CMV Diagnostic Strategies: Current and Future

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Faculty/Presenter Disclosure

- **Relationships with commercial interests:**
  - **Grants/Research Support:** Qvella, bioMerieux
  - **Speakers Bureau/Honoraria:** Merck, Sunovion
  - **Advisory Board:** Merck, Pfizer, Qvella, Microbix, Roche

Mitigating Potential Bias

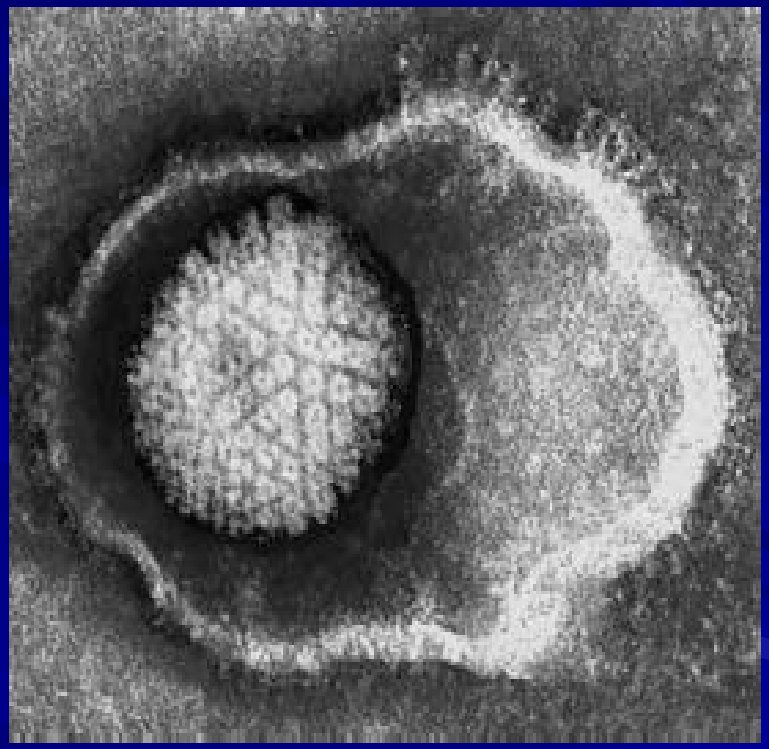
- **No** financial or other support has been received from any commercial entity for the preparation or presentation of this talk

Learning Objectives

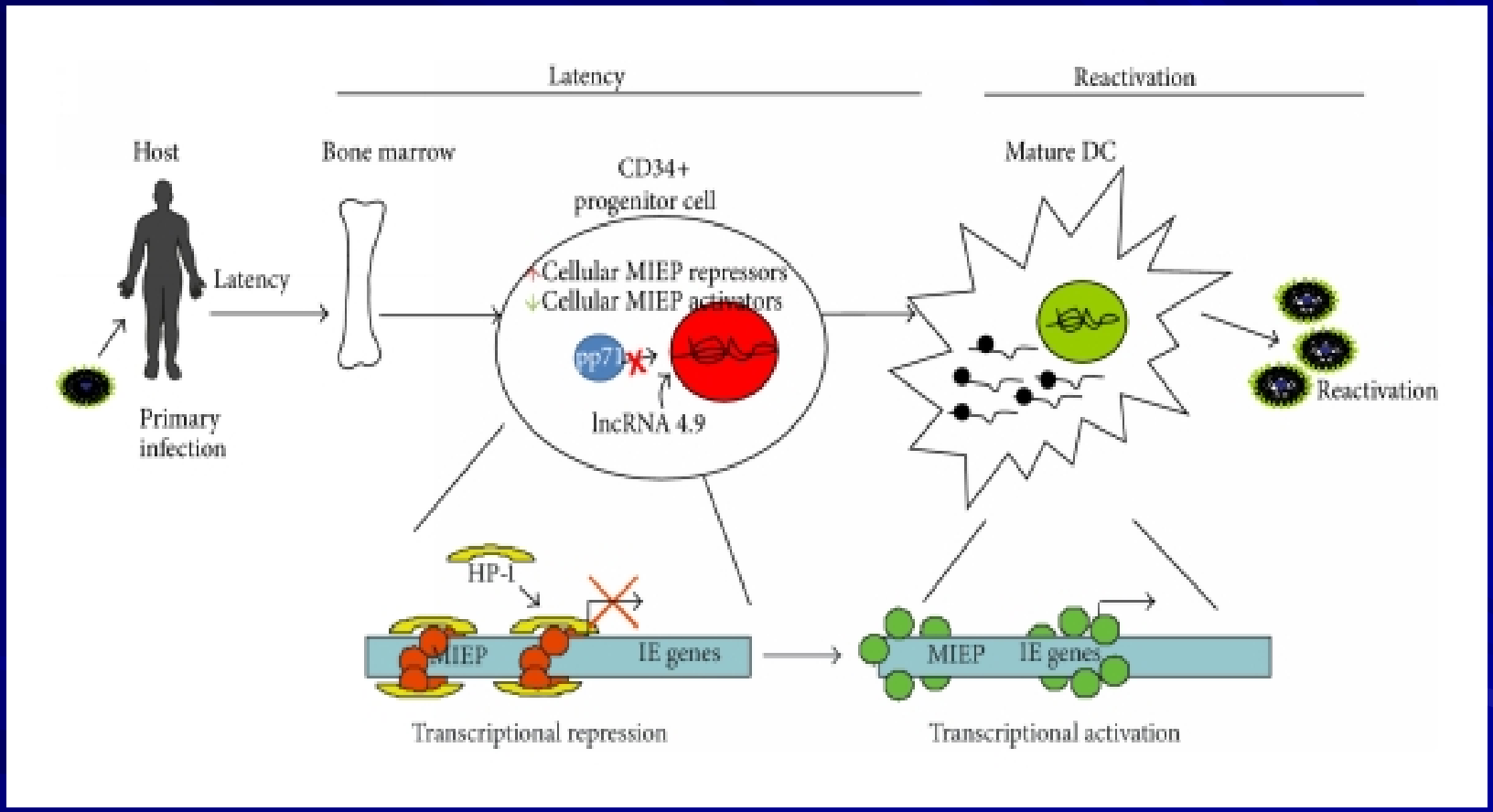
- At the end of this presentation, participants will be able to:
  - Cite current tests available for the diagnosis and management of CMV disease in SOT and HSCT patients including resistance testing
  - Interpret results of resistance testing
  - Recognize future testing options

CYTOMEGALOVIRUS

- Betaherpesvirus:
  - ds DNA
  - Icoshedral capsid
  - Lipid envelope
- Establishes latency:
  - “Once infected always infected”
- Prevalence:
  - Species specific
  - 40-70% adult pop’n

