The development of measurement procedure for quantification of human cytomegalovirus

Mojca Milavec

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Accurate Results for Patient Care Workshop 2019

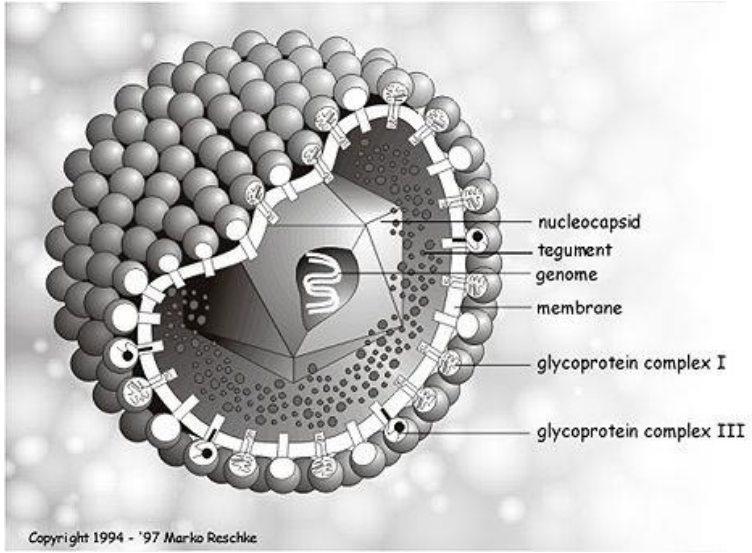
- Improved comparability and traceability of approaches used for the surveillance and monitoring of infectious diseases

- Highly accurate methodologies for the quantitative measurement of infectious agents

- Three model systems of infectious agents, tuberculosis as a bacterial model, human cytomegalovirus as a viral DNA model and influenza as a viral RNA model

hCMV as a one of the model organisms

- Human herpesvirus 5
- Linear dsDNA (~230 kbp)
- 150–300 nm in diameter
- Transmission through bodily fluids
- 50-90% seroprevalence
- In most cases mild, nonspecific symptoms in immunocompetent patients, while morbidity and mortality in **immunosuppressed** or **immunocompromised** patients (organ transplantation, HIV/AIDS, newborns).



hCMV as a one of the model organisms

- No vaccination
- Harmful antivirotics - prophylaxis not the best option
- Pre-emptive treatment - detection of viral replication (using PCR) as a trigger

CUT-OFF VALUE

- Lowest viral load (in IU/mL or cp/mL) that indicates replication of hCMV
- Defined within an individual laboratory for a group of specified patients (e.g. kidney transplant recipients)

MONITORING OF VIRAL KINETICS

- Consecutive measurements of viral load
- Clinically significant changes:
  - at least 5-fold (0.7 $\log_{10}$) for viral load values below 1000 IU/mL
  - at least 3-fold (0.5 $\log_{10}$) for viral load values above 1000 IU/mL

