Supporting Information

Electrochemical Detection of a Single Cytomegalovirus at an Ultramicroelectrode and its Antibody Anchoring

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Table SI 1. Comparison of several virus detection techniques.

Method	Advantages	Disadvantages
Presented Electrochemical Technique	• Rapid detection, assays complete within one hour • No washing step • Sub-picomolar detection • Dual measurements: ΔI and frequency	• Electrode sensitive to non-specific adsorption, can result in false positive • Currently unquantifiable
Real-Time Polymerase Chain Reaction (RTPCR)	• High Sensitivity • Detection limit of 122-1953 genomes/mL, depending on method[i]	• Detection limit varies with sample • Limits of detection not readily reproducible
Enzyme-Linked Immunosorbent Assay (ELISA)	• Can detect presence of IgM and/or IgG against HCMV (Abcam®, Calbiotech, Genway Biotech Inc.) • Specificity ~98%	• Indirect ELISAs are qualitative; cannot quantitate the amount of virus • Assay dependent on K_D of antibody
Culture Test	• "Gold standard" of CMV detection[ii] • Can quantitate titers of infectious units present in sample	• False negative viral culture results are possible[25] • Not as sensitive as PCR based detection methods
Electron Microscopy	• Direct, visual evidence of CMV • Can use morphological details to classify pathogen[iii]	• Not suitable for highly dilute samples • Not suitable for examining large numbers of samples

Table SI 1 provides a comparison of several virus-detection techniques commonly used for the diagnosis of CMV, including the new electrochemical technique. The most commonly used technique for the qualitative diagnosis of CMV is by Enzyme-Linked Immunosorbent Assays (ELISAs). These assays are highly specific and somewhat quantifiable. Perhaps the most sensitive technique, Real-Time Polymerase Chain Reaction (RTPCR), can determine the presence of cytomegalovirus genome. De Vries *et al.* refer to the culture test as the 'gold standard' in CMV detection; however, this technique takes extensive sample preparation. Electron microscopy techniques may provide evidence for a specific virus and morphological details; however, the technique requires significant experimental workup. The electrochemical system shows promise over currently used techniques for many reasons. By observing a reference particle, such as a PSB, the effect of the virus on aggregation should be similar over a range of virus sizes. This implies that the technique could be extended to other viral families and is not limited to CMV or viruses found in the herpes family. Also, the technique has the advantage of no washing step. Once the 'background' of PSBs is known (i.e., the current step size distribution), monitoring for large aggregates and a decrease in frequency can signal the presence of the antigen of interest. Despite the advantages to the electrochemical technique, there are also disadvantages. Non-specific adsorption of species in the solution to the electrode can provide false positives; however, by monitoring the frequency of collision, false positives should be ruled out. Also, electrode fouling due to dissolved organic matter may affect the electrochemical and collision response. Because the technique was used to show selectivity to a particular virus of interest, it may also be extended to possibly screen for different types of antibodies in a system.

Figures:

