Digital PCR as a reference measurement procedure for HIV quantification

Jim Huggett

Principal Scientist (NML) & Senior Lecture (University of Surrey)

Metrological traceability

analytical chemistry

Article

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DNA Copy-Concer
Digital Polymeras

Hee-Bong Yoo,[†,‡] Sang-Ryc

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NIST Special Publication 260-191

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Clinical Chemistry 64:9
1296–1307 (2018)

Special Report

Assessment of Digital PCR as a Primary Reference Measurement Procedure to Support Advances in Precision Medicine

Alexandra S. Whale,[1†] Gerwyn M. Jones,[1†] Jernej Pavšič,[2,3] Tanja Dreo,[2] Nicholas Redshaw,[1] Sema Akyürek,[4] Müslüm Akgöz,[4] Carla Divieto,[5] Maria Paola Sassi,[5] Hua-Jun He,[6] Kenneth D. Cole,[6] Young-Kyung Bae,[7] Sang-Ryoul Park,[7] Liesbet Deprez,[8] Philippe Corbisier,[8] Sonia Garrigou,[9] Valérie Taly,[9] Raquel Larios,[10] Simon Cowen,[11] Denise M. O'Sullivan,[1] Claire A. Bushell,[1] Heidi Goenaga-Infante,[10] Carole A. Foy,[1] Alison J. Woolford,[1] Helen Parkes,[1] Jim F. Huggett,[1,12*†] and Alison S. Devonshire[1*†]

Article

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on of *Mycobacterium*

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Foy,[†] and Jim F. Huggett*[,†,‡]

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University College London, London NW3 2PF,

Result

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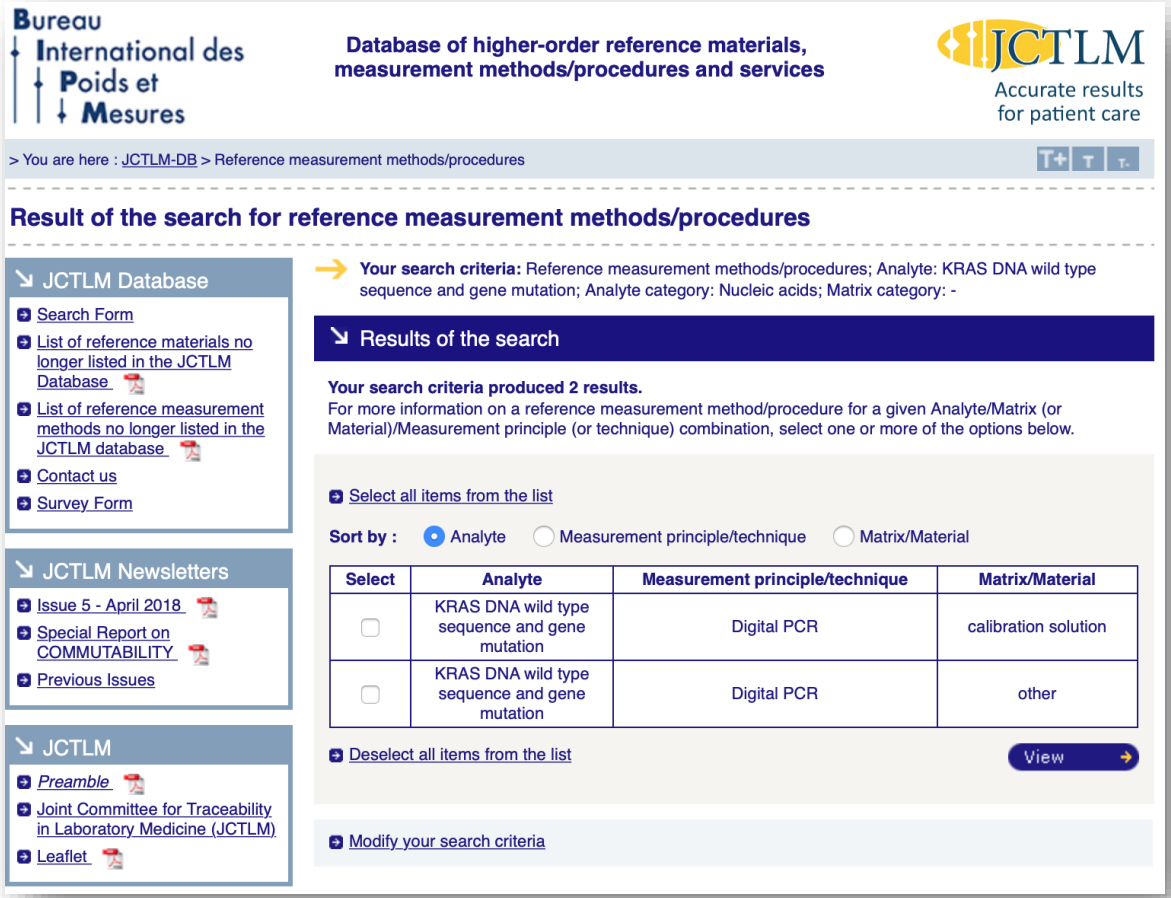
LGC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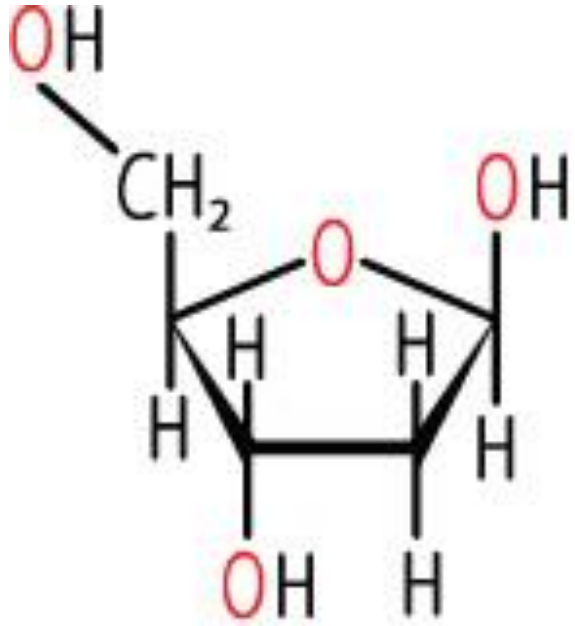
Leaflet
Your search criteria: Reference measurement methods/procedures; Analyte: KRAS DNA wild type sequence and gene mutation; Analyte category: Nucleic acids; Matrix category: -
Results of the search
Your search criteria produced 2 results.
For more information on a reference measurement method/procedure for a given Analyte/Matrix (or Material)/Measurement principle (or technique) combination, select one or more of the options below.
Select all items from the list
Sort by : Analyte Measurement principle/technique Matrix/Material
Select | Analyte | Measurement principle/technique | Matrix/Material
KRAS DNA wild type sequence and gene mutation | Digital PCR | calibration solution
KRAS DNA wild type sequence and gene mutation | Digital PCR | other
Deselect all items from the list
View
Modify your search criteria