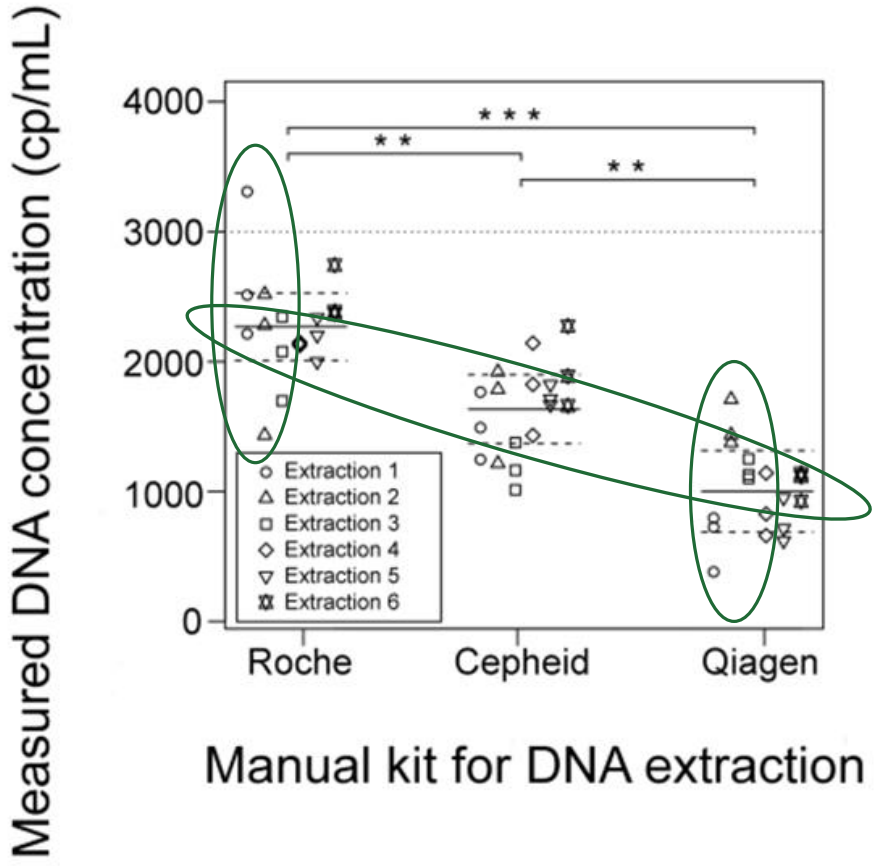
Up to 100-fold differences in results among laboratories
In INSTAND's EQA schemes results within ± 0.8 $\log_{10}$ of target value are valid

What are the reasons for the differences between laboratories?

- Methods for extraction of DNA from hCMV (from different matrices e.g. plasma, whole blood)
- Target sequences (e.g. UL83, UL54, UL123)
- In-house and commercial methods
- Chemicals used for amplification and detection of DNA
- Reference/control materials

Comparison of different extraction methods

1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) – low concentration (diluted in PBS)

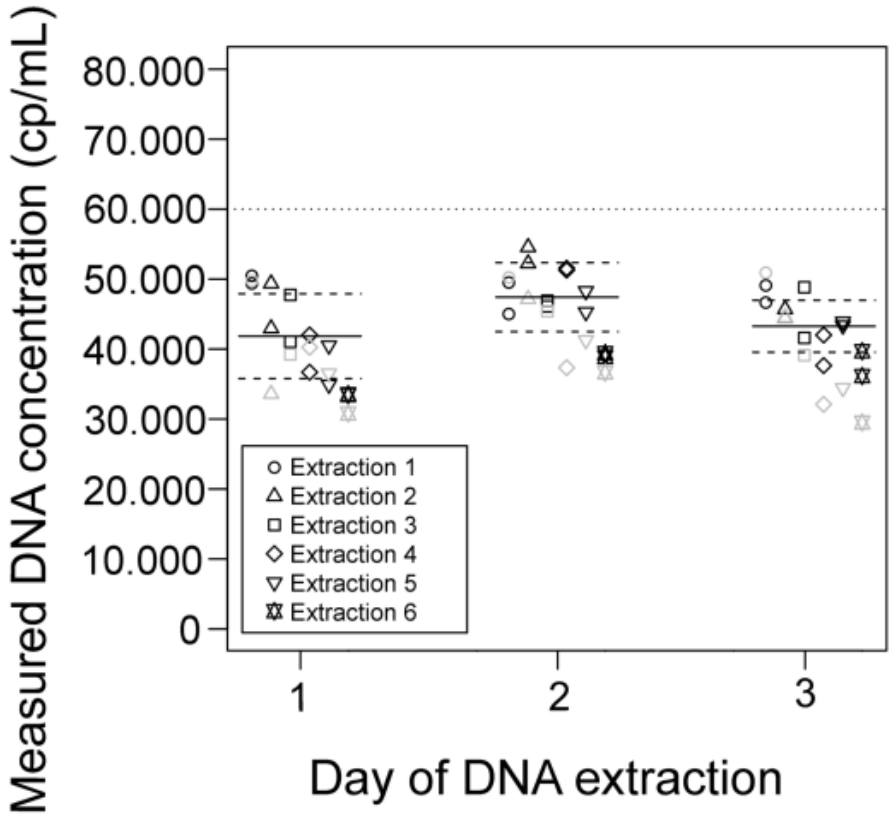


- More than 2-fold difference between extractions kits (average)
- More than 2-fold differences within the same experiment