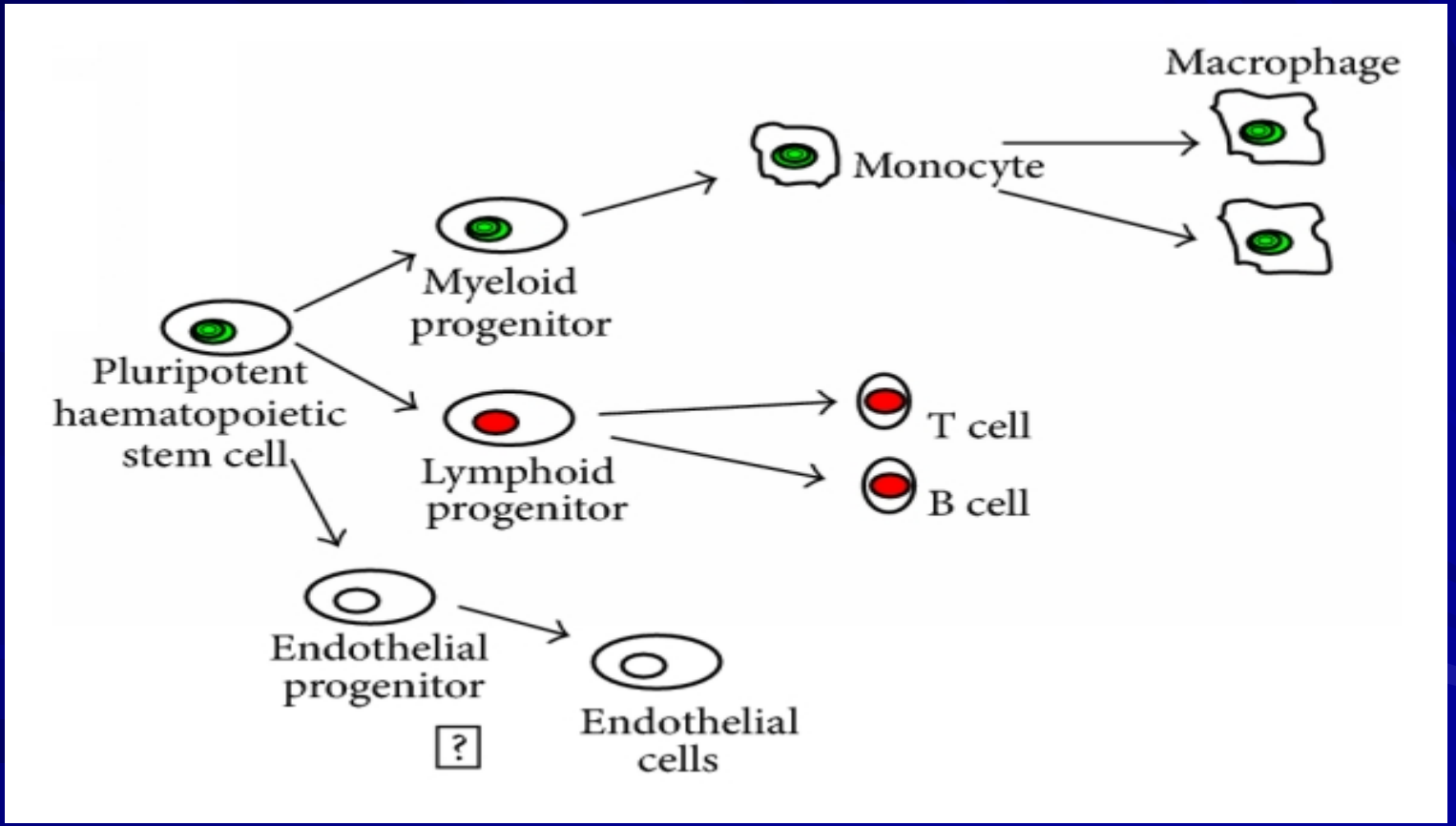


Cytomegalovirus Latency



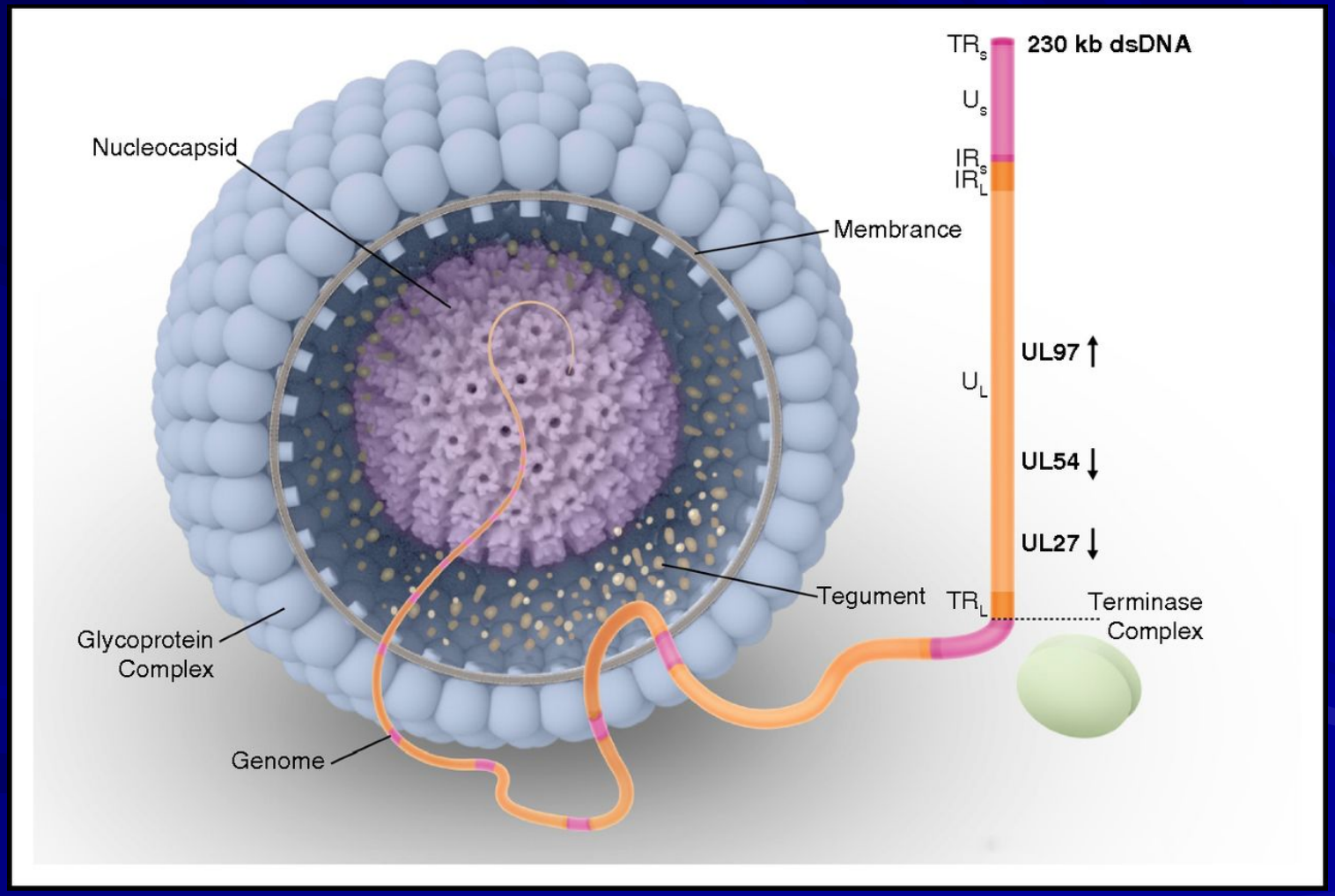
Poole E, et al. New Journal of Science. Volume 2014 (2014)

Cytomegalovirus Latency



Poole E, et al. New Journal of Science. Volume 2014 (2014)

Human CMV Virion Structure



Firas El Chaer et al. Blood 2016;128:2624-2636

Available Tests for Detection, Diagnosis, & Monitoring of CMV

- CMV serology (IgG, IgM, and avidity testing)
- Direct detection – EM, In-situ hybridization, Antigenemia
- Culture: Tube and Shell vial
- Molecular – Quantitative and Qualitative detection of CMV DNA
- Antiviral Resistance testing - sequencing the UL97 and/or UL54 (polymerase) genes directly from a plasma specimen (genotypic); plaque reduction assays (phenotypic)

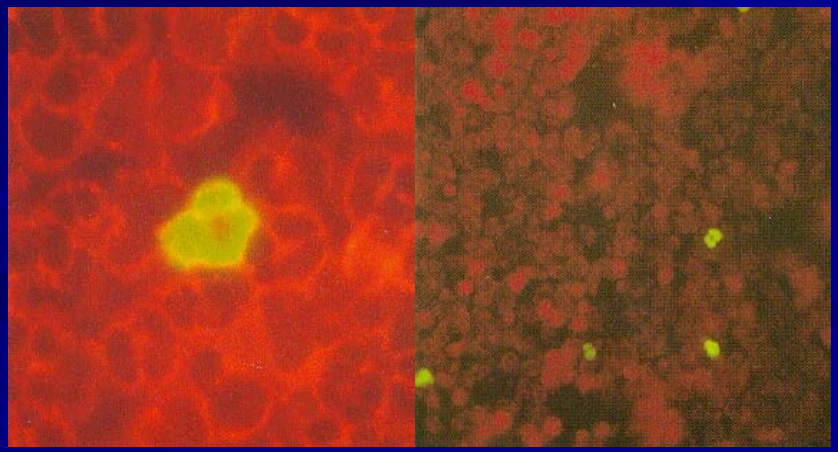
CMV Diagnosis and Monitoring in Transplantation

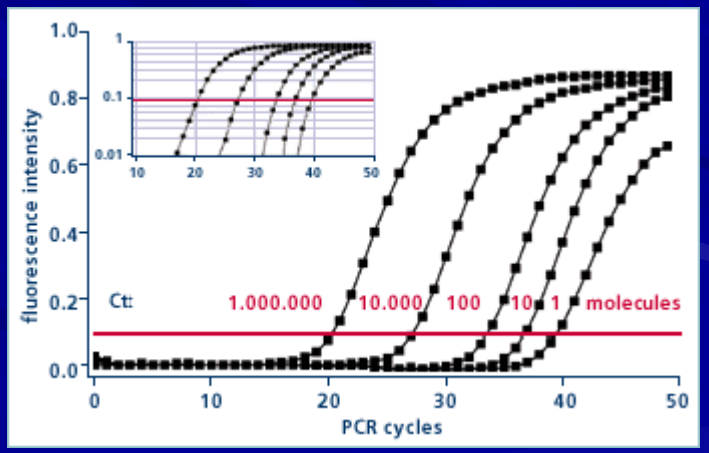
Antigenemia

(Mid/Late 1990s)

Molecular

(Early/Mid 2000s)