DNA vs RNA



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PLOS ONE

Evaluation of Digital PCR for Absolute RNA Quantification

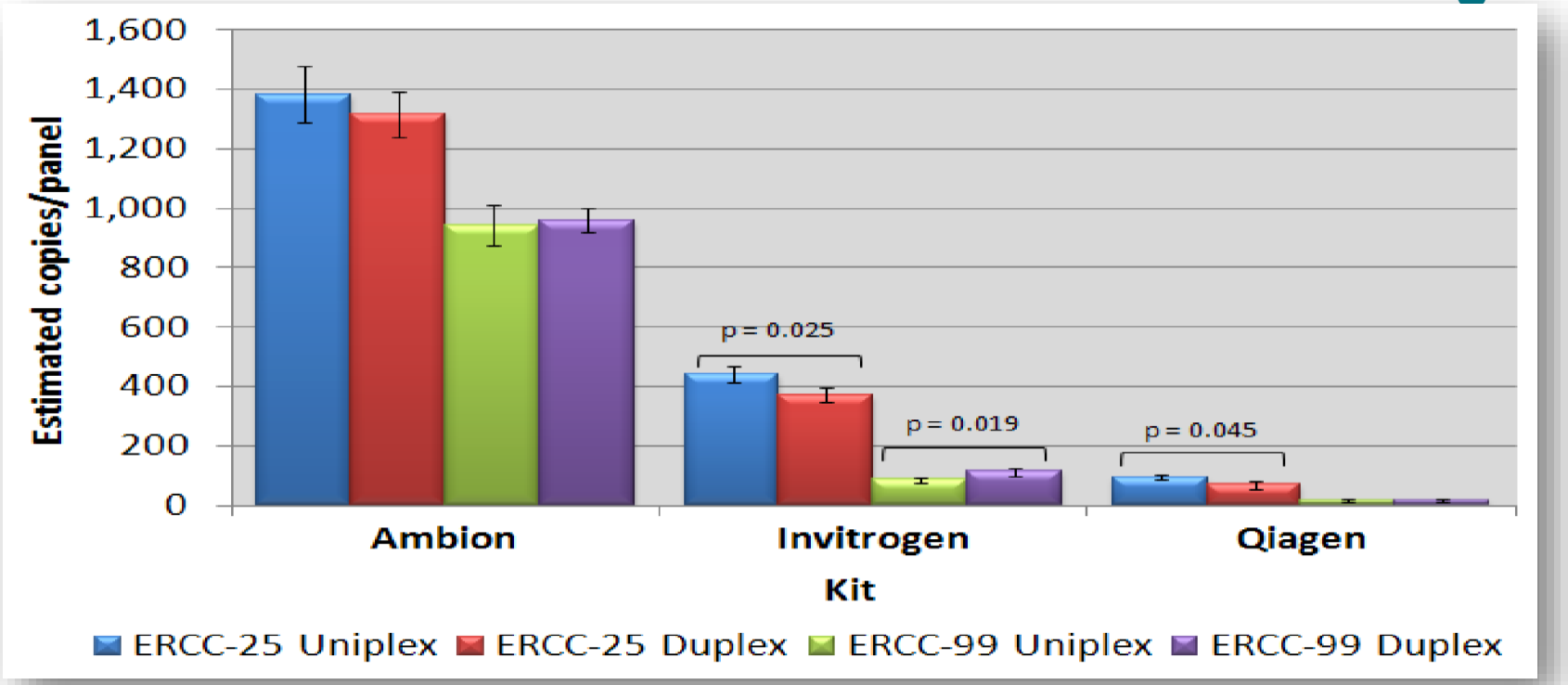
Rebecca Sanders[1,2]*, Deborah J. Mason[2], Carole A. Foy[1], Jim F. Huggett[1]

1 Molecular and Cell Biology, LGC, Teddington, United Kingdom, **2** Cardiff School of BioSciences, The Sir Martin Evans Building, Cardiff, United Kingdom

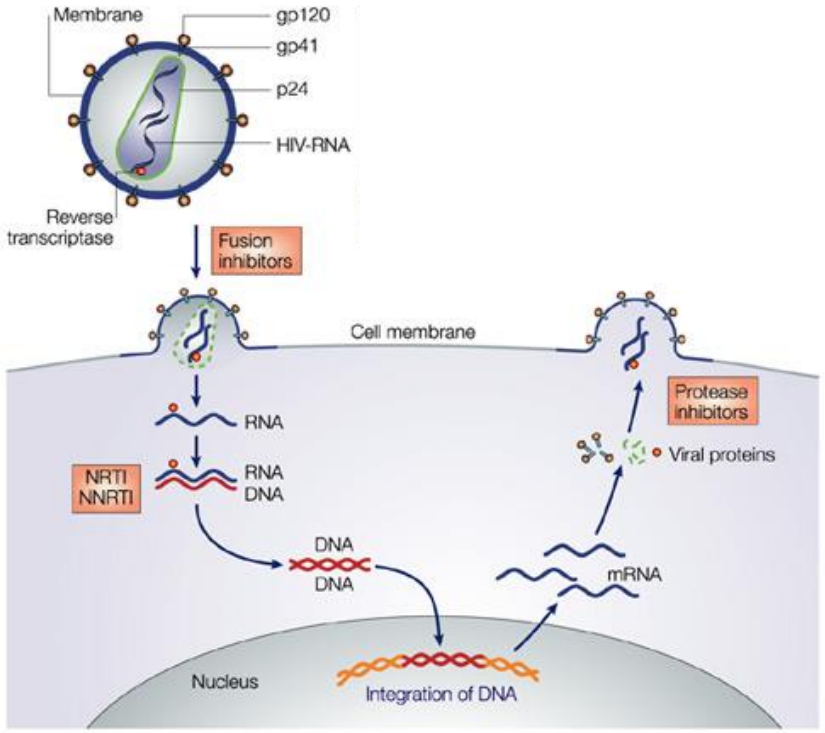
Abstract

Gene expression measurements detailing mRNA quantities are widely employed in molecular biology and are increasingly important in diagnostic fields. Reverse transcription (RT), necessary for generating complementary DNA, can be both inefficient and imprecise, but remains a quintessential RNA analysis tool using qPCR. This study developed a Transcriptomic Calibration Material and assessed the RT reaction using digital (d)PCR for RNA measurement. While many studies characterise dPCR capabilities for DNA quantification, less work has been performed investigating similar parameters using RT-dPCR for RNA analysis. RT-dPCR measurement using three, one-step RT-qPCR kits was evaluated using single and multiplex formats when measuring endogenous and synthetic RNAs. The best performing kit was compared to UV quantification and sensitivity and technical reproducibility investigated. Our results demonstrate assay and kit dependent RT-dPCR measurements differed significantly compared to UV quantification. Different values were reported by different kits for each target, despite evaluation of identical samples using the same instrument. RT-dPCR did not display the strong inter-assay agreement previously described when analysing DNA. This study demonstrates that, as with DNA measurement, RT-dPCR is capable of accurate quantification of low copy RNA targets, but the results are both kit and target dependent supporting the need for calibration controls.