Dotted line represents expected concentration

Comparison of different extraction methods

1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) – high concentration (diluted in PBS)



- With higher concentrations measurement results are closer (average)
- Less than 2-fold difference within one day

Dotted line represents expected concentration

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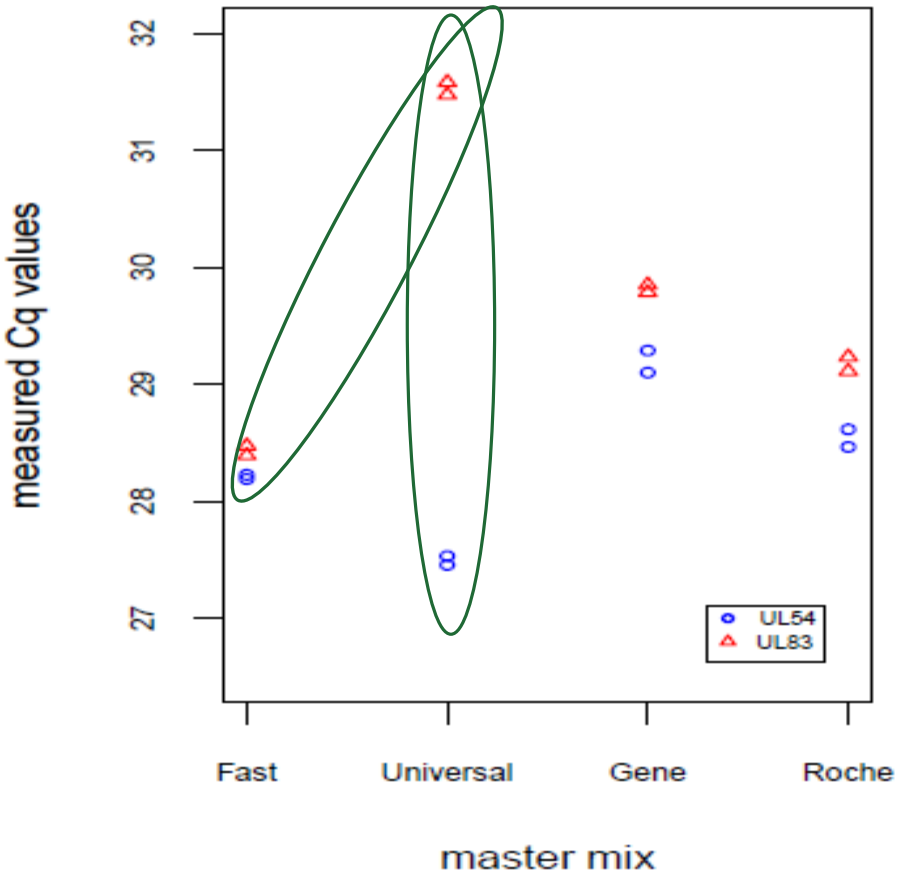
Influence of master mixes and assays on qPCR

Genomic DNA (gDNA) extracted from 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162)



- Up to **8-fold** difference between mastermixes (Universal and Fast)
- Up to **16-fold** difference between assays (UL54 and UL83)
- Fast and Universal were selected for further analysis