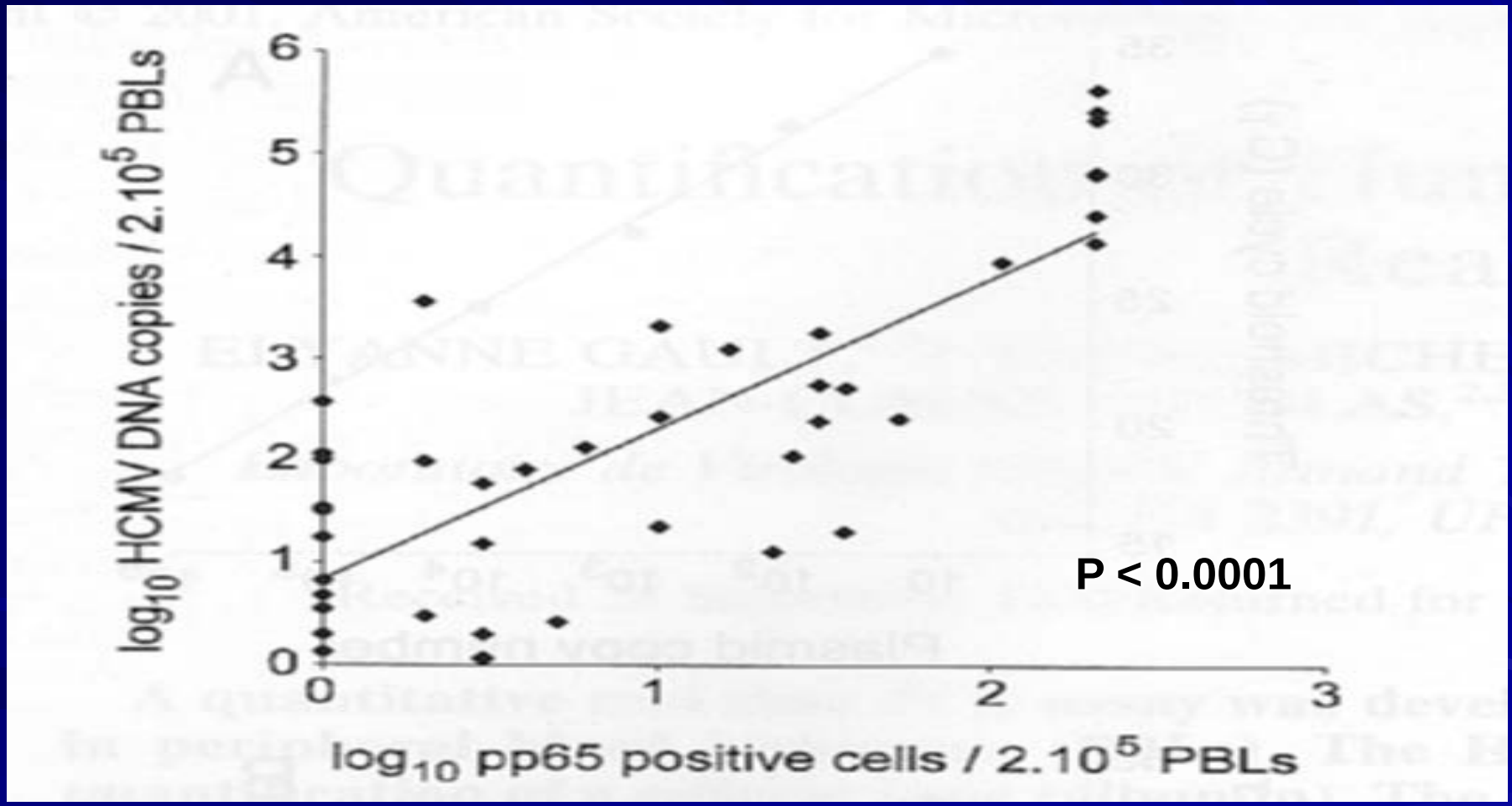




CMV PCR vs Antigenemia

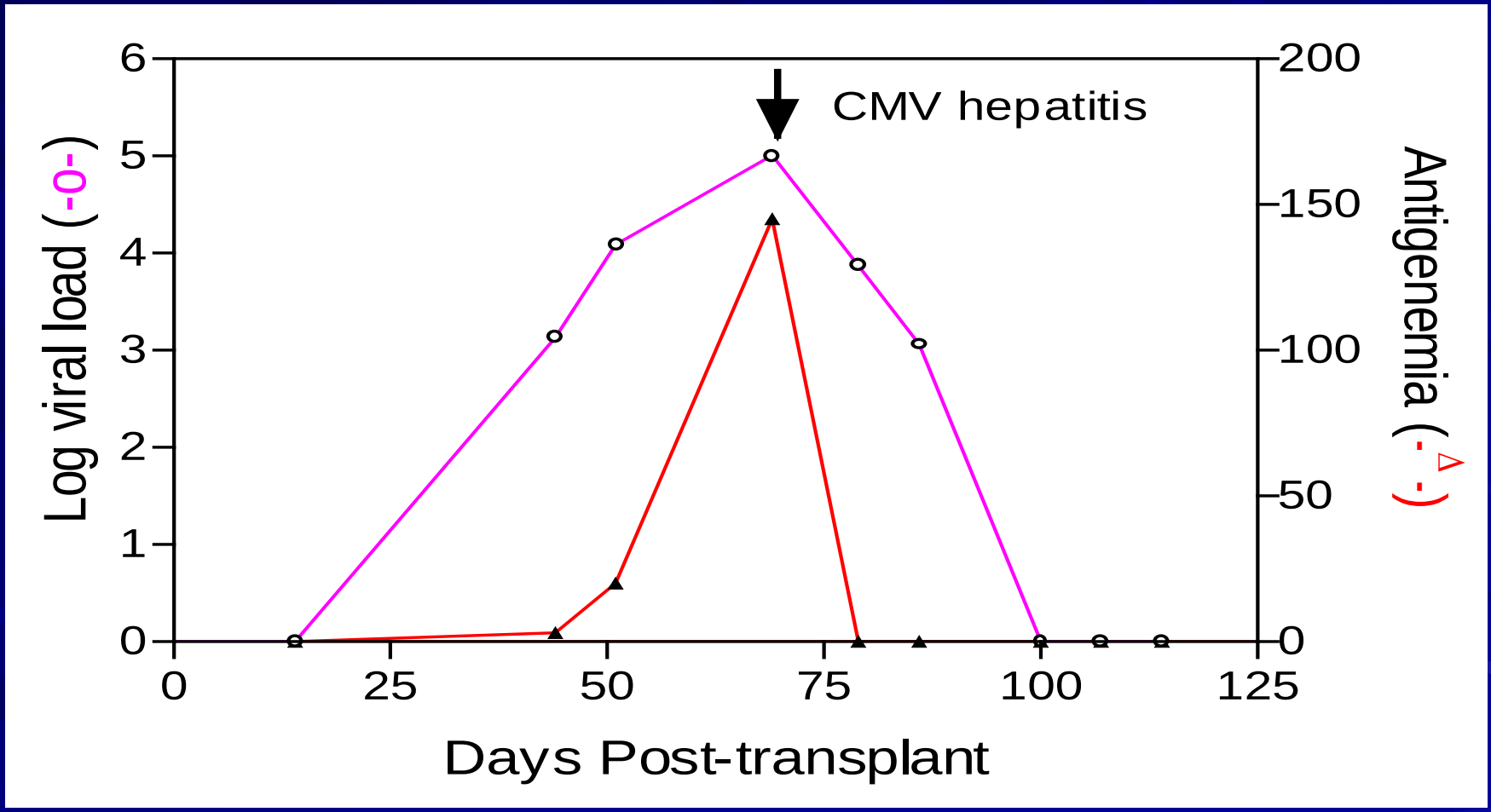
CMV antigenemia		CMV PCR	
CMV antigenemia	# specimens	# samples Negative	# samples Positive
Negative	44	30 (68%)	14 (32%)
0-2 pos cells/10^5	37	2 (5%)	35 (95%)
3-5 pos cells/10^5	14	0	14
>5 pos cells/10^5	16	0	16
Total	111	32	79