Anal Bioanal Chem. 2014 Oct;406(26):6471-83


1,600
1,400
1,200
1,000
800
600
400
200
0
Estimated copies/panel
p = 0.025
p = 0.019
p = 0.045
Ambion
Invitrogen
Qiagen
Kit
ERCC-25 Uniplex
ERCC-25 Duplex
ERCC-99 Uniplex
ERCC-99 Duplex

The HIV Life Cycle



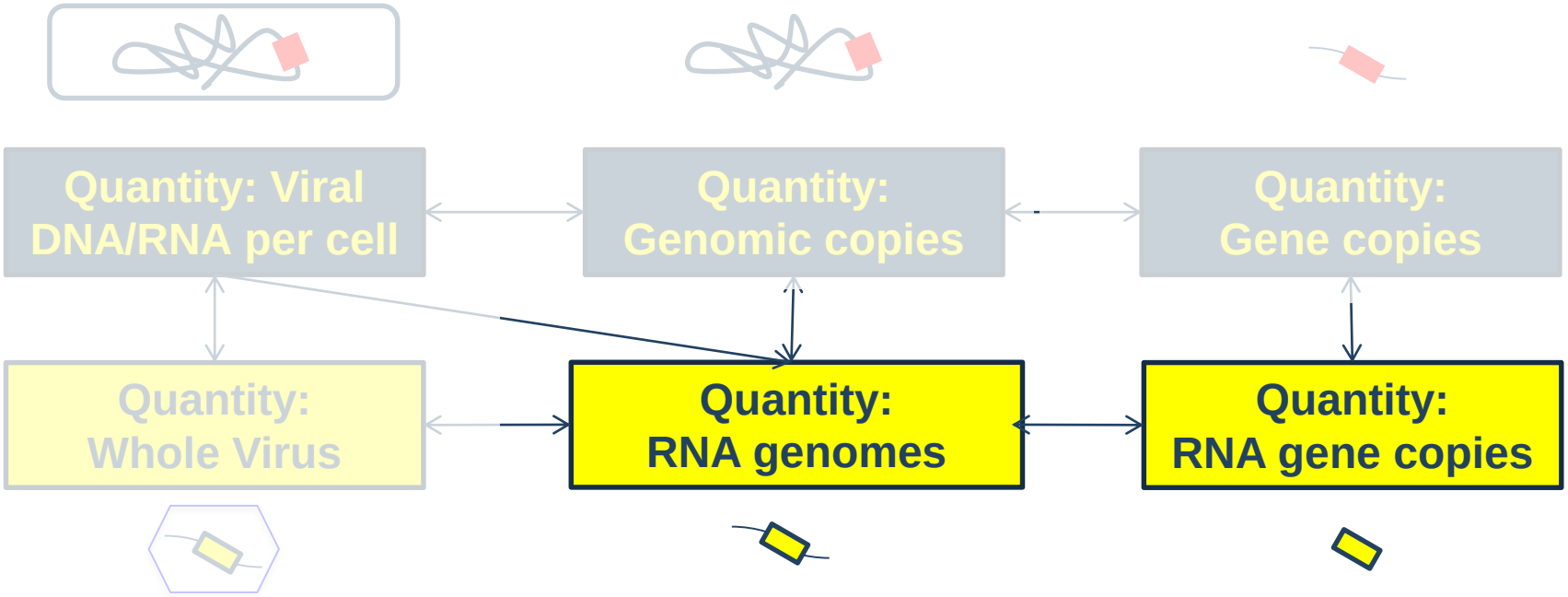
Nature Reviews | Microbiology

HIV-1

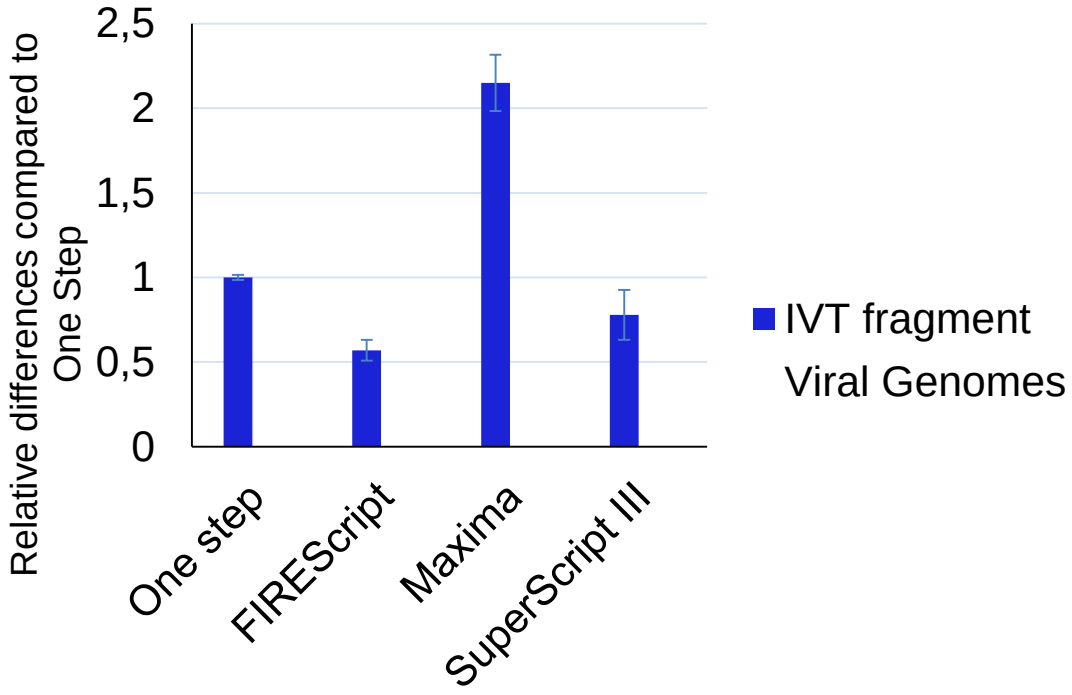
- Retrovirus (single stranded RNA genome)