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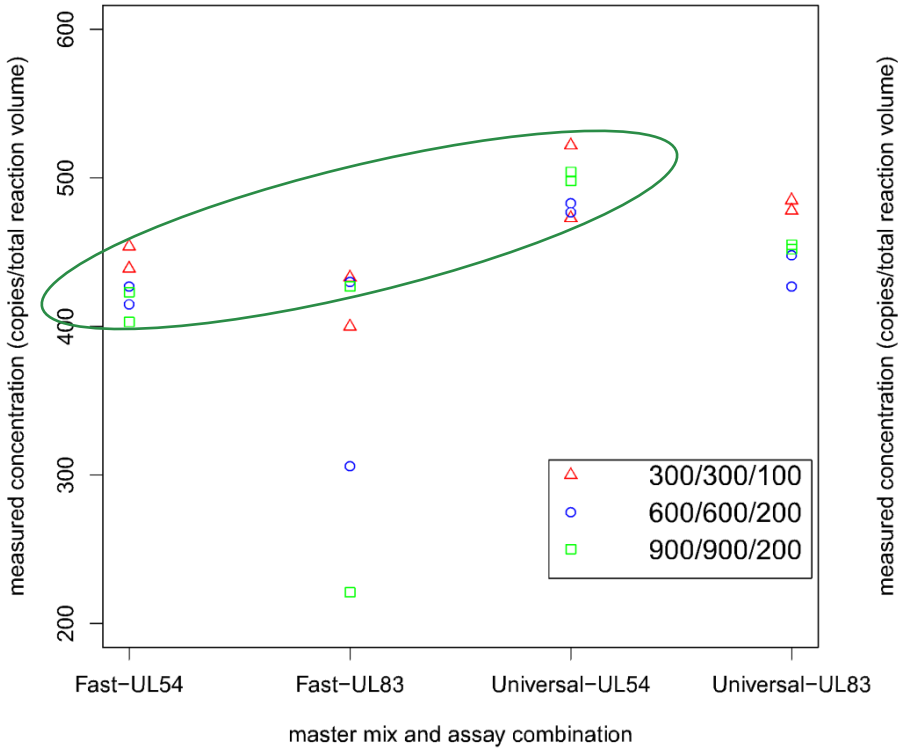
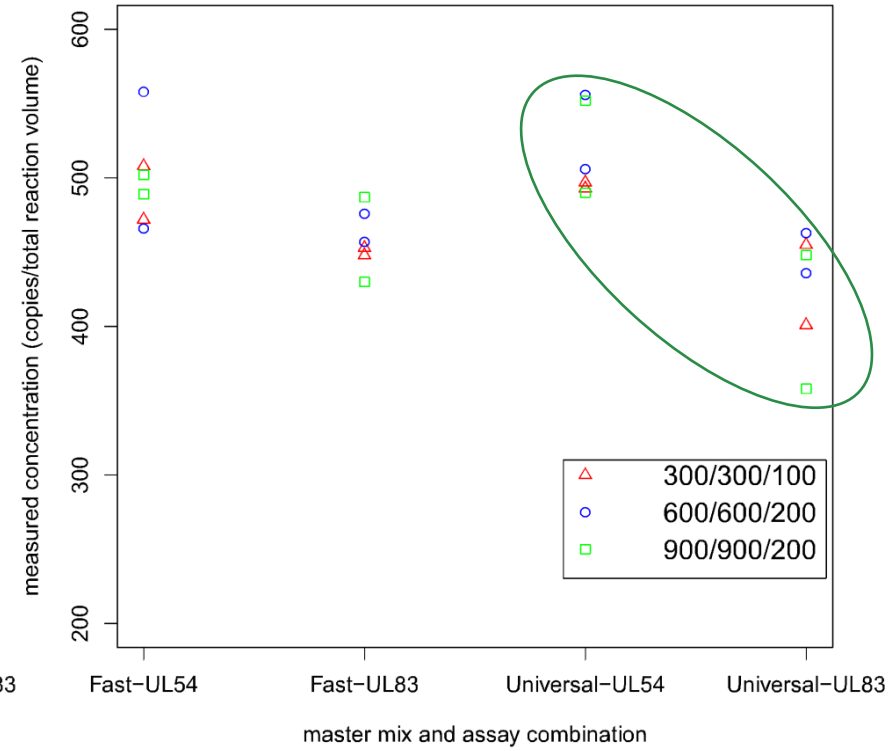
Comparison of master mixes and assays with chamber based dPCR

Plasmid DNA (NIST)*



gDNA (WHO)



Up to 19% difference in copy number estimation between master mixes (Fast and Universal)

