improved the agreement between laboratories.*
- *HCVcc does not significantly worsen the agreement between laboratories.*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



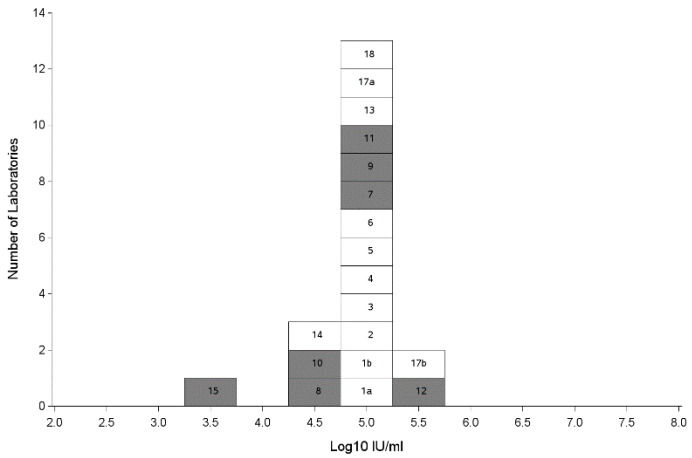
Biological Standardisation allows assay improvement to be quantified

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

Raw data returned by participants of collaborative study for 1st IS for HCV RNA



Raw data returned by participants of 5th IS HCV RNA



- 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)

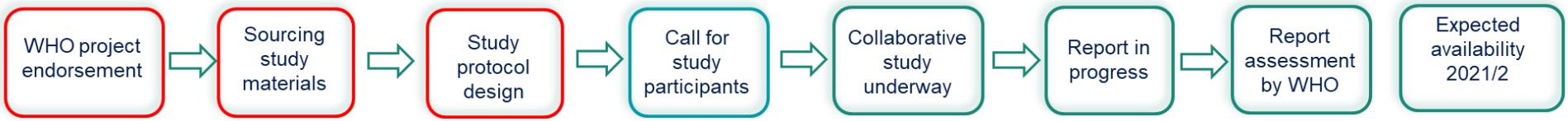
Point of Care/Impact Testing

Influenza types A and B, RSV (1st IS's)

Complexity of Studies increasing – need for Pilot studies

Pilot study samples:

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



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.