- Quantified clinically:
  - Guide treatment
  - Monitor resistance

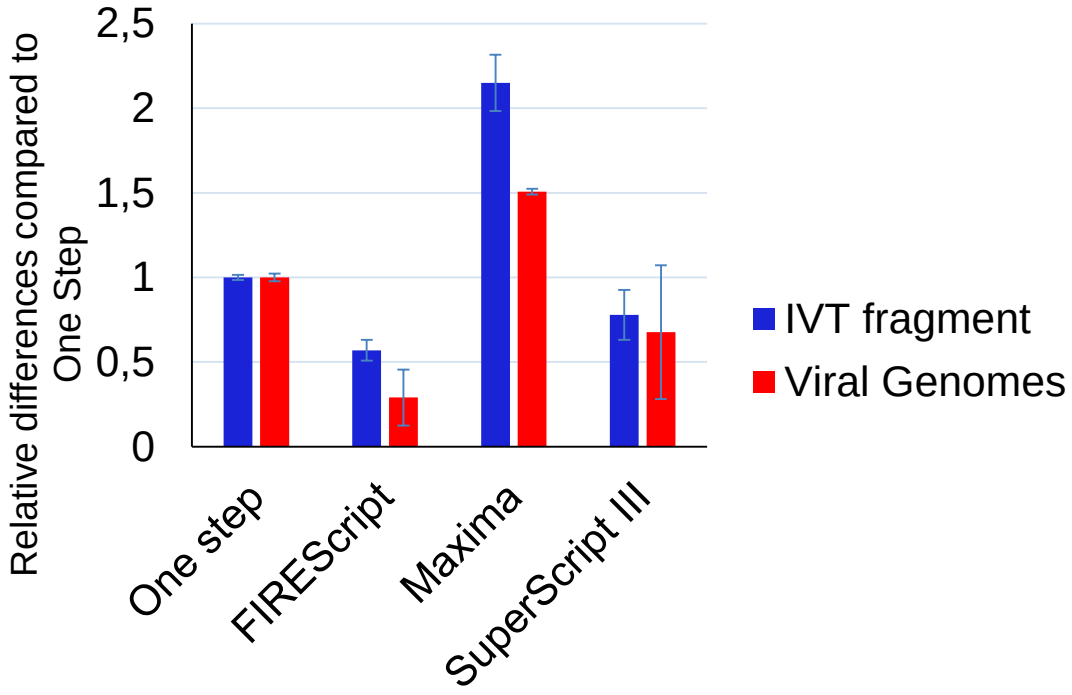
- Measured internationally as copies(IU)/ml blood, plasma, serum
  - <50->100,000 copies/ml
  - RT-qPCR

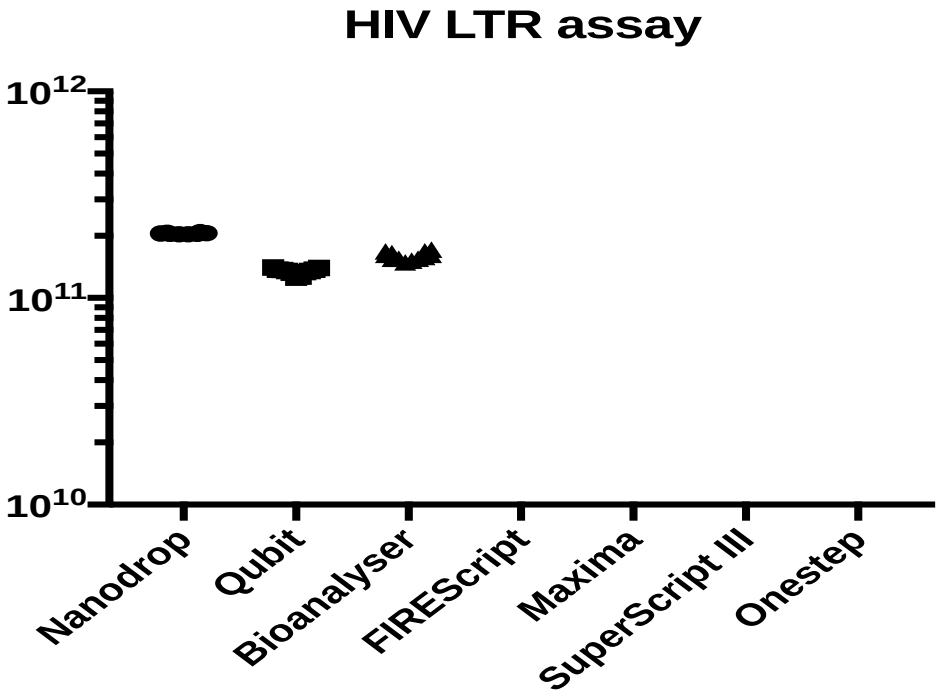

Quantity: Viral DNA/RNA per cell
Quantity: Genomic copies
Quantity: Gene copies
Quantity: Whole Virus
Quantity: RNA genomes
Quantity: RNA gene copies

Comparison of Reverse Transcriptase choice (LTR/GAG assay)

