SoGAT and the Standardisation of NAT for Blood-Borne Pathogens

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SoGAT and the Development of Standards

- NIBSC and EPFA organised Workshop on use of nucleic acid-based techniques (NAT) for blood safety in Helsinki in 1994
- Identified need for forum to discuss NAT and their standardisation
- Led to the setting up of the SoGAT Working Group:
  - Standardisation of Gene Amplification Techniques for blood borne viruses (SoGAT)
- First met in April 1995 at NIBSC
- Co-sponsored by WHO until 2003
- SoGATs objectives (revised 2003) include:
  - Development, evaluation and provision of reference reagents and International Standards for NAT for blood borne pathogens
  - Organise collaborative studies to evaluate candidate standards, methods and laboratory performance
  - Exchange information on technical and scientific aspects of NAT
  - Discuss new developments in technology (eg synthetic standards, multiplex standards, micro-arrays etc)
- SoGAT provided forum in which the views of all groups (control labs, fractionators, kit manufacturers, reference labs etc) could be voiced and could influence development of standards

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SoGAT and the Development of Standards

- Over the last 10 years great strides made in providing International standards for main blood pathogens

- NAT standards developed in collaboration with WHO:
  - International Standards – in International Units (IU)
  - Reference Reagents – less characterised, usually not given IU
  - International Reference Panels – collection of reagents

- SoGAT, WHO and NIBSC have collaborated in establishing range of International Standards:

  - 1992: 1st International Reference Reagent for HIV-1 p24 Ag (1000 IU/amp)
  - 1997: 1st International Standard for HCV RNA (50,000 IU/vial)
  - 1999: 1st International Standard for HIV-1 RNA (100,000 IU/vial)
  - 1999: 1st international Standard for HBV DNA (500,000 IU/vial)
  - 2000: 1st International Standard for B19 DNA
  - 2002: 1st International Standard for HAV RNA
  - 2003: 1st International Reference Panel for HIV-1 Genotypes
  - HBsAg, subtype adw2 genotype A (33 IU/vial) 2nd IS 2003

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Retrovirology Standards and Working Reagents

SoGAT and the Development of Standards

- **Standardisation of biologicals – problems and difficulties:**
  - **Inherent variability of biological systems, including biological and immunological assays**
  - **Complex macromolecules / systems (eg viruses)**
  - **Cannot be adequately characterised by chemical and/or physical means alone**
  - **Used in prophylaxis, therapy or diagnosis of human diseases**
  - **Many biologicals exist in both active and inactive states in plasma**
  - **Activity (IU) rather than content (mol) may better reflect the clinical situation**
  - **Calibration in less precise biological units (IU) may be more appropriate than calibration in more precise, but clinically less relevant SI units**

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SoGAT and the Development of Standards
WHO vs ISO Standards

Since 1995, NAT standards have been established following WHO principles rather than those of the more recent prEN/ISO 17511 standard

- **WHO International Biological Standards:**
- **Arbitrary International Units assigned (IU)**
- **Multi-method collaborative study to assign value**
- **No imprecision assigned to the ampoule content**

- **prEN/ISO 17511:**
- **Calibration in SI units (eg mg) – represents higher metrological status**
- **Single defined reference method to define value**
- **Traceability to previous standard, with defined uncertainty**

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- **WHO Consultation on Global Measurement Standards and their use in the *in vitro* Biological Diagnostic Field**
- **Reviewed scientific basis for the preparation and characterization of biological reference materials.**
- **Re-affirmation that concepts used by WHO for biological standardization still appropriate**
- **Re-affirmation of the continued need and usefulness of this class of reference materials**
- **Need for improved clarity in explaining principles used to establish WHO International Standards**

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SoGAT and the Development of Standards

- **WHO Consultation:**
  - **Choice of unit – IU, SI, none**
  - **should be based on biological, medical, physicochemical information available on case-by-case basis**
  - **Where appropriate for WHO biological standard to be calibrated in SI units, principles of ISO 17511 to be followed**
  - **Issues/principles to be dealt with include:**
    - **Methods – single or multiple**
    - **Measurement uncertainty**
    - **Commutability in *in vitro* diagnostics field (matrix issues)**
    - **Traceability**
  - **Calibration – 2nd standard calibrated in terms of 1st with uncertainty – complies with metrological principles**
  - **Value assignment – 2nd standard arbitrarily assigned a value intended to preserve the value of the IU but without traceability – not compliant, but works in practice!**

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Example of SoGAT Activity: Development of International Standard for HIV-1 RNA

- **Various qualitative and quantitative assays available for HIV-1 NAT based on different technologies:**
  - **Target amplification – PCR, isothermal methods**
  - **Signal amplification – bDNA**
  - **May amplify gag, pol, LTR region of genome**