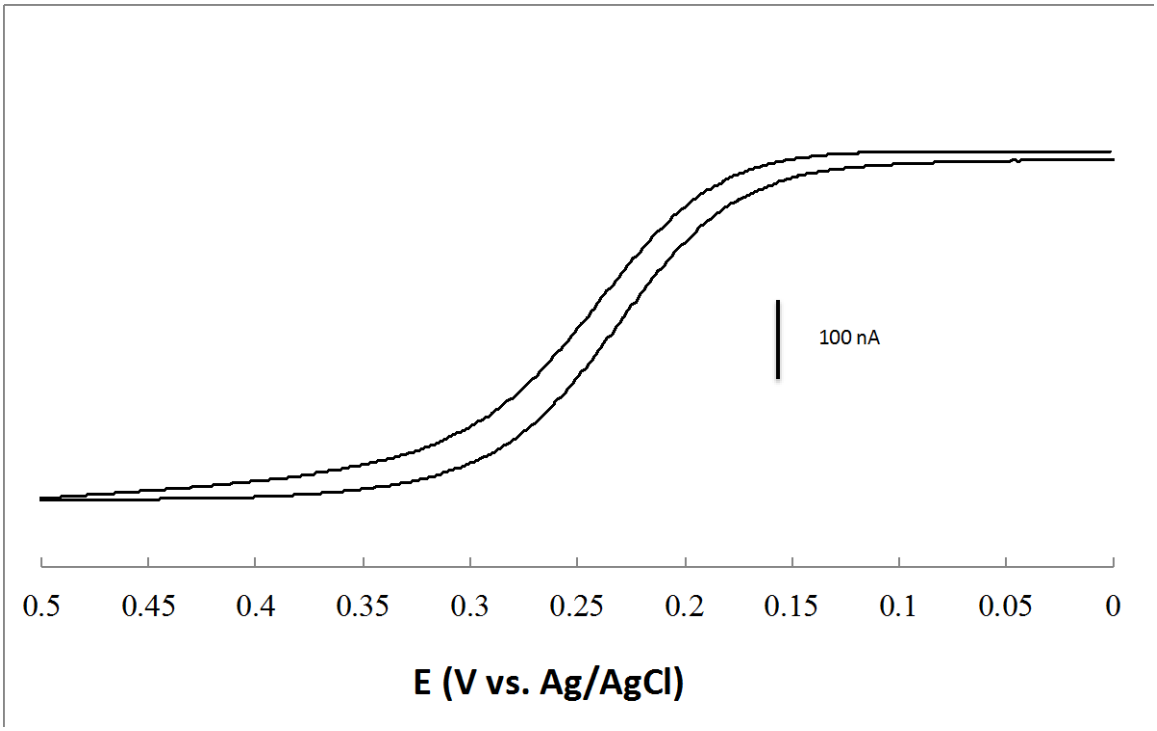


Figure S1: Cyclic voltammogram (CV) of 400 mM $K_4Fe(CN)_6$ on a 10 um Pt UME. The scan rate was 50 mV/s.