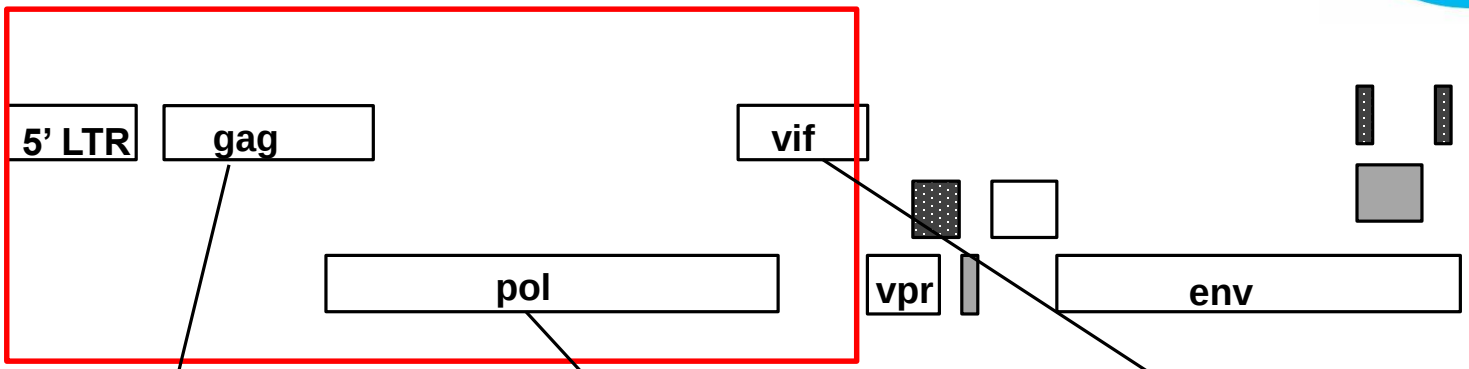


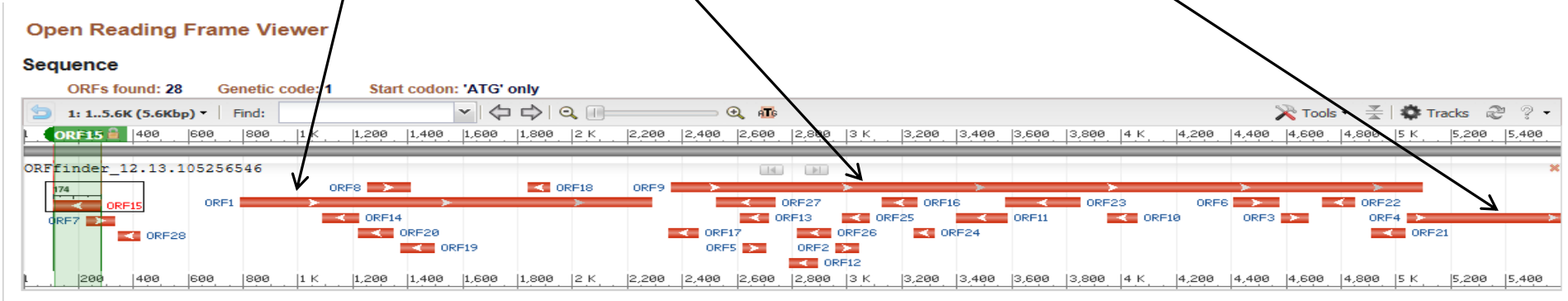
Comparison of Reverse Transcriptase choice (LTR/GAG assay)

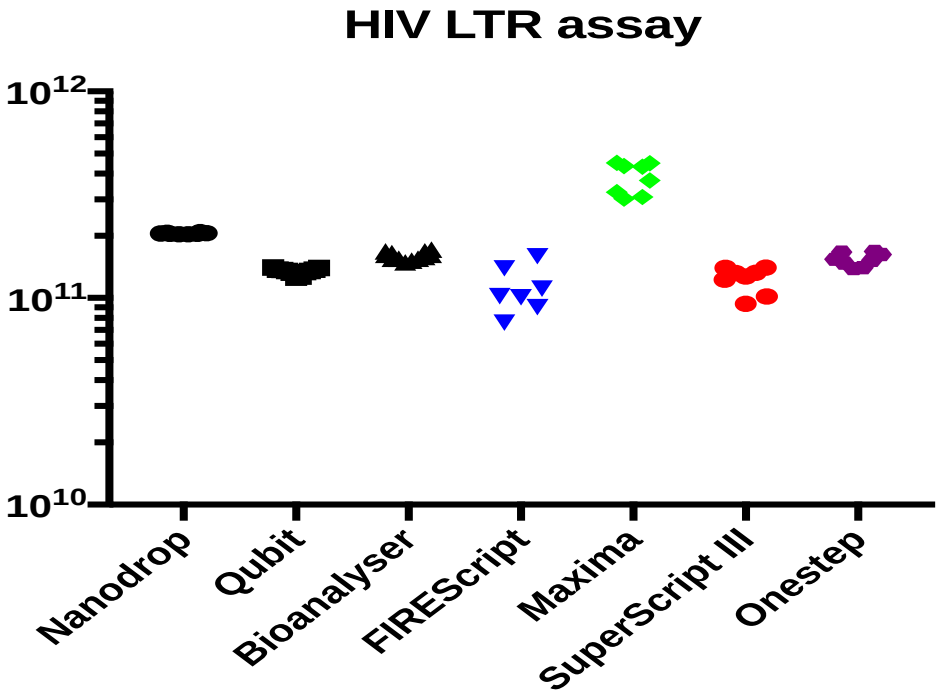


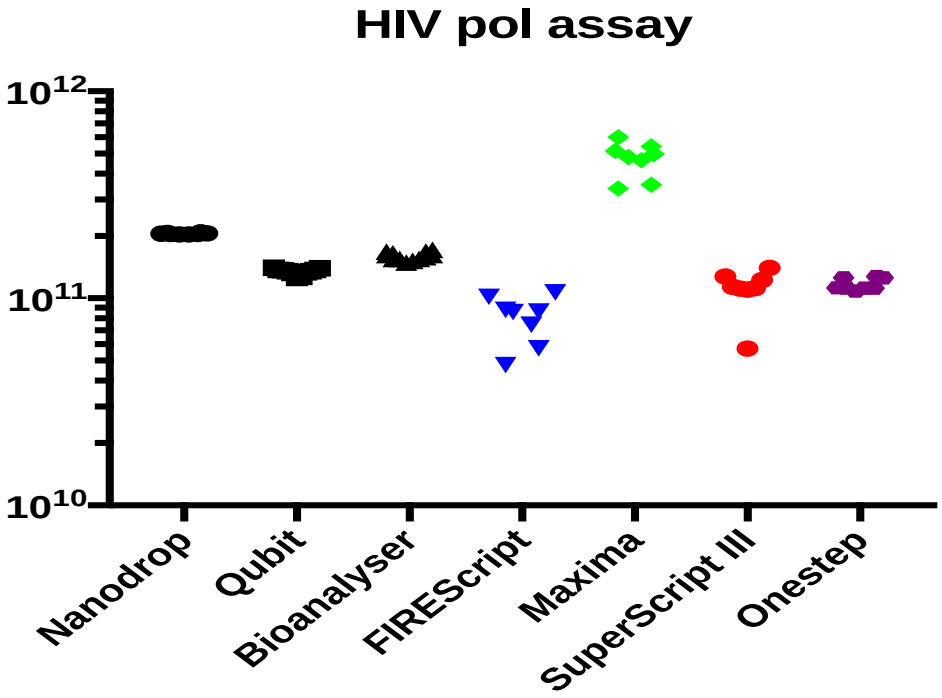

HIV LTR assay
10^12
10^11
10^10
Nanodrop
Qubit
Bioanalyser
FIREScript
Maxima
SuperScript III
Onestep

Synthetic gene fragment ‘UB49’