CMV Quantification by PCR & Antigenemia



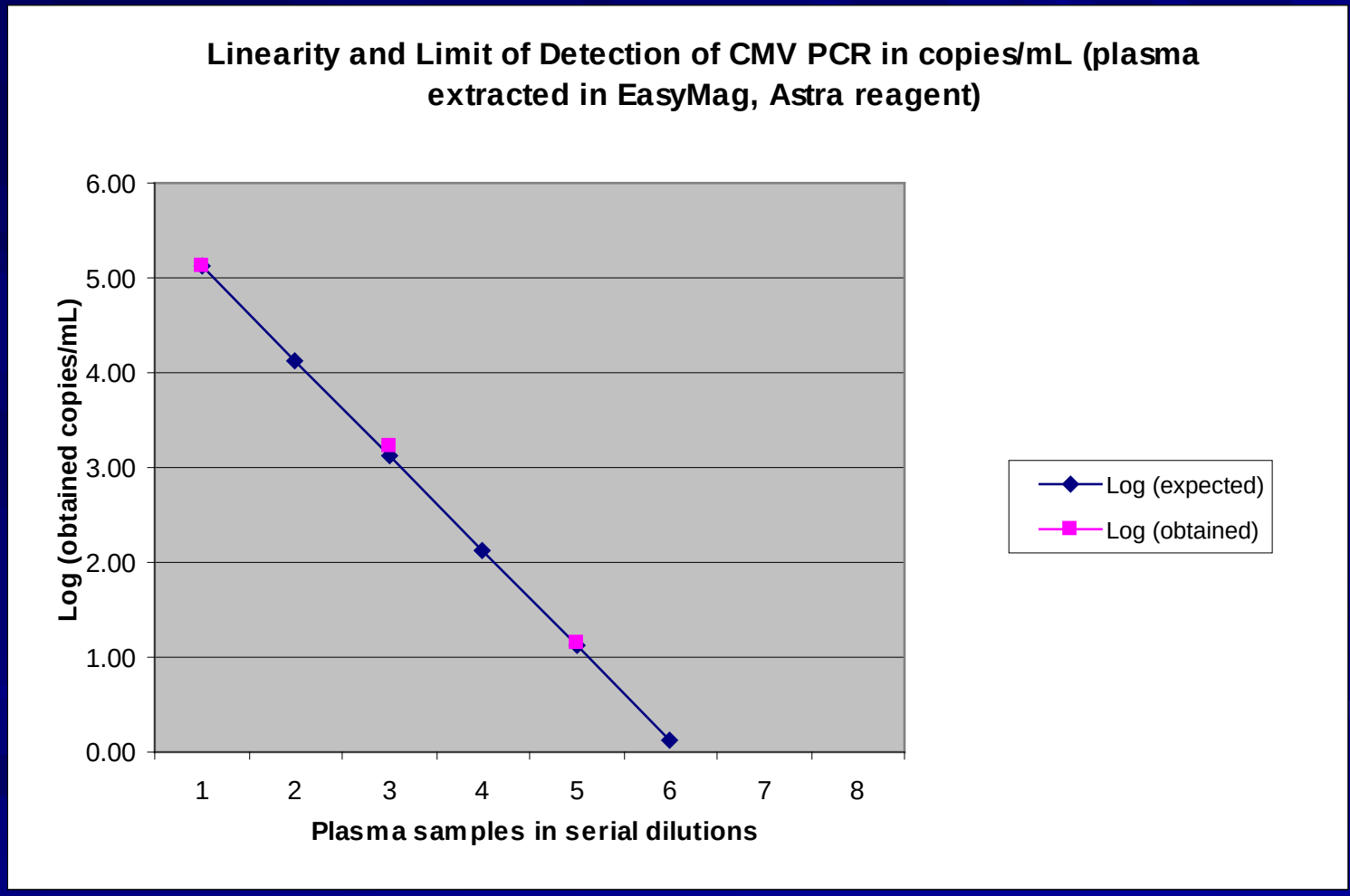
Gault et al. J Clin Micro 2001:39

CMV Quantification by PCR & Antigenemia



Mazzulli, T et al. Unpublished

Linearity of CMV PCR



Mazzulli, T et al. Unpublished

Clinical Utility of CMV DNA Testing

Clinicians may use a CMV DNA test for determining:

1. Likelihood that a symptomatic patient has active CMV disease
2. Likelihood that an asymptomatic patient will develop active disease
3. A patient's response to therapy
4. The risk of developing relapsed infection
5. The appropriate time to discontinue therapy

Performance and Interpretation of CMV DNA Testing

- Standardization – Commercial vs In-house and use of WHO International Standard
- Sample type and stability – Whole blood, serum, plasma, buffy coat
  - Viral load may be ~1 to 2 $\log_{10}$ (up to 100-fold) higher in whole blood than plasma in same patient
  - CMV DNA stable up to 14 days in plasma at 4^{o}C
- Analytical test characteristics – LOD, LOQ, Precision, Accuracy, Specificity, (Sensitivity?)
  - clinical significance of low levels of CMV DNA (100–500 copies/mL) may be difficult to interpret

Lisboa LF, et al. Transplantation 2011:91

Performance and Interpretation of CMV DNA Testing

- Determining the threshold for treatment:
  - e.g. viral load value of 2000–5000 copies/mL correlated with the development of end organ disease in liver transplant using the Cobas Amplicor Monitor Assay[1]
  - Some studies suggest both the viral load value and the rate of change in viral load are important predictors of the development of active disease[2]

[1]Humar A, et al. J Infect Dis 2002:186;
[2]Emery VC, et al. Lancet 2000:355

Performance and Interpretation of CMV DNA Testing

- Assay variability:
  - No clear difference exists between results in a patient in whom the viral load value has increased from 200 copies/mL to 500 copies/mL
  - Viral load changes of 0.5 $\log_{10}$ copies/mL in HIV and other viruses are thought to represent biologically important changes in viral replication – does this apply to CMV DNA?

Kraft C, et al. Clin Infect Dis 2012;54(12)

World Health Organization

WHO/BS/10.2138
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 18 to 22 October 2010

Collaborative Study to Evaluate the Proposed 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAT)-Based Assays

Jacqueline F. Fryer [1,3], Alan B. Heath [2], Rob Anderson [1], Philip D. Minor [1] and the Collaborative Study Group *

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E-mail Jacqueline.Fryer@nibsc.hpa.org.uk

** See Appendix 1*

Overall Mean Estimates and Inter-Laboratory Variation (log10 'copies/mL' for quantitative or 'NAT-detectable units/mL' for qualitative assays

Sample	Assay	No. data sets	Mean	SD	%GCV	Min	Max
S4	quantitative	48	7.11	0.61	307	5.06	8.81

1st WHO International Standard for HCMV:

- Whole virus preparation of 'Merlin' HCMV strain
- 5 x 10^6 copies/mL (6.7 $\log_{10}$)
  - Individual lab mean estimates ranged from 5.4 to 7.5 $\log_{10}$ copies/mL
- Uncertainty of 0.23%
- Stable for up to 8 months (freeze/thaw stable)

Clinical Infectious Diseases

MAJOR ARTICLE

Are We There Yet? Impact of the First International Standard for Cytomegalovirus DNA on the Harmonization of Results Reported on Plasma Samples

Jutta K. Preiksaitis,[1] Randall T. Hayden,[2] Yupin Tong,[1] Xiaoli L. Pang,[3] Jacqueline F. Fryer,[4] Alan B. Heath,[4] Linda Cook,[5] Astrid K. Petrich,[6] Brian Yu,[7] and Angela M. Caliendo[8]

Clinical Infectious Diseases 2016;63(5):583

Characteristics of CMV PCR Assays

Test	Lab1	Lab1	Lab2	Lab3	Lab3	Lab3	Lab4	Lab5	Lab5	Lab6
Assay characteristic	MultiCode-RTx CMV	COBAS AmpliPrep/ COBAS TaqMan CMV Test (CAP/ CTM)	RealStar CMV PCR Kit 1.0	RealStar CMV PCR Kit 1.0	CMV LC-PCR	artus CMV RG PCR Kit[b]	Quantitative TaqMan PCR (UL55/UL123-exon 4)	Abbott RealTime CMV	artus CMV RG PCR Kit	Simplexa CMV Kit
Manufacturer	Luminex Corporation, Madison, WI	Roche Molecular Systems, Pleasanton, CA	Altona Diagnostics, Hamburg, Germany	Altona Diagnostics, Hamburg, Germany	LDT, Edmonton Canada	Qiagen, Hilden, Germany	LDT Seattle, WA	Abbott Molecular, Des Plaines, IL	Qiagen, Hilden, Germany	Focus Diagnostics, Inc, Cypress, CA
Registration status	Commercial, ASR	Commercial, FDA-approved CE-marked	Commercial, CE marked	Commercial, CE marked	LDT	Commercial, FDA-approved CE marked	LDT	Commercial, CE marked	Commercial, FDA-approved CE marked	Commercial, CE marked
Test calibrators	LD amplicon	MP Plasmid	MP amplicon	MP amplicon	LD Plasmid	MP amplicon	LD plasmid	MP Plasmid	MP amplicon	MP amplicon
Gene Target	DNA Polymerase (UL54)	DNA polymerase (UL54)	confidential	confidential	Glycoprotein B (UL55)	MIE	Glycoprotein B (UL55) and IE (UL123)	UL34 UL80.5	MIE	UL83
Amplicon size(s)(bp)	52	340	<100	<100	254	105	64 (UL55), 76 (UL123)	95 (UL80.5) 105 (UL34)	105	86
Probe Chemistry	FRET labeled primers	TaqMan	TaqMan	TaqMan	FRET	TaqMan	TaqMan	TaqMan	TaqMan	FAM-labeled Scorpion
Extraction method	Qiagen EZ1 Advanced XL	Roche COBAS AmpliPrep	King Fisher FLex	Qiagen QIAcube	Qiagen QIAcube	Qiagen EZ1 Advanced	Roche MagNa Pure 96	Abbott m2000	Qiagen EZ1 Advanced XL	Roche MagNa Pure LC
Extraction Kits	Qiagen EZ1 Advanced XL DNA Tissue Card	Roche COBAS AmpliPrep	King Fisher MagMAX Viral Isolation Kit	Qiagen DNA Mini Kit	Qiagen DNA Mini Kit	Qiagen EZ1 DSP Virus Kit	Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit	Abbott Sample Preparation System DNA KIt	Qiagen EZQ Virus Mini Kit v2.0	Roche MagNa Pure LC Total Nucleic Acid Isolation Kit
Plasma input volume	200 µL	500 µL	100 µL	200 µL	200 µL	400 µL	200 µL	600 µL	200 µL	200 µL
Detection system	ABI Prism 7500	COBAS TaqMan 48 Analyzer	Rotor-Gene 3000/6000	ABI 7500	LightCycler 1.0	Rotor-Gene Q	ABI Stepone	Abbott m2000	Rotor-Gene Q	3M Integrated Cycler
LOD, $\log_{10}$ IU/mL	3.35	2.14	2.40	1.61	1.64	1.76	0.78	NA	1.76	2.85
LOQ, $\log_{10}$ IU/mL	3.35	2.14	3.00	2.18	1.94	2.48	1.30	1.49	2.48	2.85