- **NAT assays for HIV-1 were:**
  - **standardised against in-house control materials**
  - **varied in their sensitivity**
  - **varied in their estimation of RNA concentration**

- **SoGAT agreed that need for International Standard for HIV-1 RNA**

- **Three candidate standards evaluated in collaborative study**

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Development of 1st International Standard for HIV-1 RNA

Candidate:	XX	YY	ZZ
Subtype:	B	B	B
Virus:	Primary isolate	Plasmapheresis donation	T-cell line virus
Freeze-dried:	Yes	Yes	No
Diluent:	Plasma	Plasma	Plasma
Volume:	1ml	1ml	0.5ml

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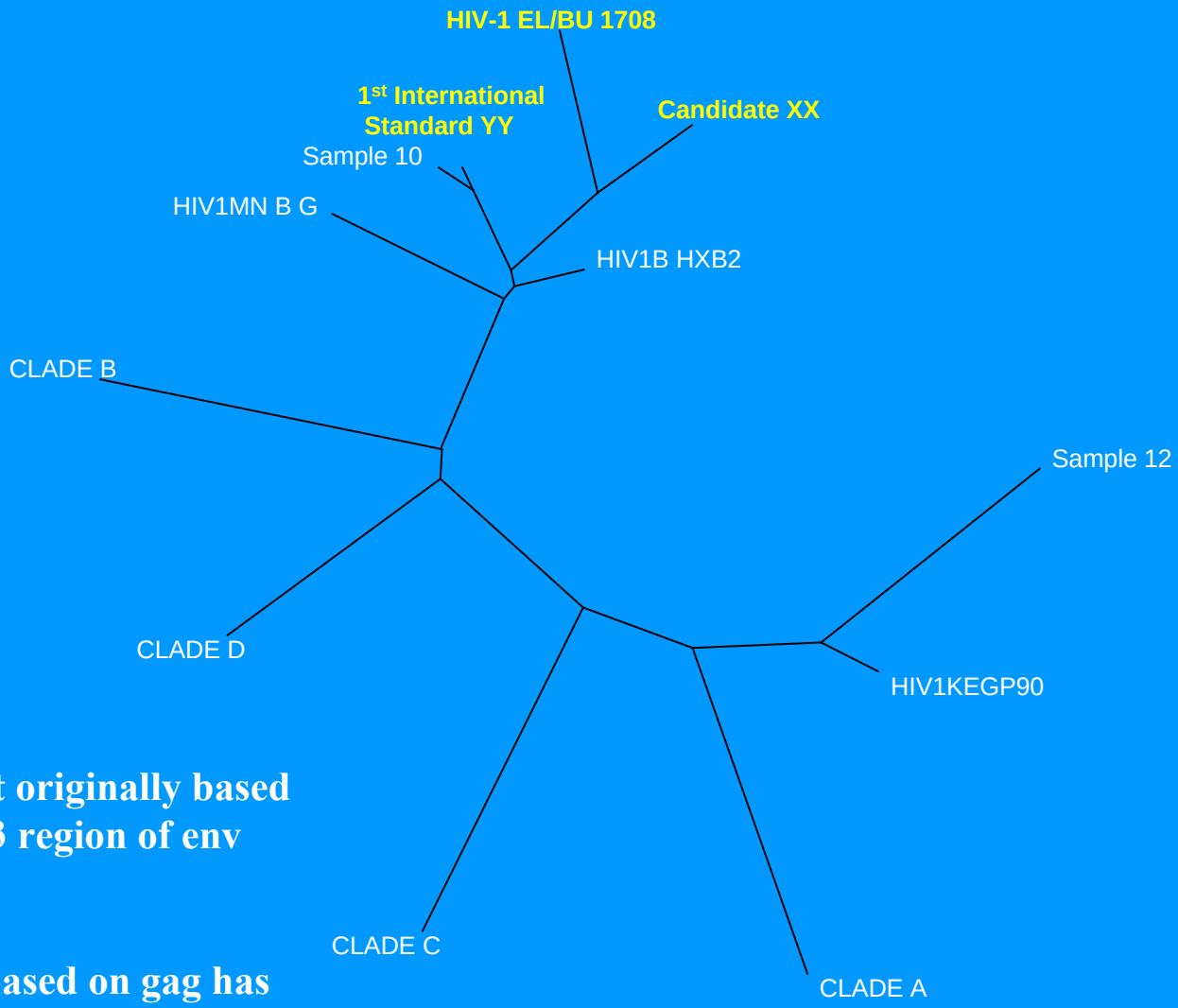
Unrooted phylogenetic tree of HIV-1 gag



Subtype assignment originally based on sequencing of V3 region of env

Sequence analysis based on gag has confirmed that virus is subtype B

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Development of 1st International Standard for HIV-1 RNA