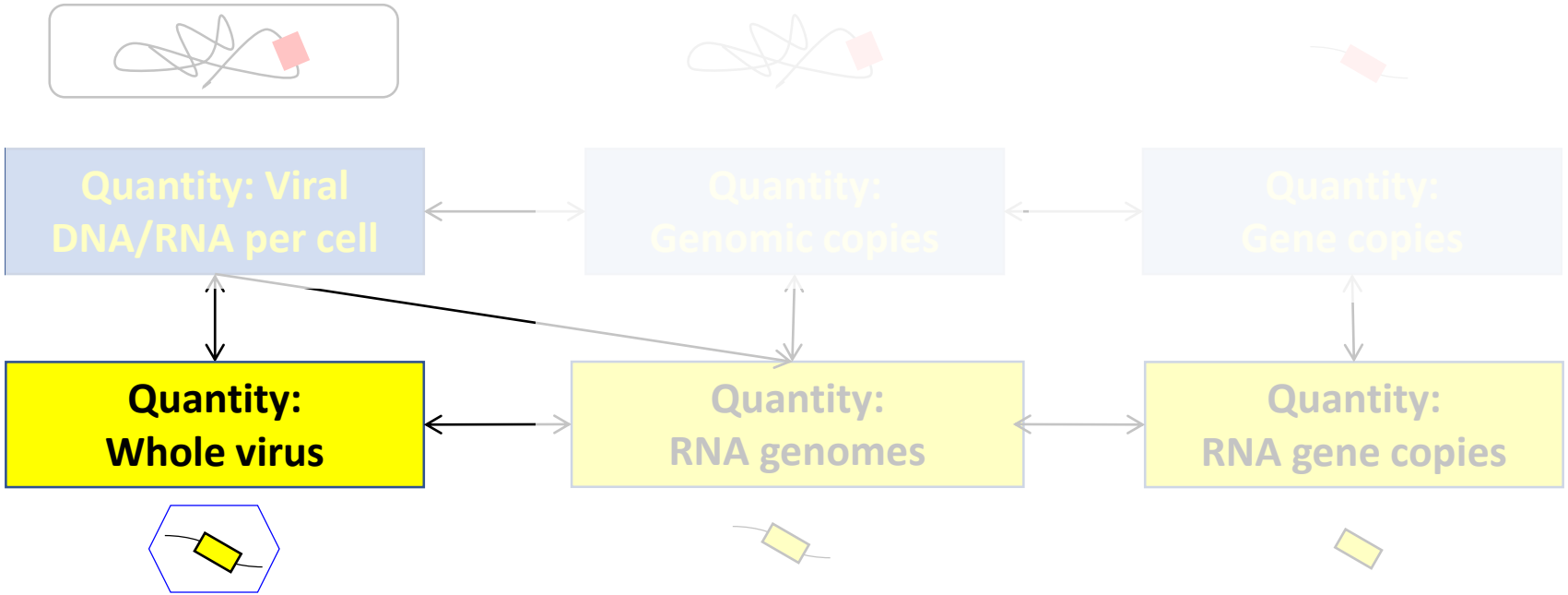




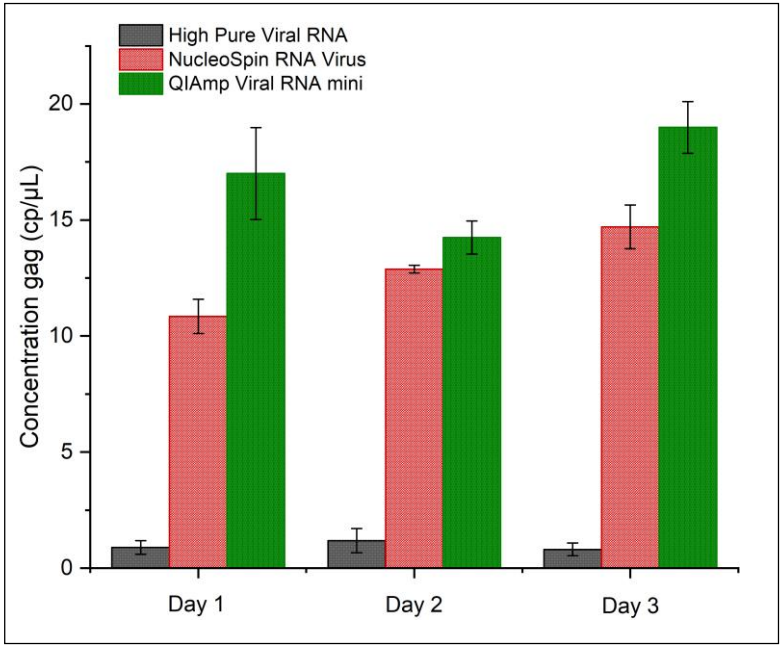

HIV LTR assay
10^12
10^11
10^10
Nanodrop
Qubit
Bioanalyser
FIREScript
Maxima
SuperScript III
Onestep


HIV pol assay
10^12
10^11
10^10
Nanodrop
Qubit
Bioanalyser
FIREScript
Maxima
SuperScript III
Onestep

Materials for full analytical workflow



HIV-1 RNA Extraction Results

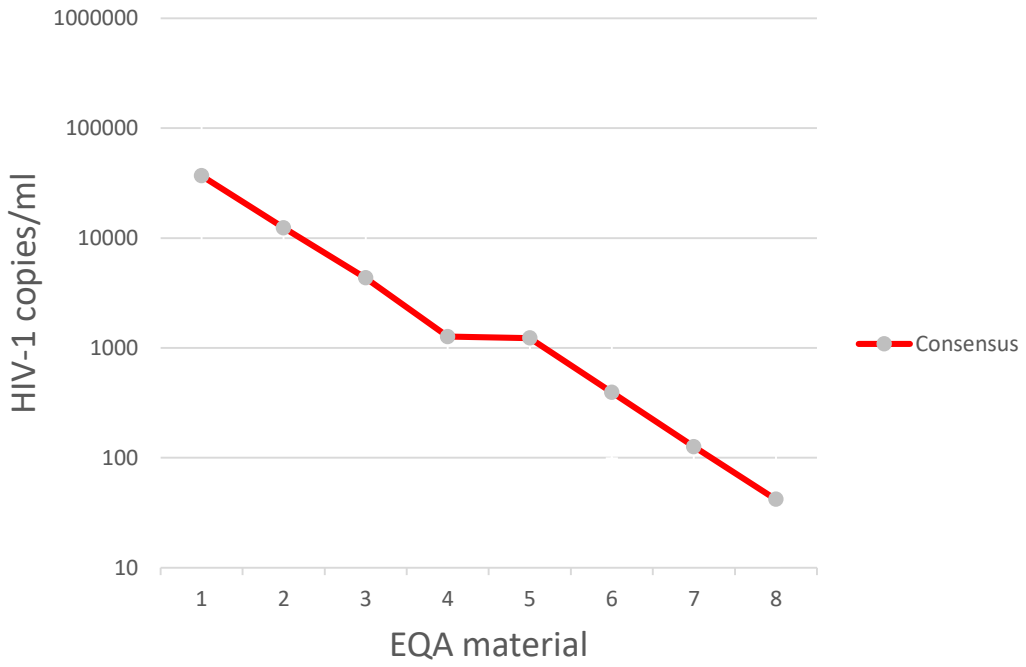


HIV-1 (RNA) Ringtrial 2019

- **HIV-1 (RNA) Virus Genome Detection Program (360)**
- **Additional HIV-1 (RNA) Training Program (382)**
- **PTB and LGC participated using dPCR**
- **Other 140 laboratories used qPCR**