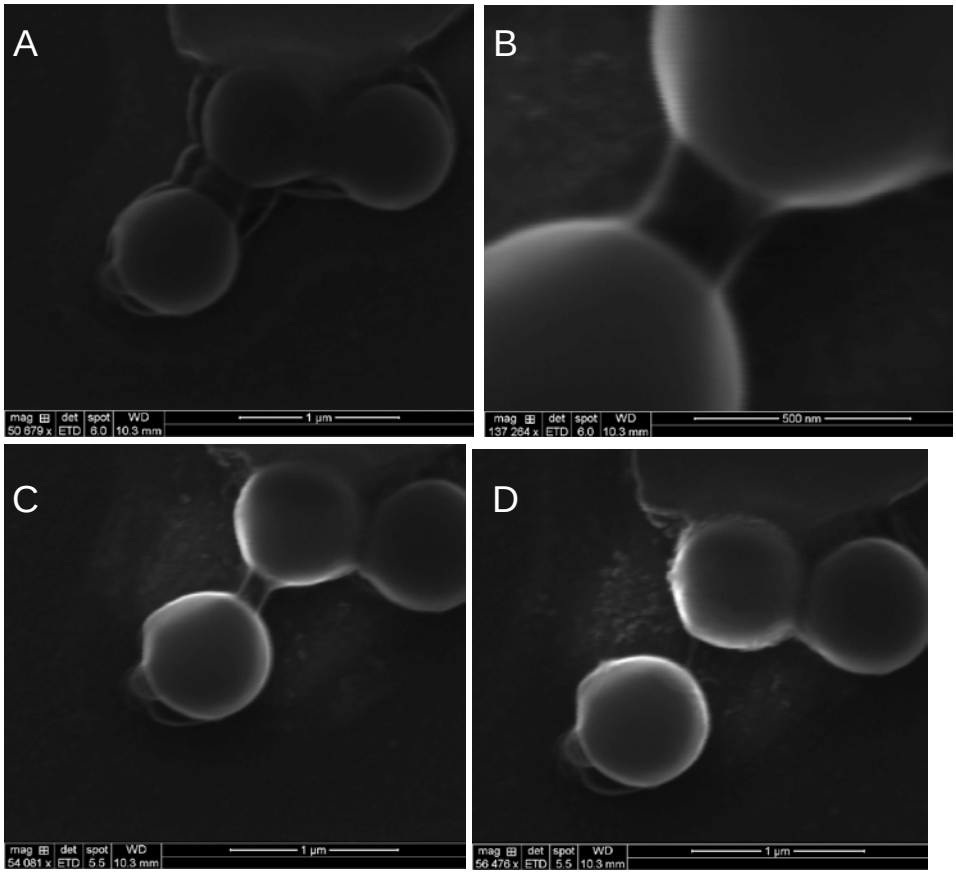


Figure S2: SEM Images of PSBs in the presence of virus. Upon interrogation with the electron beam, the beads were pulled apart (C-D). Clearly, a 200 nm wide bridge holds the beads together, which is consistent with the size of MCMV (A-C). This bridge was eventually severed due to extended exposure to the electron beam.