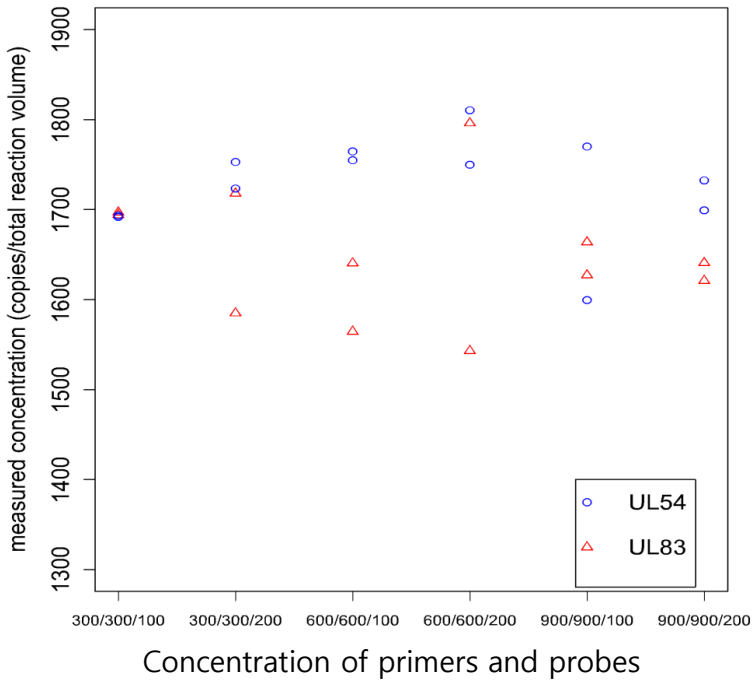
Up to 15% difference in copy number estimation between assays (UL54 and UL83)

Primers and probe concentration does not affect the result

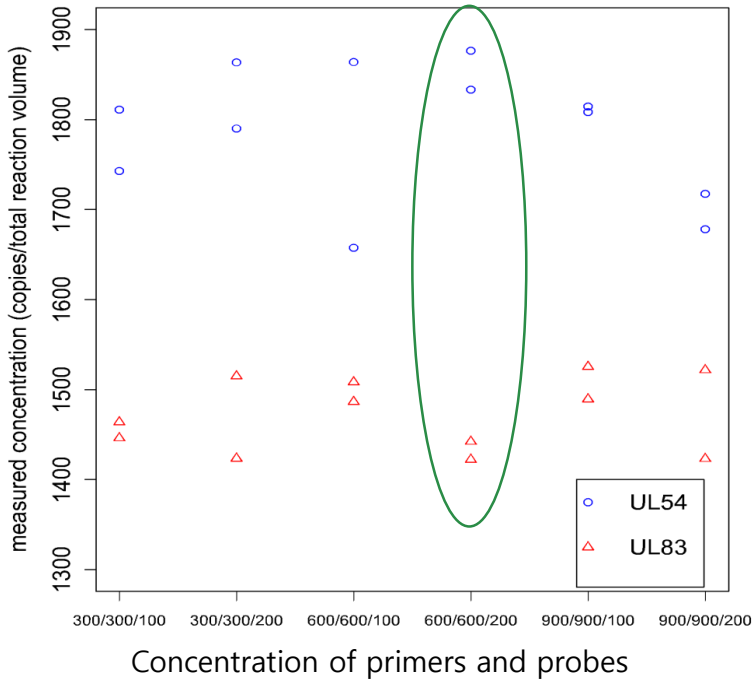
* Standard reference material 2366 Cytomegalovirus (National Institute of Standards and Technology)

Comparison of assays with droplet based dPCR

Plasmid DNA (NIST)



gDNA (WHO)



Up to 20% difference in copy number estimation between assays (UL54 and UL83)

Primers and probe concentration does not affect the result

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Assessment of methods

- **One extraction method** was selected (High Pure Viral Nucleic acid Kit, Roche).
- **One target sequence** was selected (UL54*).
- Relative and absolute quantification of target sequence was assessed on **all three platforms (one qPCR, two dPCR)** in terms of precision, limits of detection and quantification and robustness .

*J. Sassenscheidt, J. Rohayem, T. Illmer, D. Bandt, *J. Virol. Methods* **2006**, *138*, 40–48.