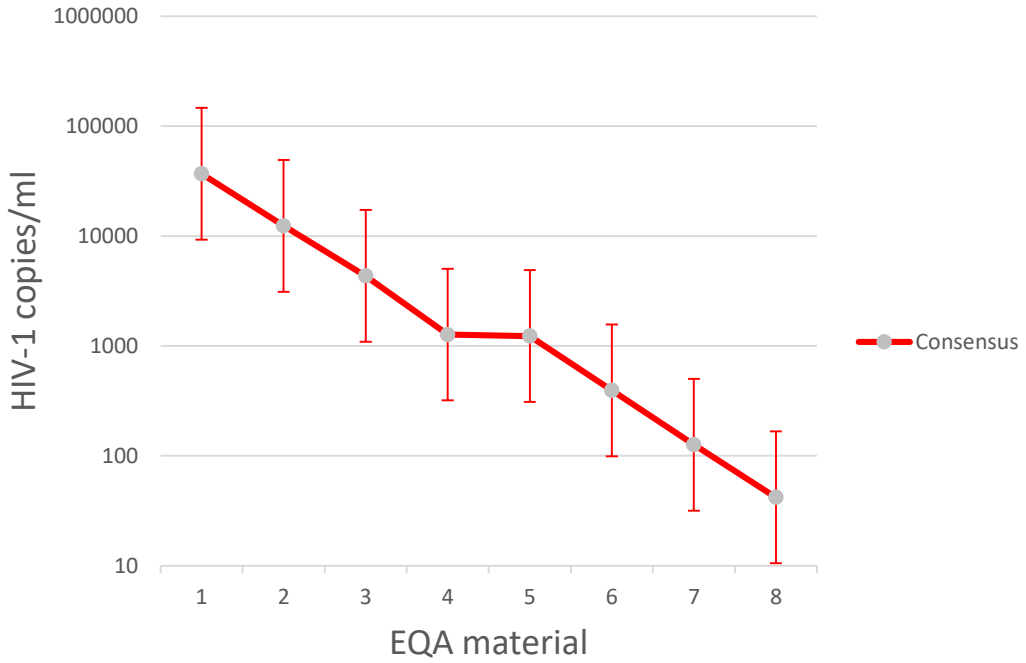
HIV-1 (RNA) Ringtrial 2019

Acceptance range ±log(0.6) for HIV Rili-BAEK



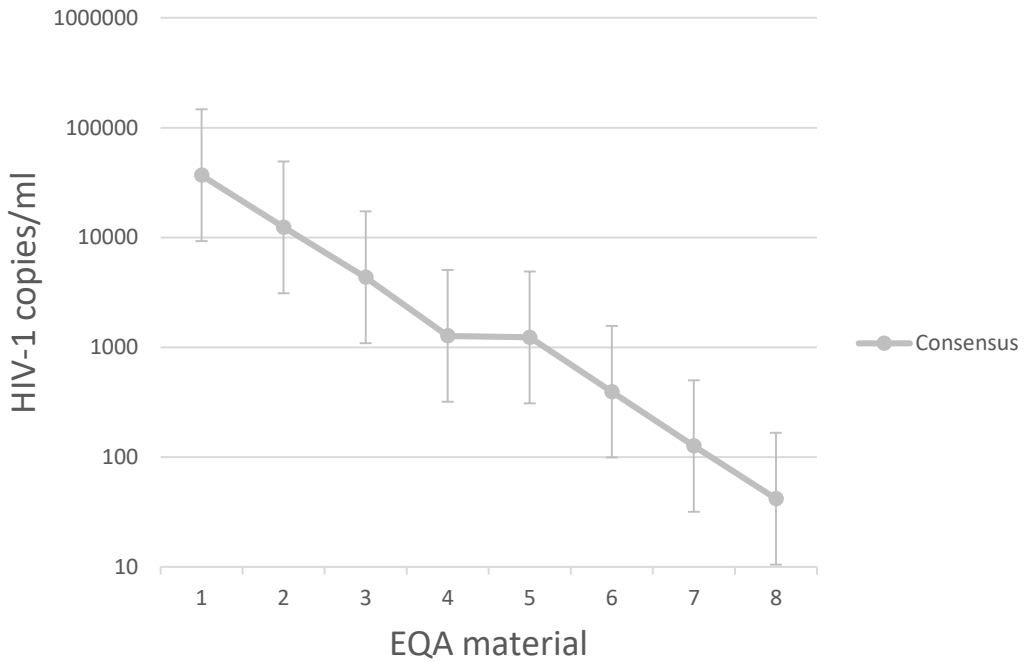
HIV-1 (RNA) Ringtrial 2019

Acceptance range ±log(0.6) for HIV Rili-BAEK



HIV-1 (RNA) Ringtrial 2019

Acceptance range ±log(0.6) for HIV Rili-BAEK



HIV-1 (RNA) Ringtrial 2019

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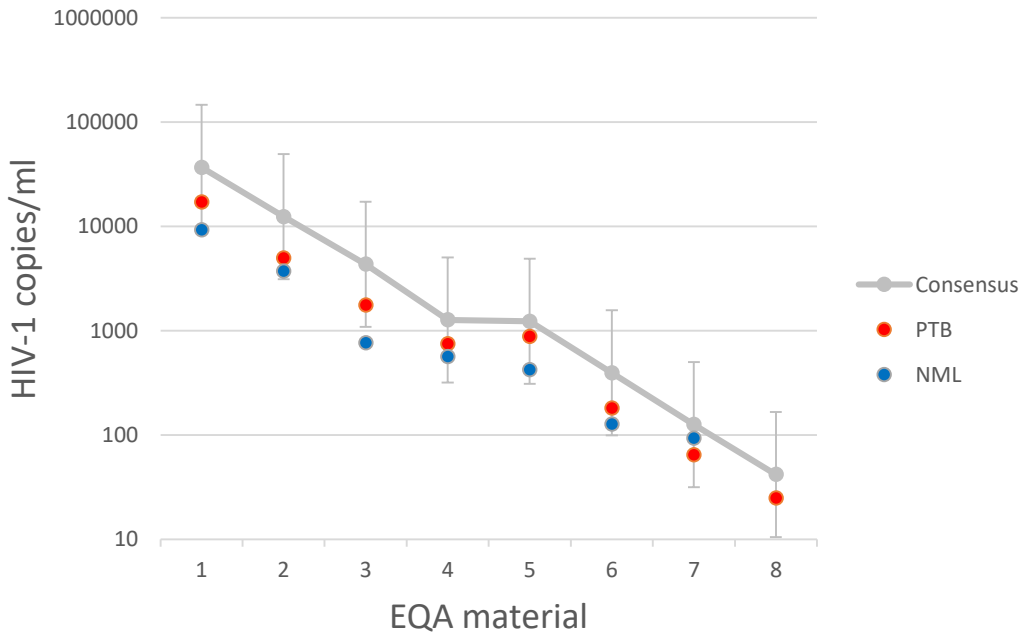
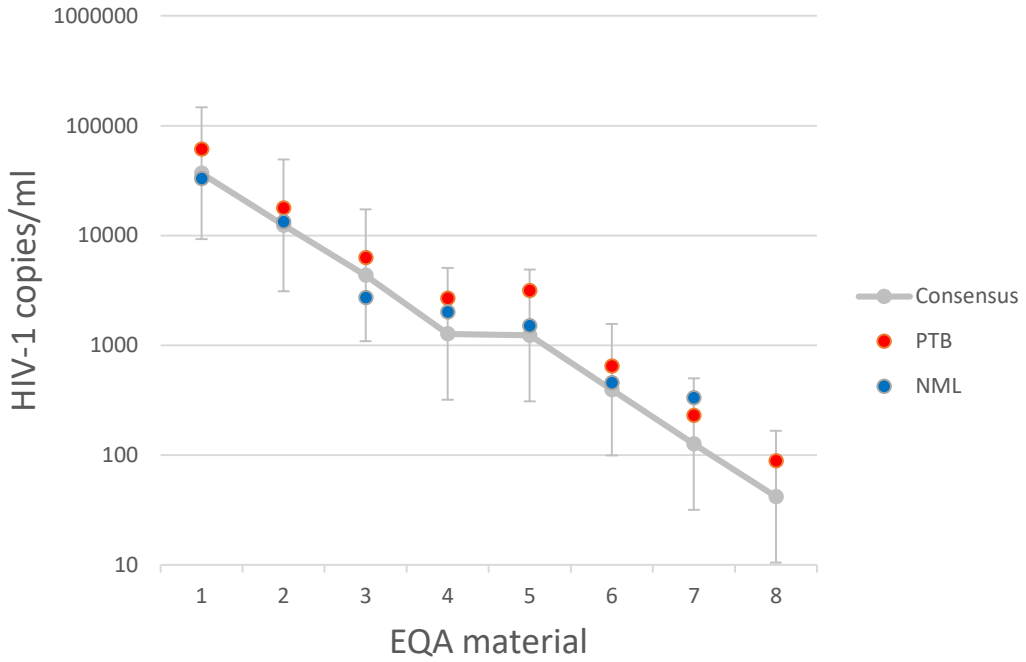


HIV-1 (RNA) Ringtrial 2019

Acceptance range ±log(0.6) for HIV Rili-BAEK



Conclusion

- dPCR shows good potential as a reference measurement procedure for HIV-1 RNA quantification

- Further work is required to explore impact of
  - Instrument
  - Assay (PCR & RT)
  - Sequence complexity
  - Continue and expand assessment of pre analytical steps
  - SI traceable orthogonal comparisons

P199 –HIV-1 RNA

- Stakeholder relevance
  - Viruses (e. g. HIV, Hep C) pose a global problem with severe health effects
  - HIV-1 viral load monitoring is directly relevant to clinical management of patient treatment
- Proposed study plan (Duration: Sept 2018 –March 2020)
  - Three materials:

1. Low concentration genome fragment