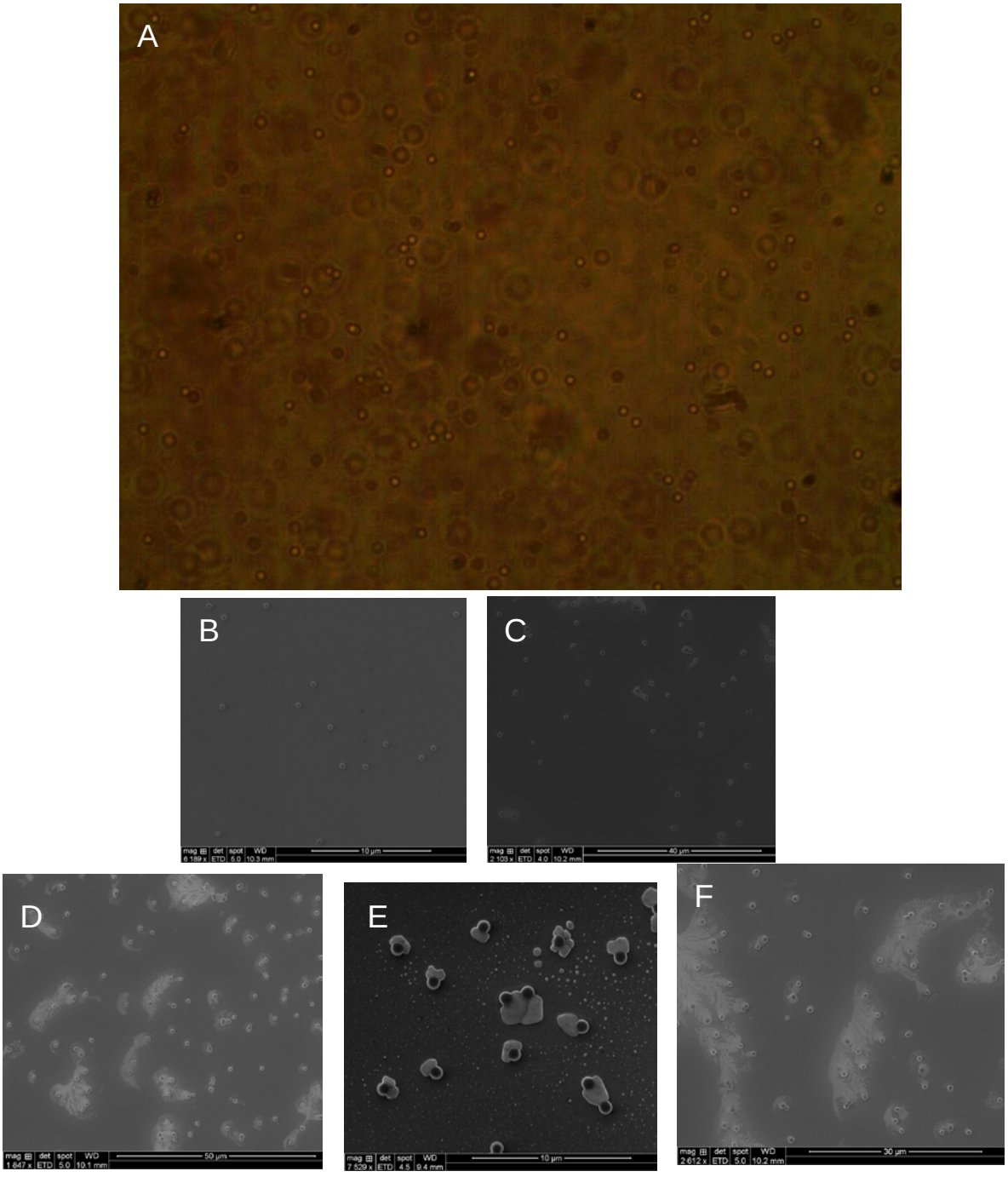
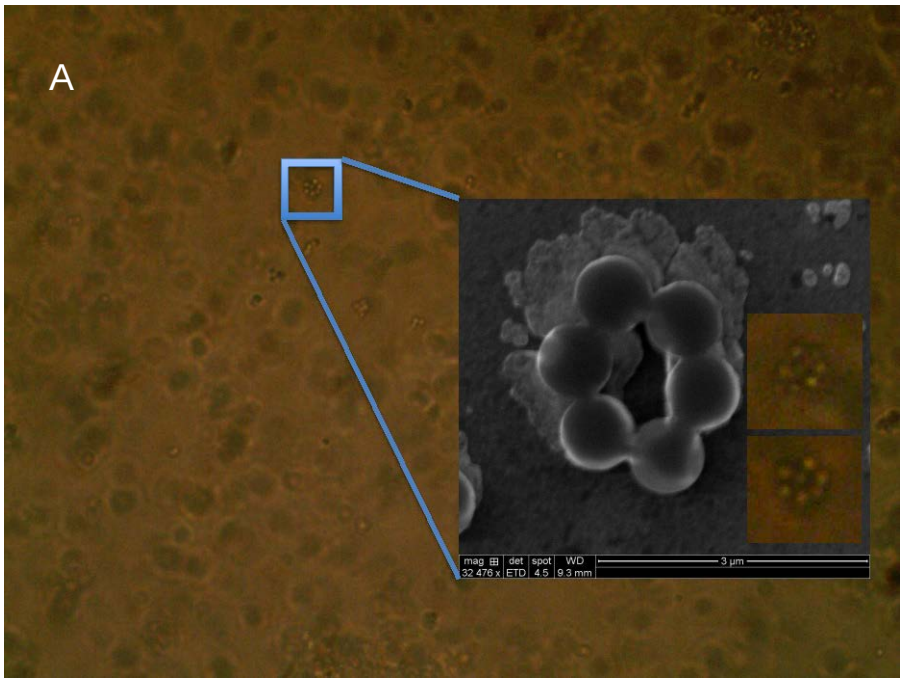
