Medicines & Healthcare products Regulatory Agency

NML
UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

COMET

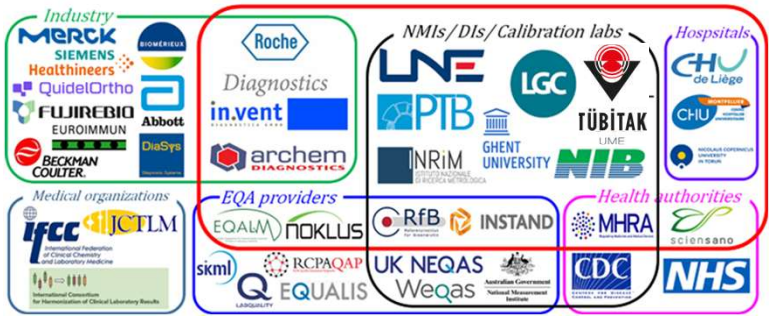
Redesigning Reference Materials for Human Cytomegalovirus

EQALM Annual Symposium, Leuven 14 Oct 2026

NIB
NACIONALNI INŠTITUT ZA BIOLOGIJO
NATIONAL INSTITUTE OF BIOLOGY

INRiM
ISTITUTO NAZIONALE
DI RICERCA METROLOGICA

TÜBİTAK
UME



NIBSC at MHRA, UK

We are the UK's Official Medicines Control Laboratory

- Independent batch release of biological medicines (e.g. vaccines, blood products)

We are a World Health Organization International Laboratory for Biological Standardisation

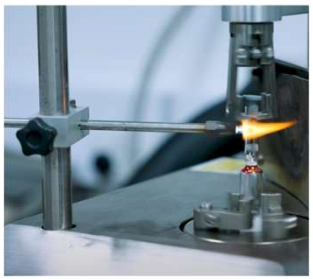
- Prepare, Curate and Distribute >90% of WHO International Biological Reference Standards (IBRS)
- Supply NIBSC Reference Reagents for monitoring performance of assays
- Research reagent repository

We perform applied research that assures the quality of biological medicines

- Quality = Both Potency and Safety







COMET

1st WHO International Standard (IS) for HCMV for NAT

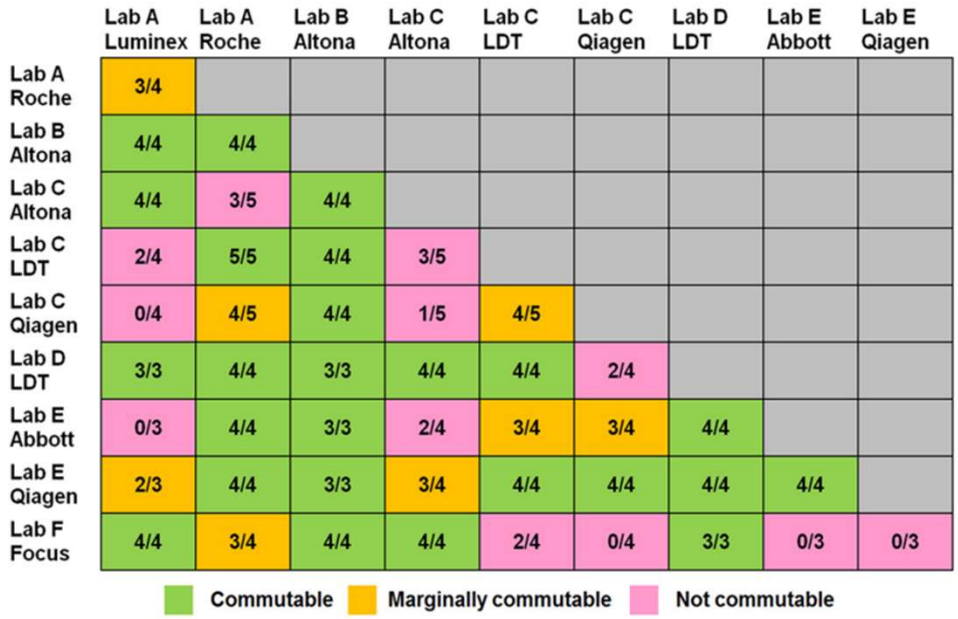
- Serial measurement of CMV DNA in blood by QNAT guides initiation and efficacy of antiviral treatment.
- Lack of common reference material contributed to variability in the performance CMV QNAT, prevented comparison of results and establishment of consensus thresholds for initiation of antiviral therapy.
- Proposal for 1st WHO IS for HCMV for NAT; Merlin strain derived from cell culture, formulate in 'universal' buffer, concentration for standard curve. Endorsed by WHO ECBS Oct 2008.
- Candidate 09/162; clarified culture supernatant Merlin virus, ultracentrifuged, formulated in 'universal buffer' (10mM Tris buffer 0.5% HSA), lyophilised.
- Candidate evaluated in range of HCMV QNAT assays in multicentre study.
- Tested alongside virus preparations (*in vitro* culture of Merlin & AD169 strains formulated in universal buffer), and BAC-cloned Merlin DNA in nuclease-free water. Harmonisation of virus preparations demonstrated.
- 09/162 established by ECBS (Oct 2010) as 1st WHO IS for HCMV for NAT, assigned unitage $5x10^6$ IU/mL. Intended to calibrate secondary references for HCMV NAT in IU/mL.