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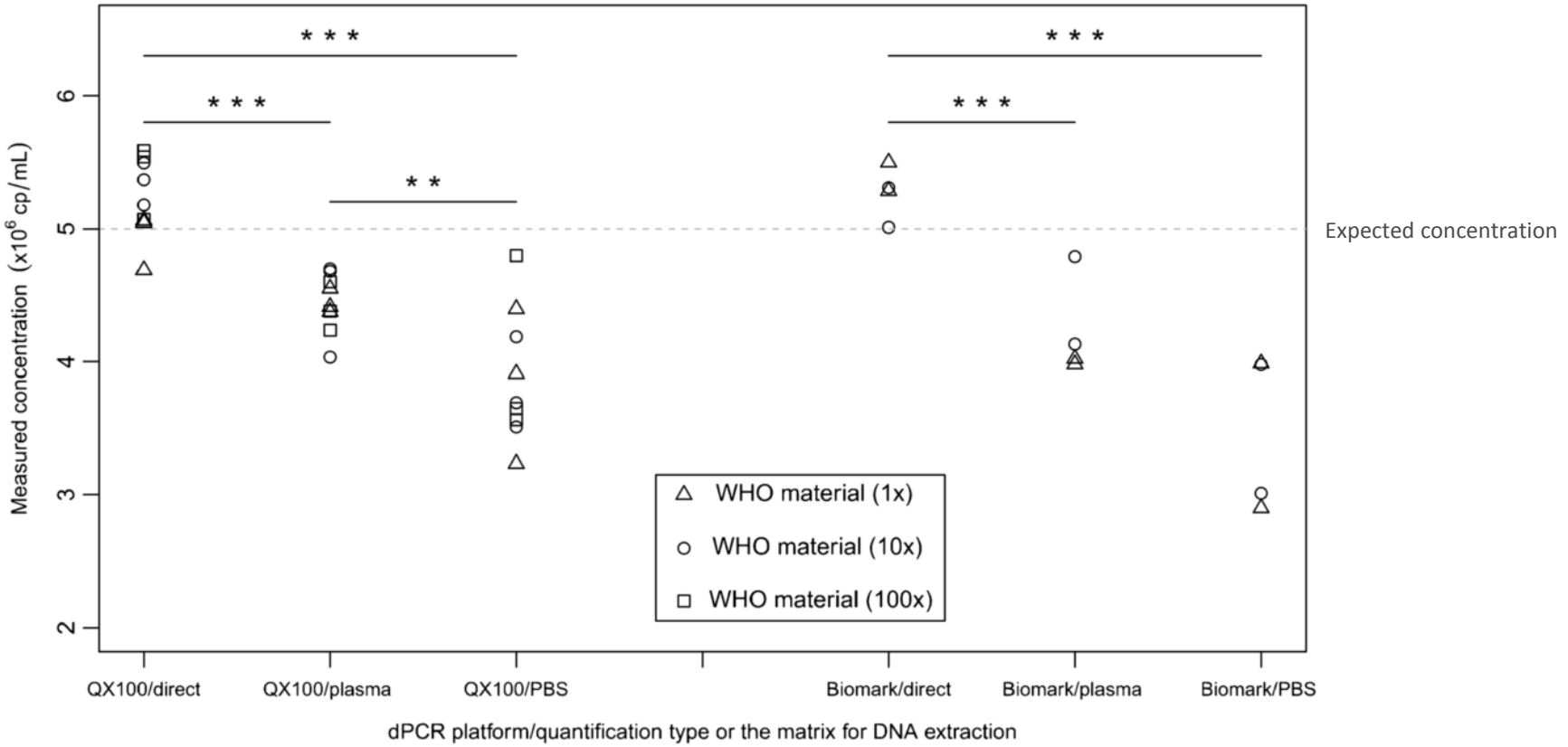
Direct quantification of hCMV with dPCR

1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162)