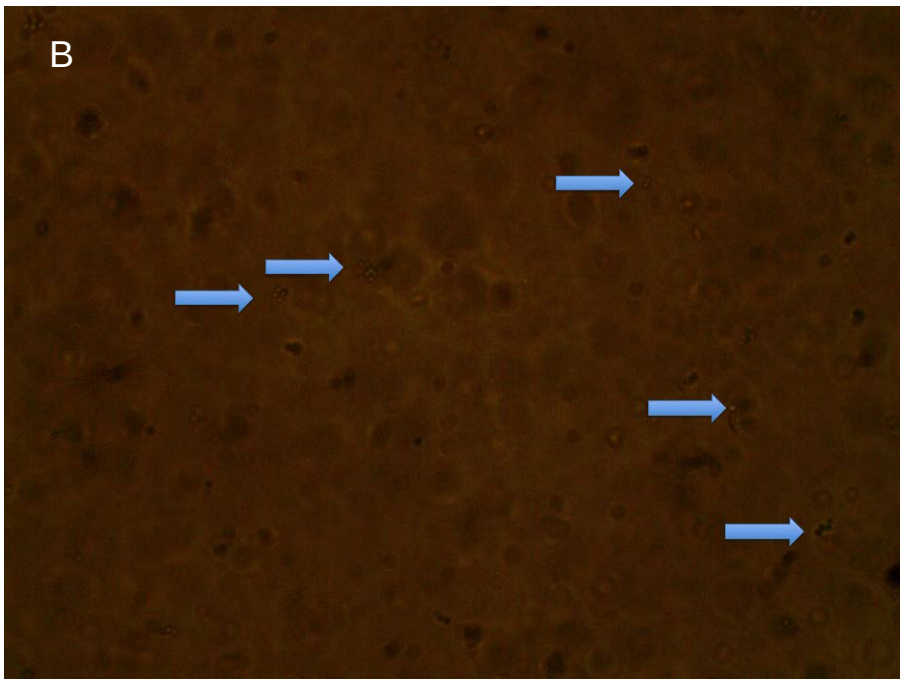