COMET

Investigation of commutability of 09/162 post-establishment

- Hayden *et al*, JCM 2015 & Preiksaitis *et al*, CID 2016; investigated commutability of WHO IS with CMV patient plasma:

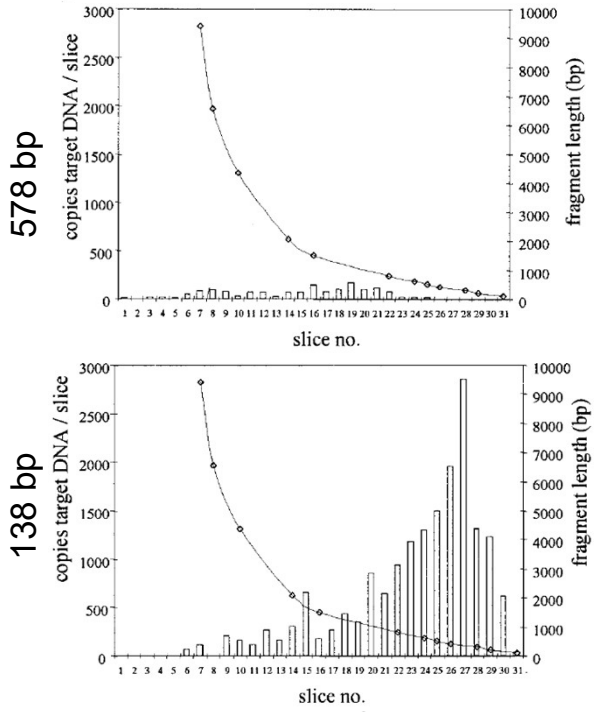
- 40 genotyped CMV +ve plasma samples (pooled from individual transplant patients), 10 –ve plasma samples tested in 6 labs using 10 qPCR assays calibrated to 09/162.
- Demonstrated non-commutability with some assay systems.
- Non-commutability noted with assays with amplicon size > ~100 bp.



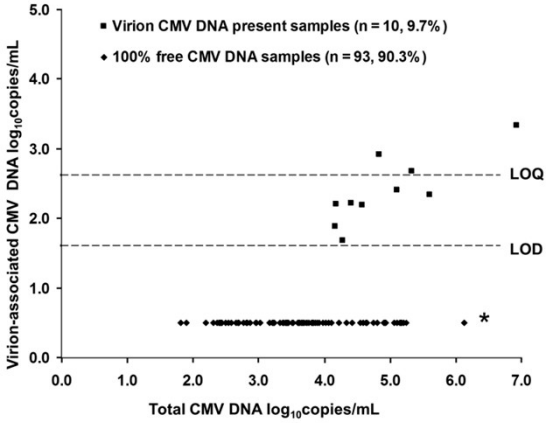
Hayden *et al*, JCM 2015

What are we measuring when monitoring CMV in blood?