--- bDNA

--- Monitor

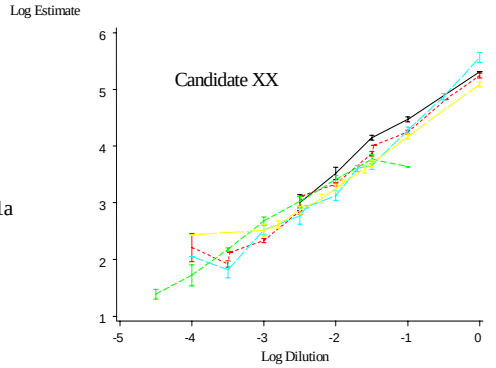
--Nuclisens

--- Abbott

--- In-house

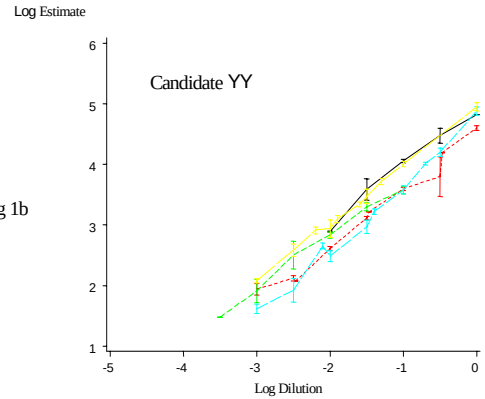
Figure 1

Fig 1a



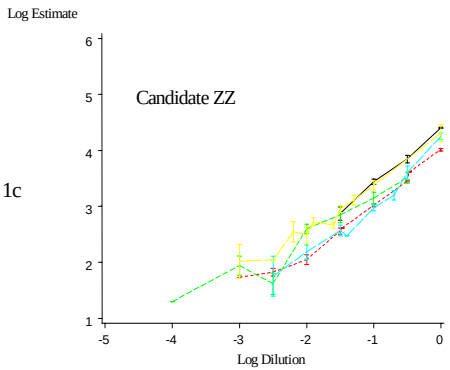
Candidate XX

Fig 1b



Candidate YY

Fig 1c



Candidate ZZ

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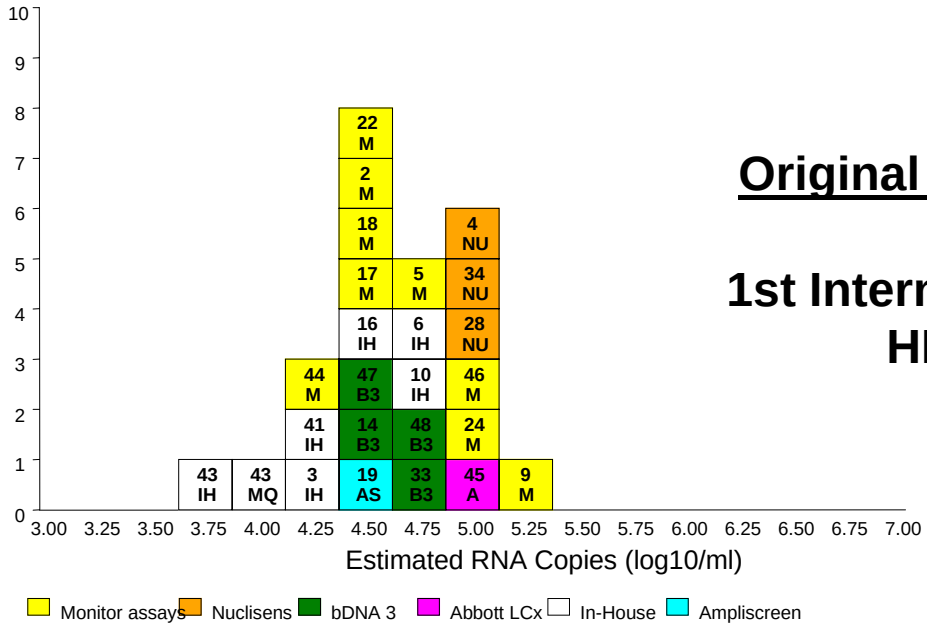
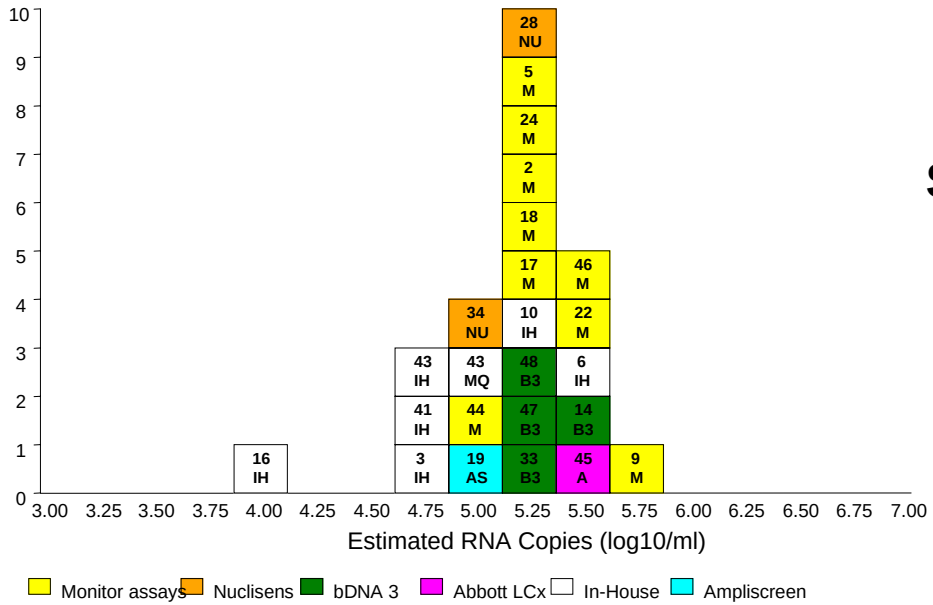