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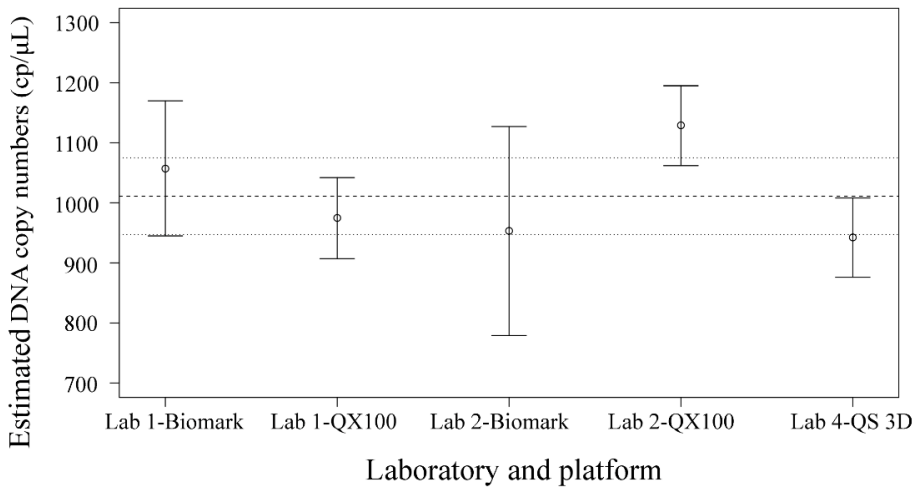
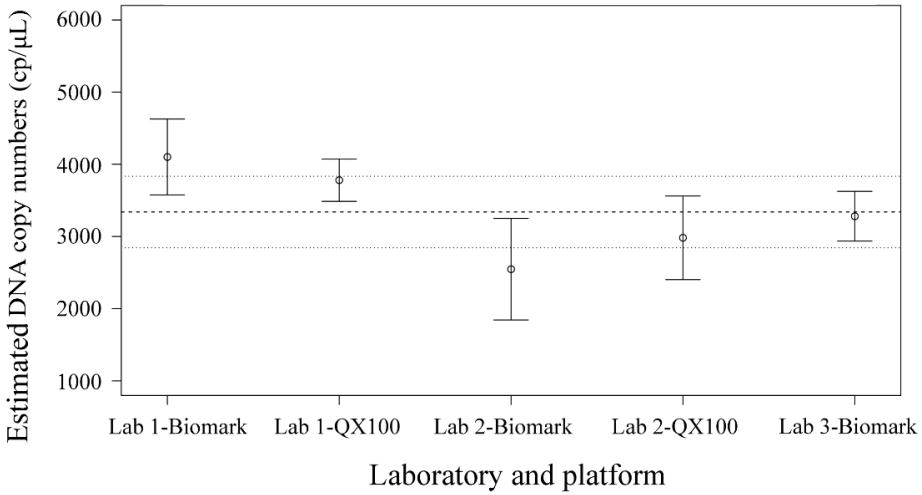
Interlaboratory comparison of dPCR platforms

1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162)

Quantification of gDNA



Extraction + quantification of gDNA



Less than 20% difference between 3 laboratories (NIB, JRC and TÜBITAK UME) and 3 different platforms (QX100 – droplet based dPCR, Biomark and QS3D – chamber based dPCR) with gDNA

Less than 2-fold difference between 3 laboratories (NIB, JRC and LGC) and 2 different platforms with whole virus (including extraction)