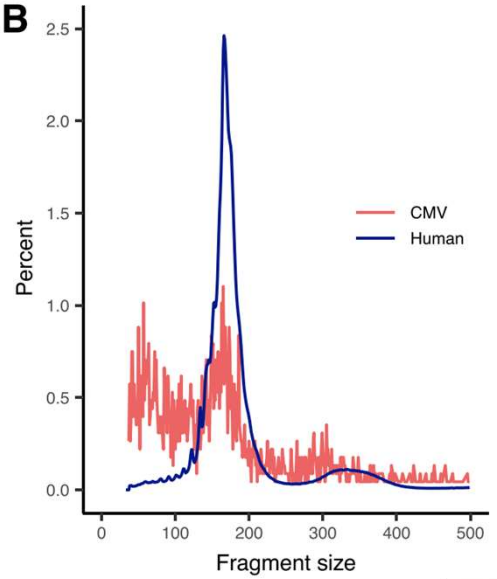
- HCMV DNA in plasma/serum specimens of transplant recipients is highly fragmented (Boom et al, 2002).
- CMV DNA in SOTR plasma exists almost exclusively free DNA, highly fragmented (Tong *et al*, JID 2017).
- Median fragment length of 149 bp [IQR 86–194 bp] in SOTR by sequencing cfDNA (Peddu *et al*, Nature 2020).



Boom et al, JCM 2002



Tong et al, JID 2017



Peddu et al,
Nature 2020

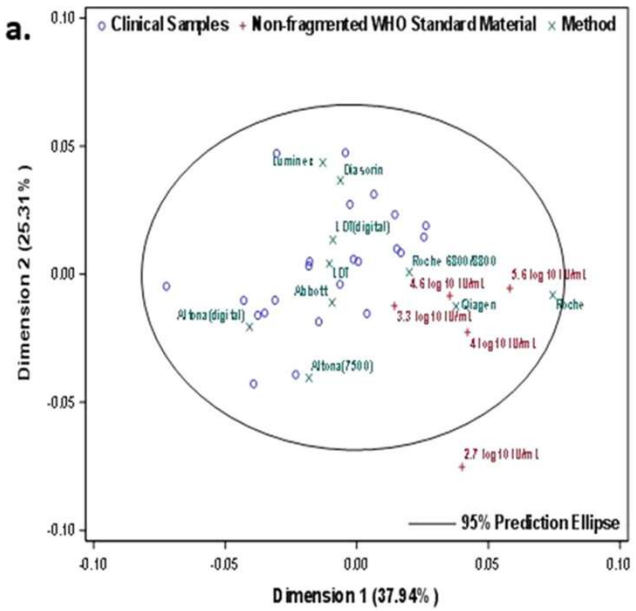
COMET

Does fragmenting the WHO IS improve commutability?

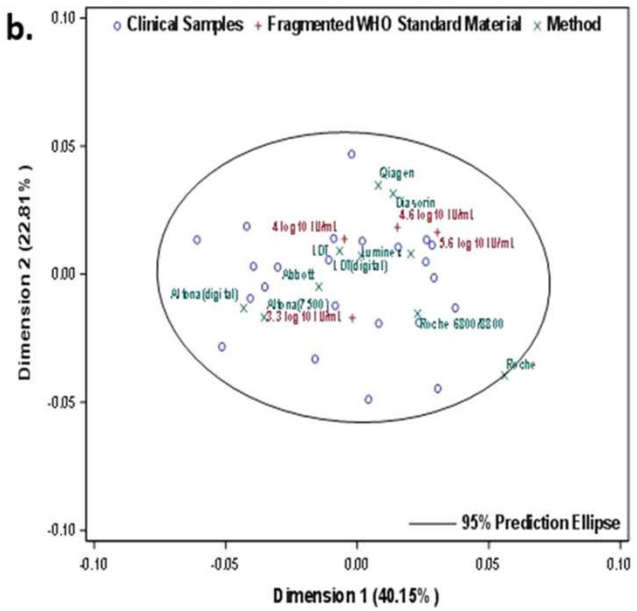
- Fragmentation of 09/162 and secondary calibrator improved commutability with CMV patient plasma (Hayden *et al*, JCM 2020).

- Dilutions of 09/162 sheared by ultrasonication
- Average fragment size of 166 bp.
- Commutability assessed with 42 patient samples and 10 commercial/LDT QPCR.

Non-fragmented



Fragmented



Use of the 1st WHO IS for HCMV for NAT

Common specifications for class D CE IVD medical devices

- 09/162 for validation of NAT sensitivity and NAT LOD.

4th International Consensus Guidelines on the Management of CMV in SOT (Kotton et al, Transplantation 2025)

- Calibration of CMV-QNAT assays with the WHO International Standard has improved agreement across tests.