Abbreviations: ASR, analyte-specific reagents; CAP/CTM, COBAS AmpliPrep/COBAS TaqMan CMV Test; CE, Conformité Européenne; CMV, cytomegalovirus; FAM, fluorescein; FDA, US Food and Drug Administration; FRET, fluorescence resonance energy transfer; LD, laboratory developed; LDT, laboratory-developed test; LOD, limit of detection; LOQ, limit of quantitation; MIE, major immediate early; MP, manufacturer provided; NA, not available; PCR, polymerase chain reaction.

Preiksaitis J et al. Clin Infect Dis 2016;63(5):583

Characteristics of CMV PCR Assays

- Gene Target:
  - DNA polymerase (UL54), glycoprotein B (UL55), UL34/UL80.5, UL83, Major immediate antigen (MIE), others
- Amplicon size: 52 to 340 bp
- Plasma input volume: 100 to 600 uL
- LOD (log_{10} IU/mL): 0.78 to 3.35
- LOQ (log_{10} IU/mL): 1.30 to 3.35
- Different probe chemistry, extraction method, detection method, etc.

Preiksaitis J et al. Clin Infect Dis 2016;63(5):583

Characteristics of CMV PCR Assays

- Assay variance:
  - Individual CMV DNA-positive samples: median, 1.5 [range, 1.22-2.82] $\log_{10}$ IU/mL
  - International standard (IS) dilutions: median, 0.94 [range, 0.69-1.35 $\log_{10}$ IU/mL ($p < 0.001$)
- 58.9% of all clinical sample results and 93.6% of IS dilution results fell within $\pm$0.5 $\log_{10}$ IU/mL of the mean
- Result variability not impacted by genotype or quantitative levels of CMV DNA

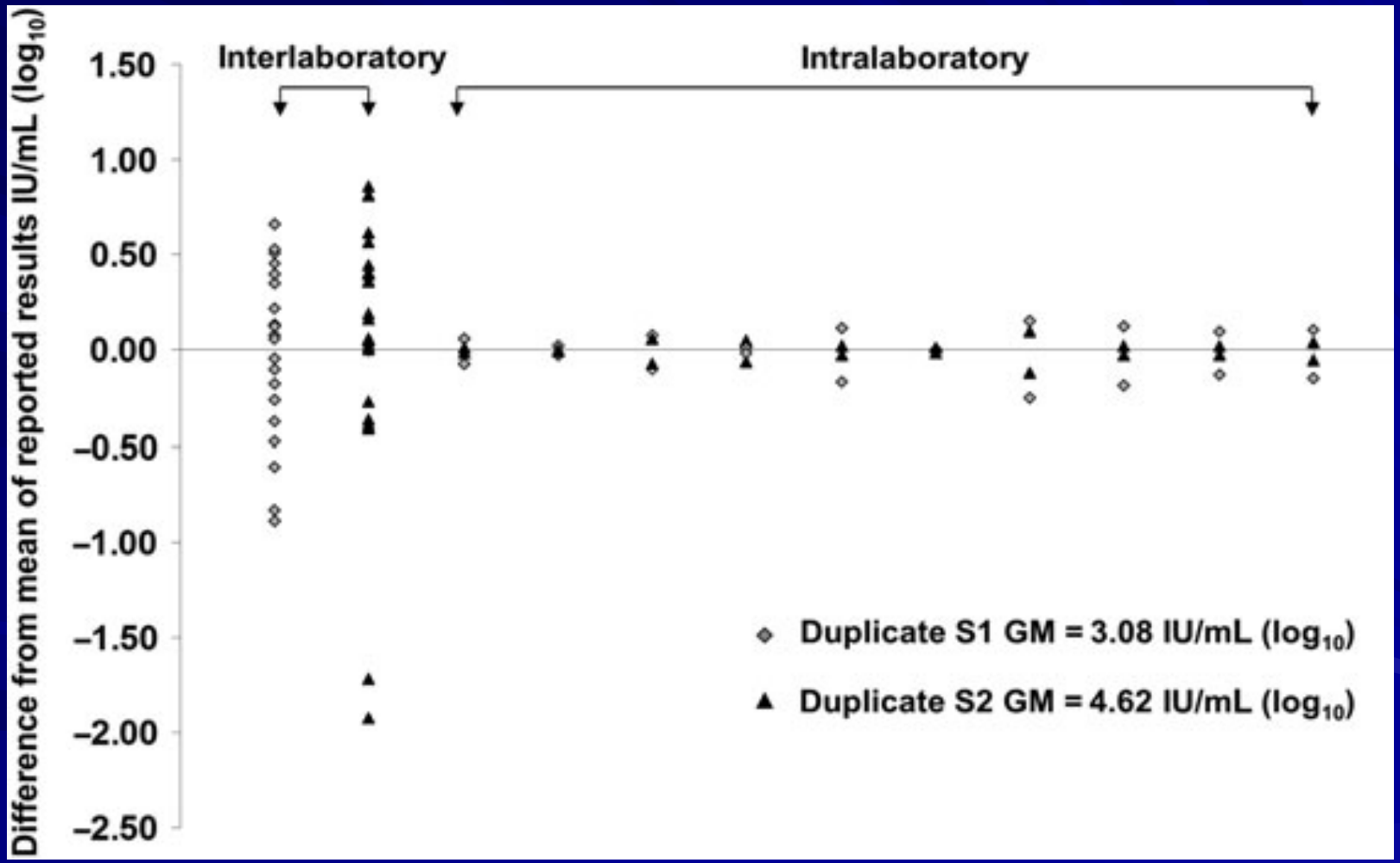
Preiksaitis J et al. Clin Infect Dis 2016;63(5):583

Characteristics of CMV PCR Assays

- For clinical samples all assays demonstrated result bias ($p < 0.008$)
- Assays with amplicon sizes $\leq$ 86 bp had significantly higher results compared to those with amplicon sizes $\geq$ 105 bp ($p < 0.001$)

Preiksaitis J et al. Clin Infect Dis 2016;63(5):583

Characteristics of CMV PCR Assays



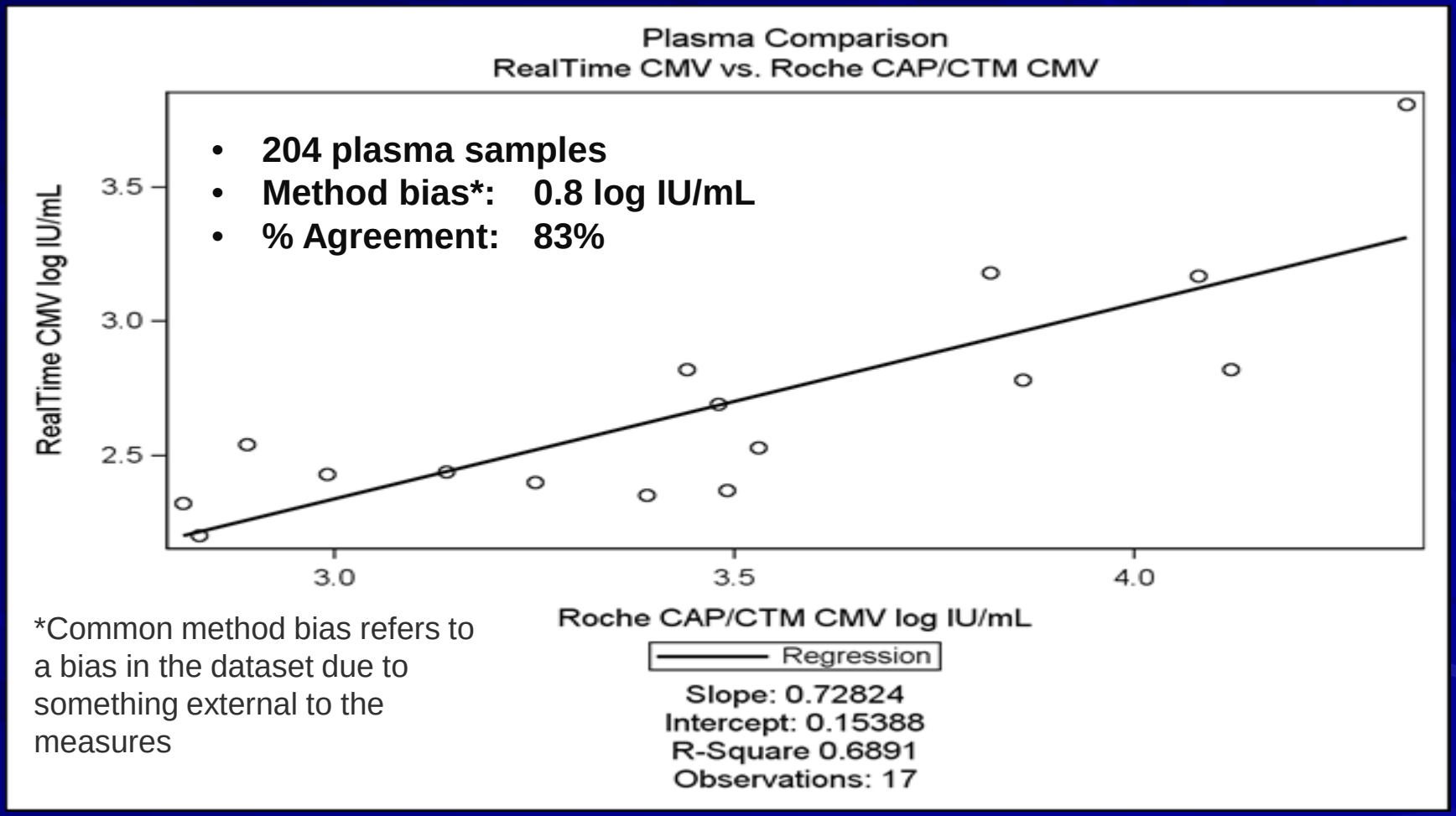
Preiksaitis J et al. Clin Infect Dis 2016;63(5):583