2. High concentration genome fragment for orthogonal SI traceable verification
3. Low concentration whole genome material

- Coordinator: LGC with NIBSC contribution
- 13 NMIs participating. Results presented April 2020

Future

Explore the role of dPCR in ensuring traceability in laboratory medicine

EN | Official Journal of the European Union

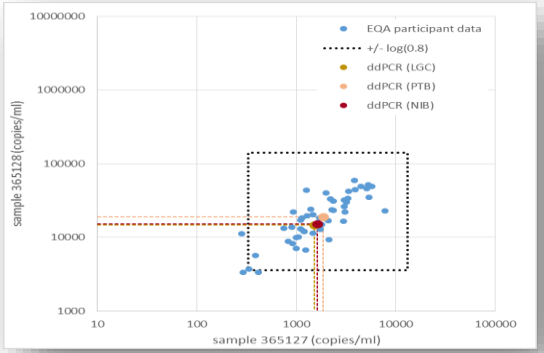
REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 5 April 2017

on *in vitro* diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU

(Text with EEA relevance)

Xpert MTB/RIF



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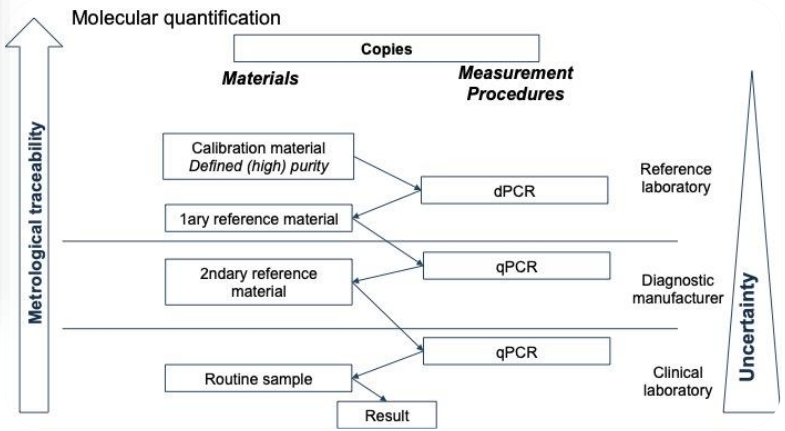

Acknowledgements

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EMPIR

EURAMET

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- Carole Foy

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- Hans-Peter Grunert
- Martin Kammel

NIBSC

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- Clare Morris
- Mei Mei Ho

NIB

- Mojca Milavec
- Jana Zel

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- Eleni Nastouli
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- Tim Mchugh

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