> • We recommend that individual patients are consistently monitored with the same assay. CMV DNAemia values should not be directly compared across centers and/or laboratories unless identical testing reagents and procedures have been assured or equivalence has been documented (strong, high).

CoMET Task 1.3: Purity & identity assessment of 1º reference and QA materials for HCMV

Activity	Description
A1.3.1 ✓	Identify & predict target uncertainties for 1º/2º reference materials & RMPs.
A1.3.2 ✓	Survey to clinical labs to define the measurand and explore use of NAT in guiding management of transplant patients.
A1.3.3 ✓	Identify existing techniques to analyse identity of HCMV DNA (in patient samples and primary reference materials).
A1.3.4 ✓	Explore contribution of inhibitors to HCMV measurement.
A1.3.5 ~	Develop new techniques to analyse identity of HCMV DNA (in patient samples and primary reference materials).
A1.3.6 ~	Explore how sequencing can be used to define identity of HCMV DNA (in patient samples and primary reference materials).
A1.3.7	Explore how to implement the purity metrics developed in A1.3.4 /5 /6 into measurement uncertainty calculations.
A1.3.8	Prepare report.

CoMET Task 3.6: HCMV commutability study

Activity	Description
A3.6.1 ✓	Use EQA data to identify the most frequently used assays and associated reported precision.
A3.6.2 ✓	Define acceptance criteria for commutability assessment of primary and secondary materials for HCMV.
A3.6.3 ✓	List requirement for clinical specimens to be collected for commutability assessment
A3.6.4 ✓	Identify sources for clinical specimens
A3.6.5 ~	Source and/or produce candidate primary and secondary materials whose commutability will be evaluated.
A3.6.6 ~	Plan commutability study; – IVD-MDs, RMPs – Study samples; clinical specimens, (reference) materials – Acceptance criteria, statistical approaches for data analysis
A3.6.7 ~	Source the clinical specimens
A3.6.8	Perform commutability study
A3.6.9	Analyse results
A3.6.10	Prepare report

Techniques for analysing HCMV DNA fragmentation

Electrophoresis



Sequencing (Illumina & ONT)





Linkage dPCR



HCMV linkage dPCR

- Based on similar published in-house HCMV assay - Devonshire *et al*. 2025 – (builds off Regan *et al*. ‘Mile-marker’ assays).

- Primers were designed for targets at intervals from the HCMV UL54 (DNA polymerase) gene, using Merlin strain as primary reference genome:

Extraction of fragmented HCMV DNA from contrived samples

- Fragment CMV (Merlin) DNA by ultrasonication to 150 & 350bp target sizes. Mix 1:1 dilute in human plasma/whole blood to ~ 4-5 log IU/mL.
- Purify with different extraction methods
- Analyse the eluates by linkage dPCR, sequencing

HCMV DNA fragmentation profile in clinical samples

- Clinical samples extracted using QIAamp DNA Blood Mini kit, eluate divided between LGC-NML, NIB and MHRA for linkage dPCR and sequencing.

HCMV DNA fragmentation profile in plasma vs. pellet (buffy coat) by linkage dPCR



HCMV DNA fragmentation profile in plasma by paired-end sequencing (Illumina)



HCMV DNA fragmentation profile in plasma by long read sequencing (ONT)



Development of new candidate reference materials



Commutability study; samples

Dilutions of 4 candidate RM prepared in EDTA plasma:

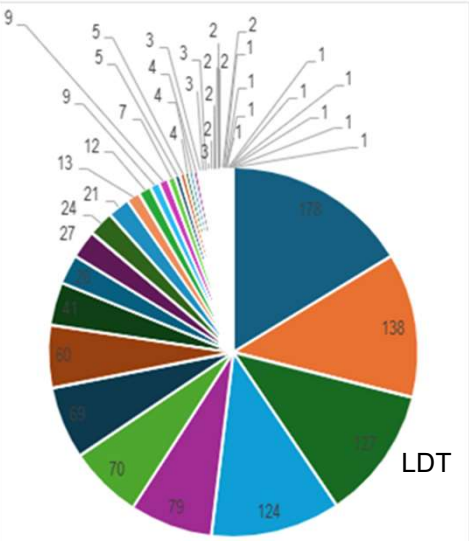
- Fragmented HCMV DNA (strain Merlin) e.g. 100-500 bp
- Fragmented HCMV DNA (strain Merlin) e.g. 100-1000 bp
- 1st WHO IS 09/162 (strain Merlin)
- Fragmented HCMV DNA (prototype secondary reference material from 3rd party)
- 5x 10-fold dilutions (~100-1E+06 IU/mL), quantified by HCMV dPCR.