Molecular Assays Licensed by Health Canada - 2017

- Abbott Realtime CMV (Abbott Molecular Inc.)
- Cobas Ampliprep/Cobas Taqman CMV Test (Roche Molecular Systems, Inc.)
- Others available (but not licensed):
  - Luminex
  - Altona
  - Artus
  - In-house assays, others

https://health-products.canada.ca/mdall-limh/dispatch-repartition.do?type=active. Accessed April 30th, 2017

Abbott RealTime vs Roche CAP/CTM CMV



*Common method bias refers to a bias in the dataset due to something external to the measures

Moussa et al. AMP Conference 2016

Abbott RealTime vs Roche CAP/CTM CMV

Abbott RealTime <LOD	Roche CAP/CTM
11	1
% Agreement @ LOD	9%
Roche CAP/CTM <LOD	**Abbott RealTime**
41	32
% Agreement @ LOD	78

Moussa et al. ECCMID Conference 2016

Clinical Interpretation of CMV DNA Tests

1. Tests have more variability near the lower LOQ - Assume that changes in viral load must exceed 3-fold (for values in the midrange of the test) to 5-fold (for values in the lower range) to represent meaningful changes in viral replication
2. Factors to consider when interpreting low viral load results include:
   - specimen type, type of transplant, degree of immune suppression, and donor/recipient CMV immune status, LOD and precision of the test

Kraft C, et al. Clin Infect Dis 2012;54(12); Humar A, Snydman D. Am J Transplant 2009; 9(Suppl 4); Kotton CN, et al. Transplantation 2010; 89

Clinical Interpretation of CMV DNA Tests

3. Monitoring post-transplant: trends in viral load may be more useful than any single viral load value, unless the viral load is very high (>10 000 copies/mL) or the patient is at very high risk (e.g. donor/recipient mismatch)
4. CMV Viral load testing should be performed the day therapy is begun, even if a recent value is available, because viral load levels may increase rapidly in patients with active disease
5. Follow-up viral load testing should be performed at 5- to 7-day intervals because the half-life of CMV DNA in the plasma ranges from 3 to 8 days. **NB**: CMV DNA may increase in 1st few days after treatment initiated

Kraft C, et al. Clin Infect Dis 2012;54(12); Humar A, Snydman D. Am J Transplant 2009; 9(Suppl 4); Kotton CN, et al. Transplantation 2010; 89

Clinical Interpretation of CMV DNA Tests

7. After initiating therapy, viral load should be followed to document clearance of CMV from plasma or whole blood
   - Depending on the initial viral load, this may take weeks or longer
8. Viral load patterns that are worrisome for drug-resistant virus include:
   - those that do not decrease after 2 weeks of adequate therapy
   - those with a plateau in the rate of viral load decline
   - those that have an initial drop and then a subsequent increase in viral load while on therapy
9. Genotypic resistance testing can be performed directly using plasma specimens

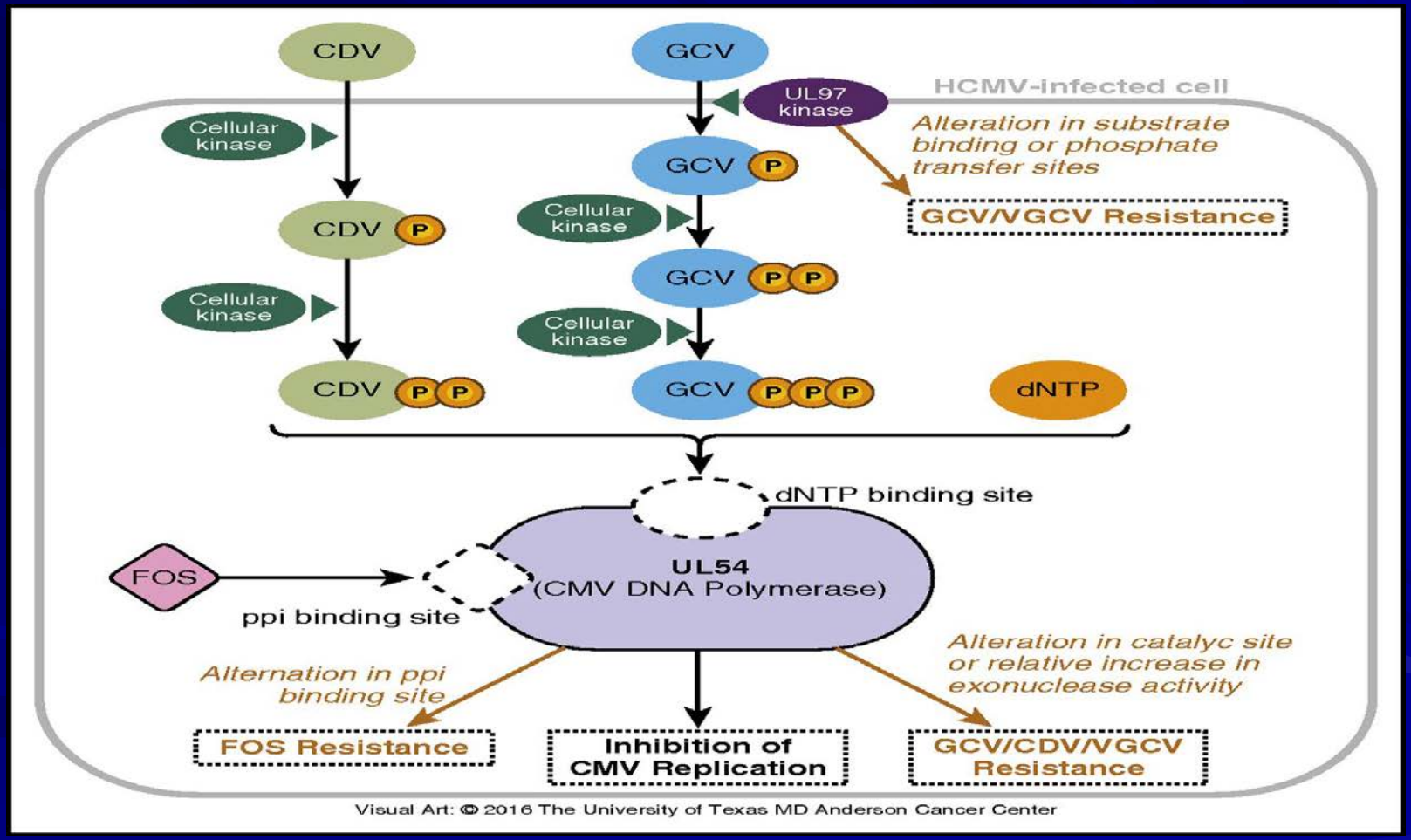
Kraft C, et al. Clin Infect Dis 2012;54(12); Humar A, Snydman D. Am J Transplant 2009; 9(Suppl 4); Kotton CN, et al. Transplantation 2010; 89

Genotypic Testing for CMV Resistance

- No commercially available
- Codons 457 to 630 of the protein kinase (UL97) and codons 393 to 1000 of the polymerase (UL54) CMV genes that harbour known anti-viral resistant mutations that confer resistance to Ganciclovir, Cidofovir and Foscarnet are amplified by PCR and sequenced
- Specimen requirement: CMV positive plasma (0.5 mL minimum), whole blood (1 mL minimum), or viral culture (0.5 mL minimum)
- If CMV viral load <1000 copies/mL may not work