Original Collaborative Study

1st International Standard for HIV-1 RNA (YY)



Sample XX

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- In 1999 WHO Expert Committee (ECBS) established candidate YY as 1st International Standard for HIV-1 RNA
- Assigned an arbitrary concentration of 5.0 $\log_{10}$ IU/ml (100,000) IU per vial
- International Standard distributed by NIBSC
- Conventional standard conforming to WHO principles
- Can synthetic RNA standards, characterised in molecular terms, allow us to move away from the WHO paradigm, towards the top end of the 17511 hierarchy?

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The biological argument:

The SI unit might promote an inference of function from structure

Mol phosphate → Genome equivalent → **Virus number** → **Infectivity**

Not all RNA molecules are found in viruses.

Not all RNA molecules found in viruses are full length and therefore infectious.

Not all viruses containing full length RNA molecules are infectious

(P Minor)

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Biological vs Synthetic Standards

Parameter	Biological	Synthetic
Actual agent determined / quantitated by assays	√	X
Matrix similar to matrix of specimen	√	X
Contains all sequences recognised by probes in all different assays	√	X
Different assays yield similar results	√	?
Long term stability	√	√

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Biological vs Synthetic Standards

Parameter	Biological	Synthetic
Homogenous material with little inherent variability	X	√
Well-characterised material	X	√
Abundant source material	X	√
Consistency between replacement lots	X	√
Ease of calibration of subsequent replacement standards	X	√

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Areas of Debate and Concern

- **Establishment of replacement International Standards**
  - **Commutability with previous IS essential to prevent 'drift' in IU**
- **Stringent requirements by regulatory authorities necessitates accurate calibration of replacement standard with regard to previous standards**
- **WHO collaborative study approach includes several laboratories and methodologies**
  - **may produce bias, inconsistent quantitation**
  - **Variation in assay results: ~ $\log_{10}$ 0.5-0.6 seen with NAT assays**
- **Limited study using proficient laboratories to establish replacement standards?**
- **Is establishment of SI standard, including reference material, reference method and reference laboratory, feasible for NAT?**
- **Feasibility of using synthetic materials as calibrators?**
  - **Being investigated by SoGAT members**

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RNA Synthetic Standard for HCV NAT

- **Initial feasibility study organised by Industrial Liaison Committee – reported to SoGAT and WHO**
- **Further study planned to compare:**
  - ***In vitro*-generated HCV RNA (Bayer)**
  - **Armoured HCV RNA (Ambion)**
  - **WHO International Standard for HCV**
- **To be evaluated using 3 commercial quantitative HCV assays**
- **Outcome:**
  - **Linearity and precision in each assay**
  - **Commutability from synthetic to conventional material**

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Summary of role of SoGAT in the Standardisation of NAT

- SoGAT focussed on development and evaluation of standards for NAT for blood borne pathogens
- SoGAT provides forum for the discussion of:
  - Technical and scientific aspects of nucleic acid assays
  - Regulatory approaches
  - Development of standards for emerging pathogens (WNV)
- Brings together professionals from many different fields
- Investigates and examines new generation of reagents and standards such as synthetic nucleic acids
- Supports generation of reagents for new technologies such as micro-arrays

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SoGAT and the Development of Standards

I would like to thank members of the SoGAT Working Group and its Scientific Organising Committee including the following:

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  - Dr Phil Minor
  - Dr Sally Baylis
  - Dr Adrian Bristow
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