Clinical samples:

- Up to 20 HCMV DNA positive EDTA plasma specimens from different transplant recipients collected as part of routine HCMV DNA monitoring.
- Pools of 2-3 consecutive HCMV DNA-positive plasma samples taken from the same patient, on/off same antiviral therapy, 2.5-3 mL required.
- HCMV DNA concentration between ~200 and ~2E+05 IU/mL

Commutability study; assays

Test	Frequency (%)	Regulatory approval	Sample type	Format (ext & amp)	CMV Quant Controls	Reporting unit	Gene Target	Amplicon length
ELITech CMV ELITe MGB kit	16.3	CE	Plasma, whole blood, CSF, Urine, buccal swab, amniotic fluid, BAL/BA	Integrated/automated	Plasmid DNA, PCR only	Cp/ml (convert to IU/ml)	MIEA UL123	112
Roche cobas® CMV for use on the cobas® 5800/6800/8800	12.6	FDA, CE	Plasma	Integrated/automated	Packaged synthetic DNA in plasma, extracted	IU/ml	gB (UL55)	135/105
bioMerieux CMV R-GENE	11.3	CE	Plasma, whole blood, Serum, CSF, BAL, Urine, Amniotic Fluid, Saliva Swabs	Not integrated (automated ext, multiple amp platforms)	Plasmid DNA, PCR only	Cp/ml (convert to IU/ml)	UL83	283
Altona RealStar® CMV PCR Kit 1.0	7.2	CE	Plasma, whole blood	Not integrated (manual ext, multiple amp platforms)	CMV DNA, PCR only	IU/ml	UL122	74
Abbott ALINITY m CMV ASSAY	6.4	FDA, CE	Plasma	Integrated/automated	Inactivated CMV in plasma/Linearised CMV plasmid in buffer, extracted	IU/ml	UL34, 80.5	98, 105
QIAGEN artus CMV RG PCR,QS-RGQ, LC PCR Kit	6.3	FDA (RGQ), CE	Plasma, (whole blood – RGQ)	Not integrated (automated/manual ext,	DNA fragments, PCR only	IU/ml or Cp/ml conversion	MIEA	105



Total; 1092 labs, 40 commercial CMV QNAT assays, LDT = 11.6%

COMET

Commutability study; protocol, participating labs and analysis

- Protocol based on Hayden et al, JCM 2020 & Boutolleau et al, JCM, 2025.
- Designed in consultation with MHRA statistician and CoMET consortium.
- Participants selected based on experience and performance with assay (in routine use).
- Samples blinded, shipped as frozen liquid.
- Requested to test study samples directly (without further manipulation).
- Report CMV concentration (IU/mL) – Ct not essential.
- Data analysis to apply calibration effectiveness, linear regression, and ?multivariate analysis.

Impact of reformulating the WHO International Standard

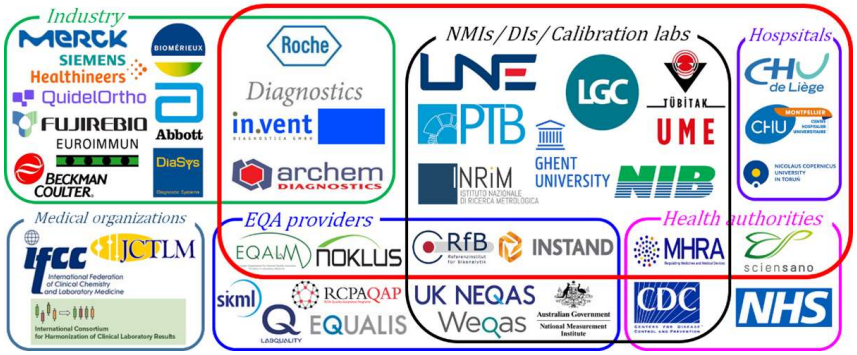
- Impact on continuity of the IU?
- Impact for existing QNAT methods calibrated to 1st IS?
- Impact on CMV monitoring in whole blood?



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