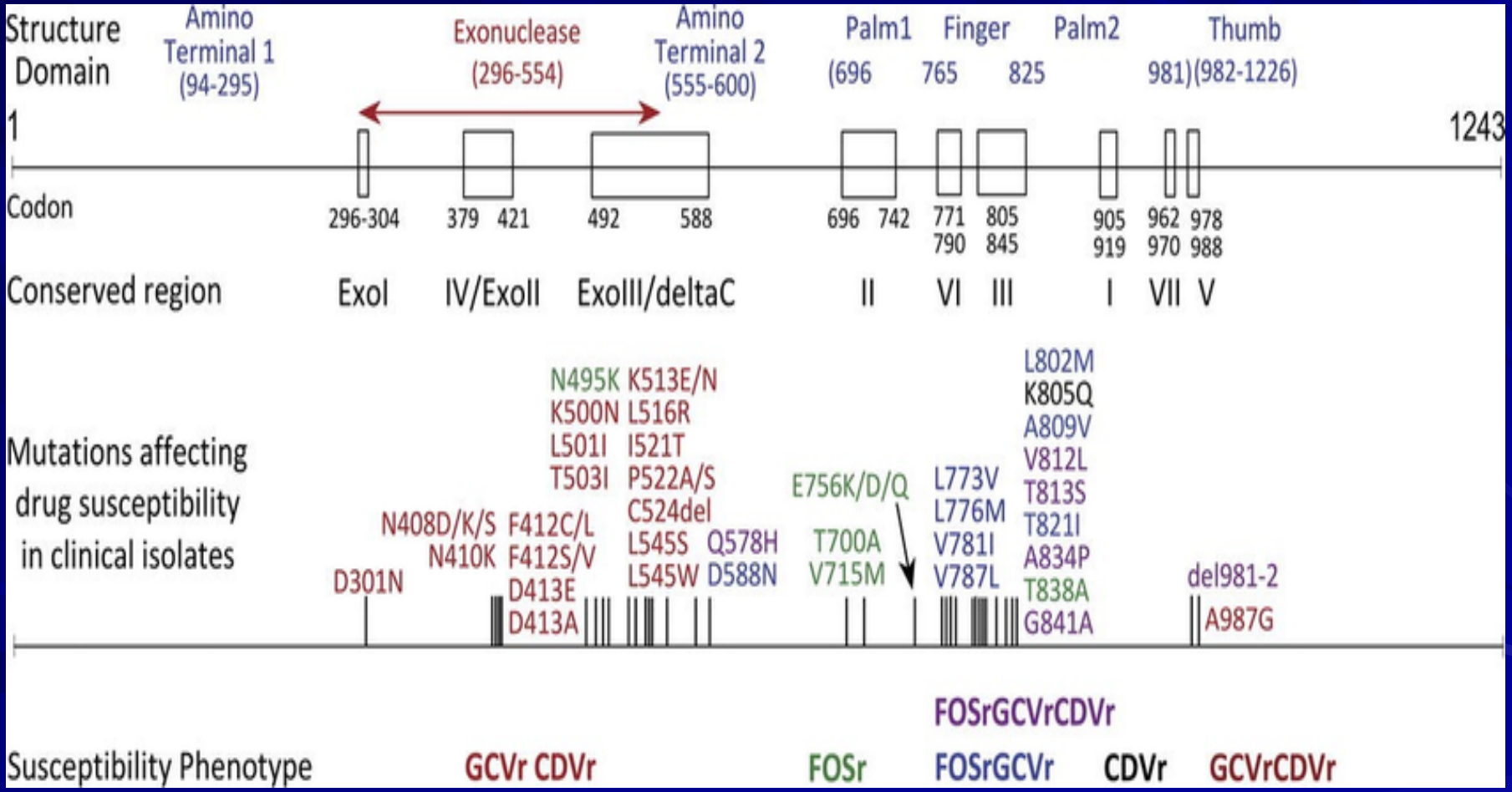
Genotypic Testing for CMV Resistance

Mechanism of Action of Antiviral Drugs for CMV



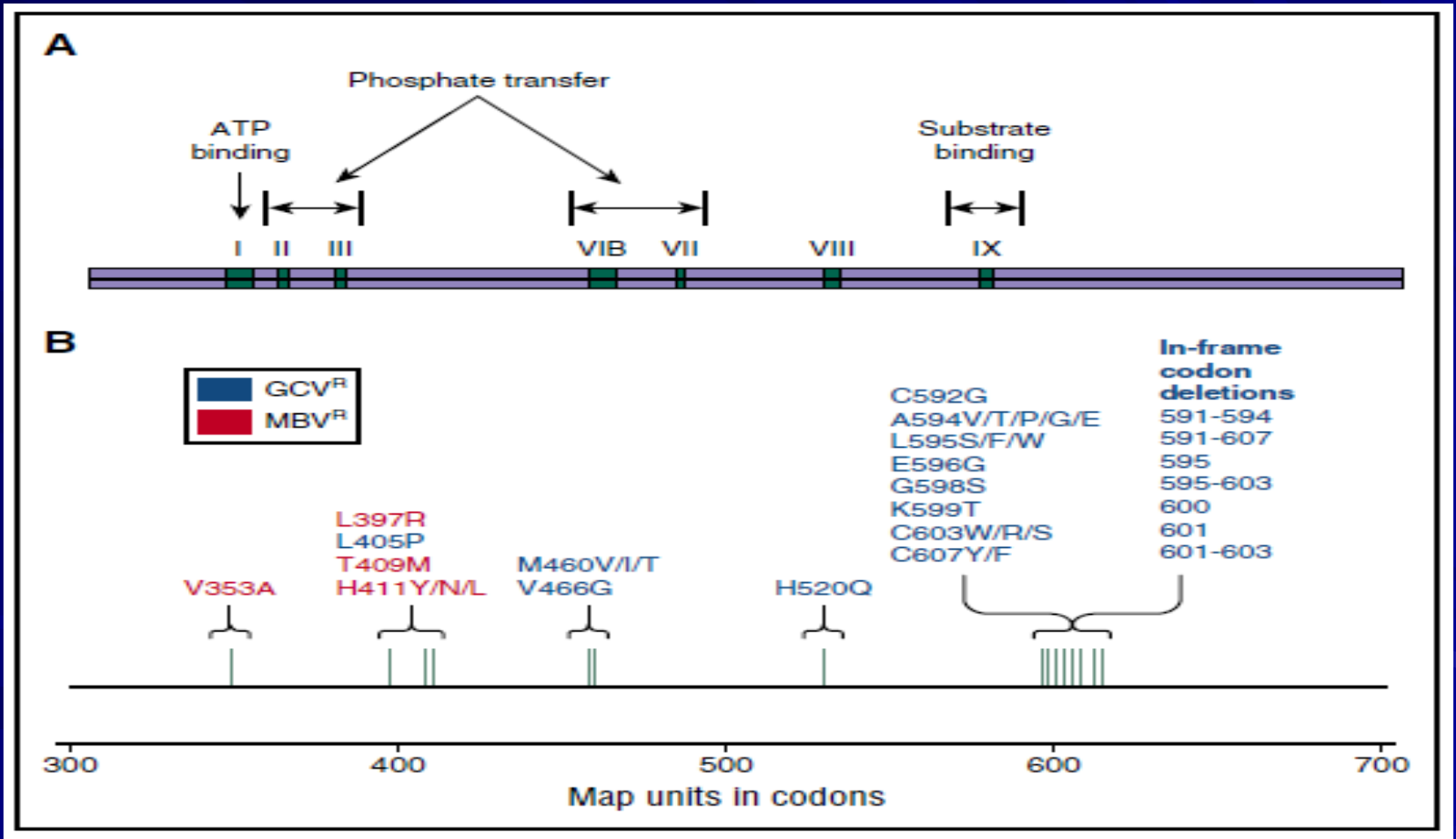
Firas El Chaer et al. Blood 2016;128:2624-2636

Mutations Conferring Antiviral Resistance to CMV



Boeckh M and Ljungman P. Blood 2009 113

Map of the CMV Kinase gene (UL97 or kinase/phosphptransferase)