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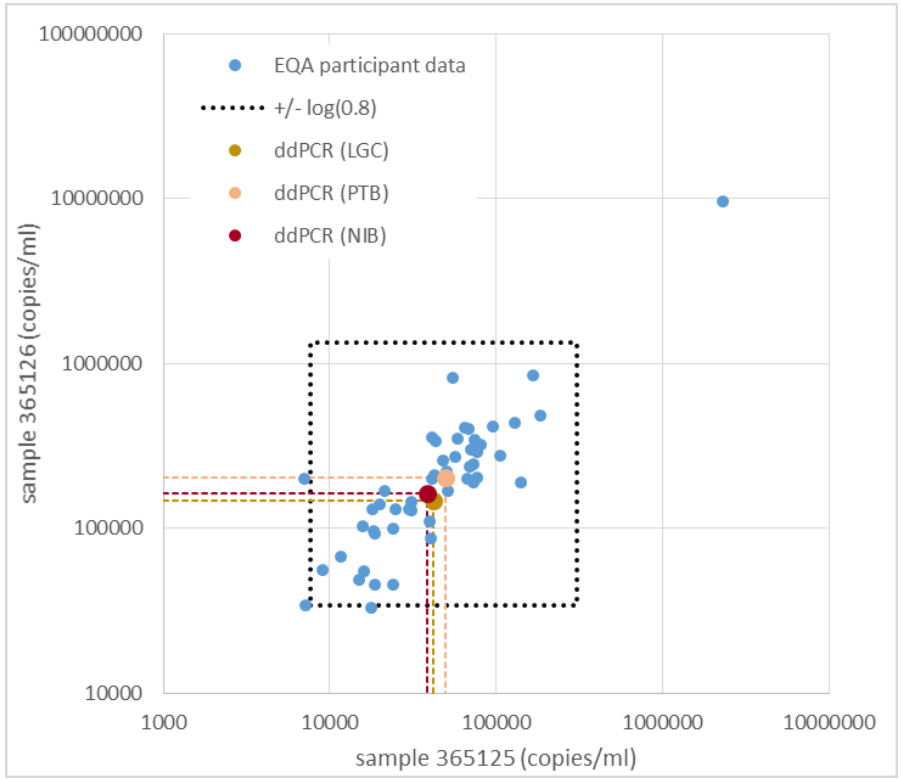
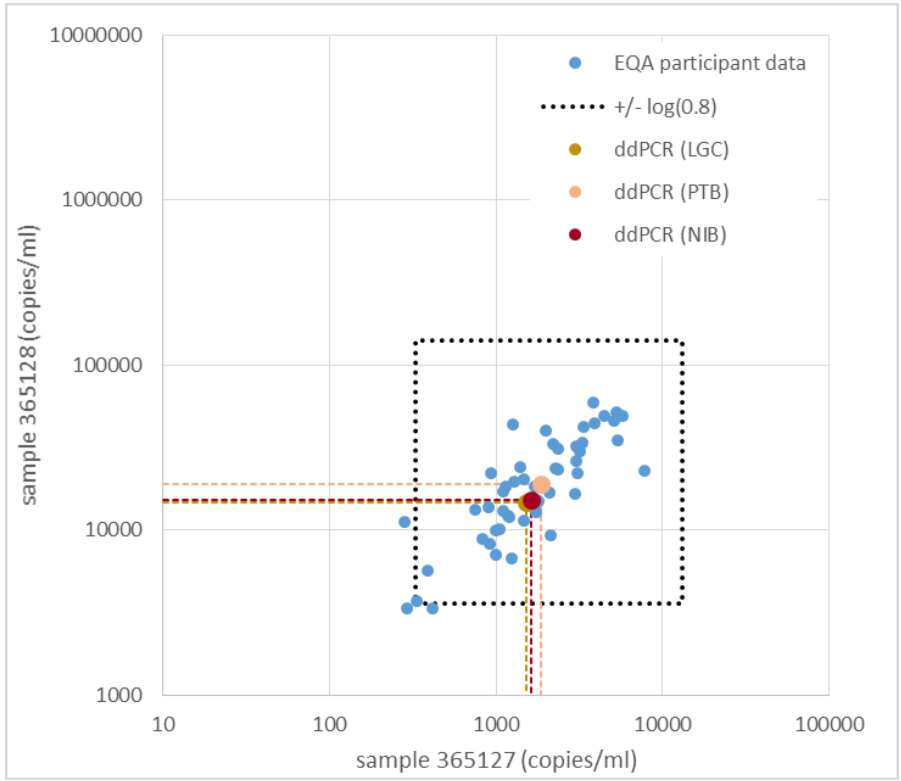
Participation in INSTAND EQA schemes – virus genome detection CMV standard program (365), March 2018





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Conclusions

- Using standard materials and metrological approaches for assessment of methods we have developed a repeatable and reproducible method for quantification of hCMV

- Digital PCR has the potential to be used as a method for diagnostics, as a reference measurement procedure (traceable to SI) as well as for value assignment of control or reference materials and materials for EQAs in different areas (infectious agents, biomarkers for cancer, genetic disorders, gene therapy…)

- Best support for clinical diagnostics can be achieved through the development of **reference measurement systems** including reference materials and reference methods

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Gene therapy

Treatment of spinal muscular atrophy (SMA) in paediatric patients

Adeno-associated virus (AAV) 9 based gene therapy designed to deliver a copy of the SMN1 gene to encode for human SMN protein

NIB applied dPCR and supported development of the downstream process (AAV purification)

NIB qualified the dPCR protocol and transferred the technology to Avexis premises

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