*MBV = Maribavir

Firas El Chaer et al. Blood 2016;128:2624-2636

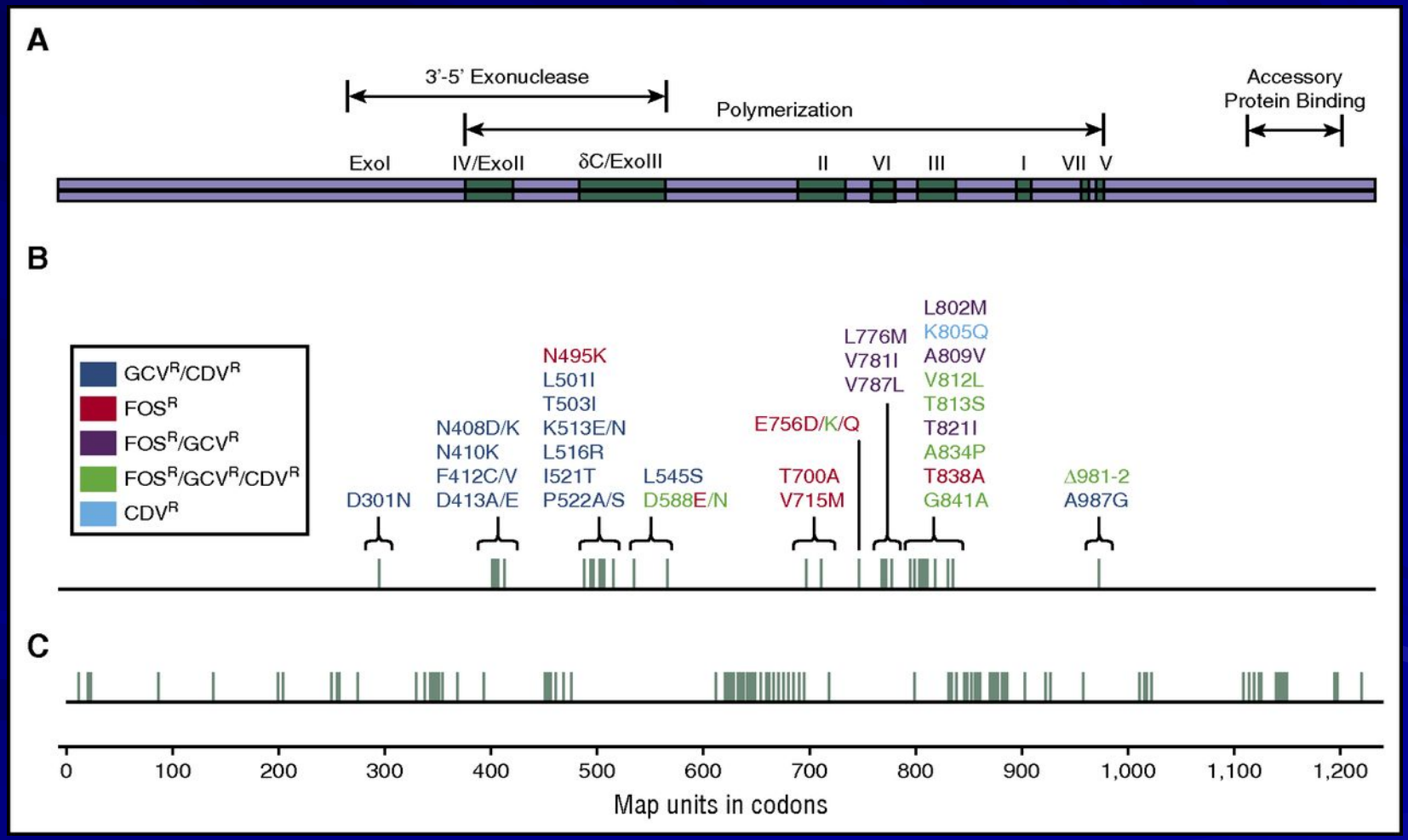
UL97 Mutations Conferring Ganciclovir Resistance to CMV (Top 7 account for 80%)

Codon number	Wild-type	Mutant	GCV ratio*	References
Canonical mutations				
460	M	I	5	47,72,80,142,152-163
460	M	V	8.3	43,47,80,82,83,142,154-156, 159-161,164-169
520	H	Q	10	47,72,83,142,154,155,159,161, 164,165,169-171
592	C	G	2.9	72,80,82,83,142,153,154,159-161,163,165,166,172,173
594	A	V	8.3	43,72,80,82,83,152-156,158-161, 164-166,171,173
595	L	S	9.2	43,72,80,82,83,153-155,158-166
603	C	W	8	72,80,83,142,153-155,159-161,163,164,171,172,174,175
Other clinically relevant substitution mutations				
595	L	F	15.7	72,156,161,175
595	L	W	5.1	72,154,155,159-161,175

*GCV ratio = Mutant IC_{50}/Wild-type IC_{50};
IC_{50} > 6uM confers resistance

Firas El Chaer et al. Blood 2016;128:2624-2636

Map of the CMV DNA polymerase gene (UL54 or pol)



Firas El Chaer et al. Blood 2016;128:2624-2636

Mutations Conferring Antiviral Resistance to CMV

Mechanism of Resistance of CMV to Current Therapies

Inhibitor	Analogue Type	Requires UL97	Resistance Due to
Ganciclovir	Nucleoside	Yes	UL97 or DNA polymerase mutations
Foscarnet	Pyrophosphate	No	DNA polymerase mutations
Cidofovir	Nucleotide	No	DNA polymerase mutations

Boeckh M and Ljungman P. Blood 2009 113

Mutations Conferring Antiviral Resistance to CMV

- In 85%–95% of patients, ganciclovir resistance results initially from UL 97 mutations
- If there are verified UL 54 resistance mutations in an isolate obtained from a patient receiving ganciclovir, the virus will be cross-resistant to cidofovir, but cross-resistance to foscarnet is uncommon
  - Mutations of foscarnet resistance will usually only be seen in patients taking foscarnet

Drew WL. Clin Infect Dis 2010:50

Mutations Conferring Antiviral Resistance to CMV

Site of resistance mutation	Current Treatment		
	Ganciclovir	Cidovovir	Foscarnet
UL97	Mediates ganciclovir resistance only	No Effect	No Effect
UL54	Mediates ganciclovir and cidofovir cross-resistance (rarely foscarnet cross-resistance)	Mediates cidofovir resistance and ganciclovir cross-resistance (rarely foscarnet cross-resistance)	Mediates foscarnet resistance (rarely ganciclovir or cidofovir cross-resistance)

Drew WL. Clin Infect Dis 2010:50

Mutations Conferring Antiviral Resistance to CMV

- Takes weeks to months for CMV to develop resistance to antivirals
- Up to two-thirds of patients may exhibit an increase in CMV DNA levels in the first 2–3 weeks of anti-CMV therapy, but this is not due to resistance and should not lead to requests for genotypic assays:
  - The explanation for this phenomenon is unclear, but it appears to be associated with corticosteroid use

Drew WL. Clin Infect Dis 2010:50

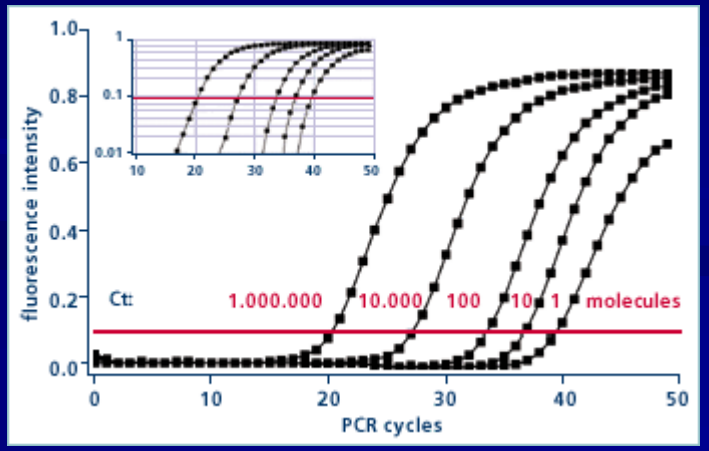
Future Testing Options

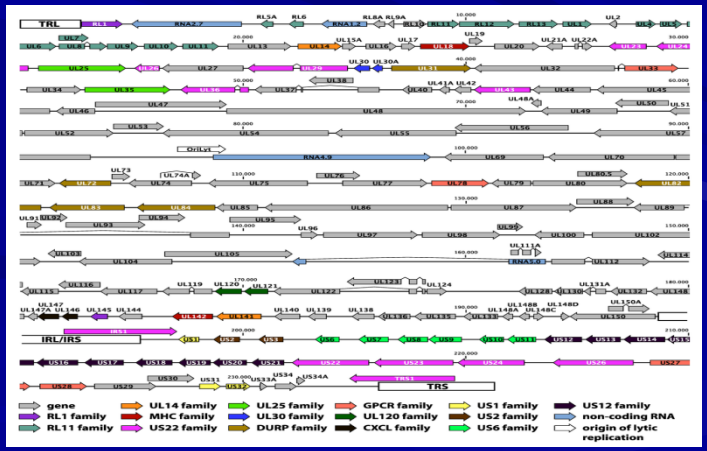
Molecular

(Early/Mid 2000s)

Improved Automation
Whole Genome/Next Generation Sequencing

(2020 and beyond)





Future Testing Options

- Molecular:
  - Faster (improved TAT)
  - Random access
  - High throughput
  - Improved standardization
  - Lower cost
- Resistance testing:
  - Deep genome sequencing to detect smaller subpopulations (e.g. currently mutant strains comprising <20 to 30% of the population may not be detected/reported; Whole genome sequencing?
  - Commercialized, standardized assays
  - New drugs with new mechanisms of action will require testing for drug resistance and correlation between genotype and phenotype


“Happy Herpes”
Thank you for your attention!