Figure S3: A.) Optical image of background. B-F.) SEM Image of background. In the SEM images, the light substance around the beads is likely part of the protenaceous tegument in which the virus and PSBs are stored.





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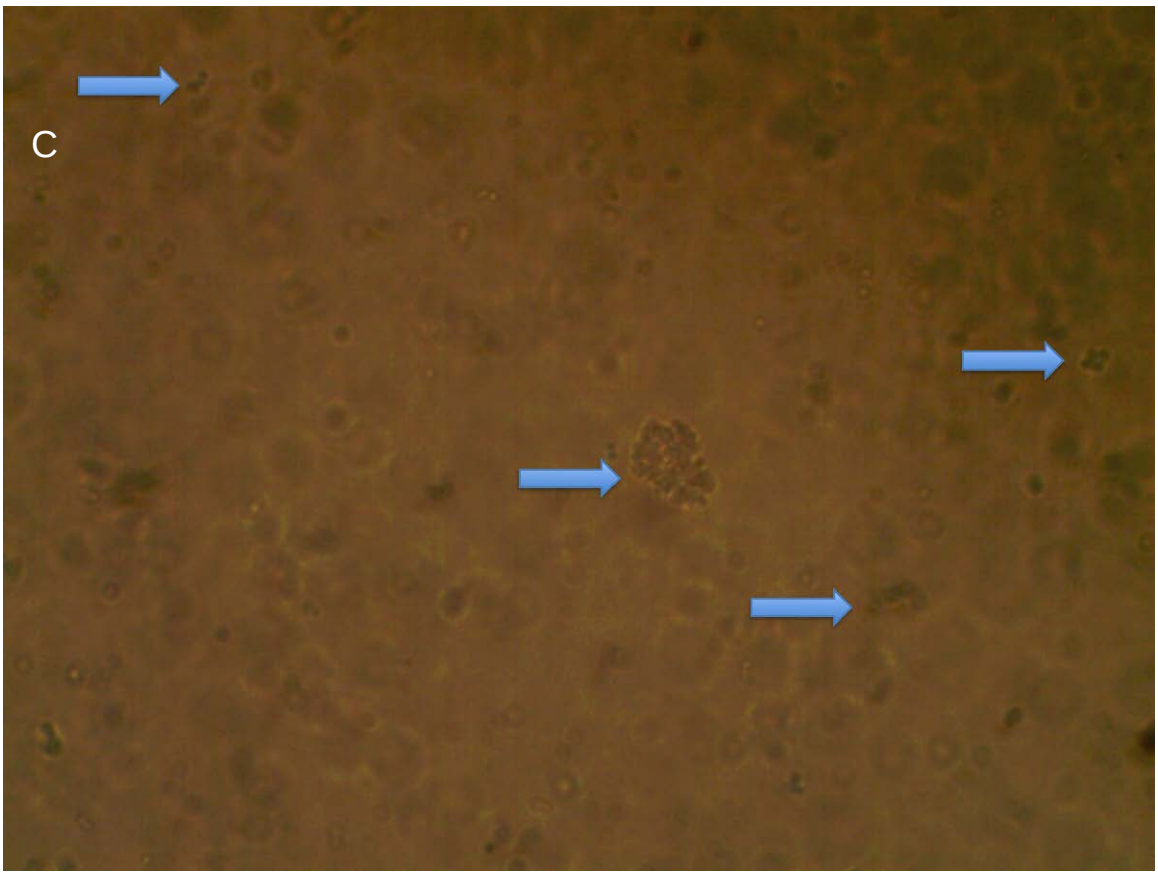
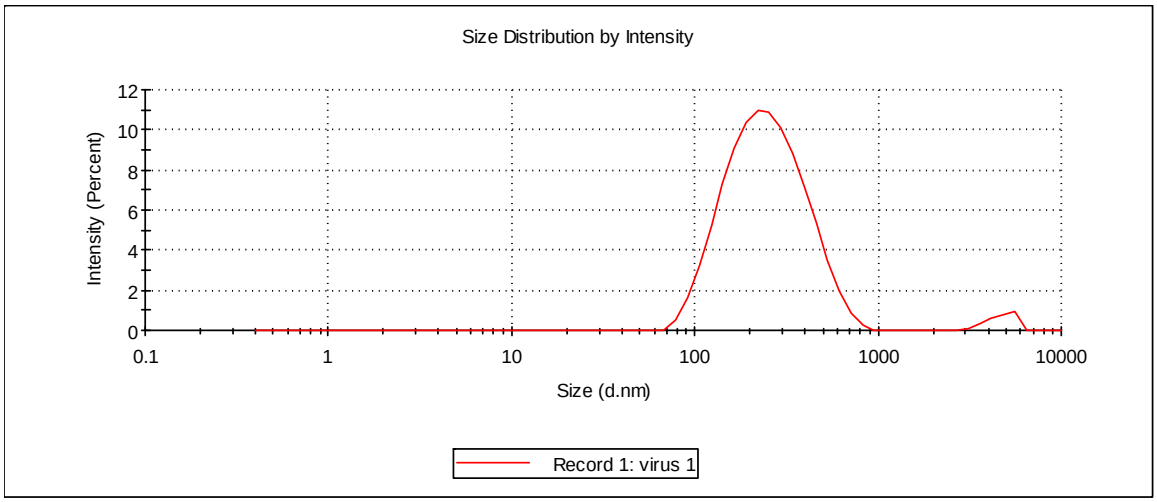


Figure S4: A.) Evidence for hexamer species, B.) Evidence for monomer, dimer, trimer, tetramer, and C.) Evidence for higher order aggregates using optical imaging techniques.

Dynamic Light Scattering:

DLS of MCMV



			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	221.1	**Peak 1:**	268.0	97.2	130.9
PdI:	0.253	**Peak 2:**	4712	2.8	774.7
Intercept:	0.956	**Peak 3:**	0.000	0.0	0.000
Result quality :	Good				

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	Virus + Antibody + Beads 1		
SOP Name:	Reference latex PSD.sop		
General Notes:			
File Name:	013114 Pt NP with hydraz...	**Dispersant Name:**	Water
Record Number:	18	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Friday, October 31, 2014 8:54:...

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	243.2	**Measurement Position (mm):**	1.25
Cell Description:	Disposable sizing cuvette	**Attenuator:**	5

Upon addition of the virus and antibody, a large peak around 5.5 um began to evolve.

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	Virus + Antibody + Beads 2 1
SOP Name:	Reference latex PSD.sop
General Notes:	

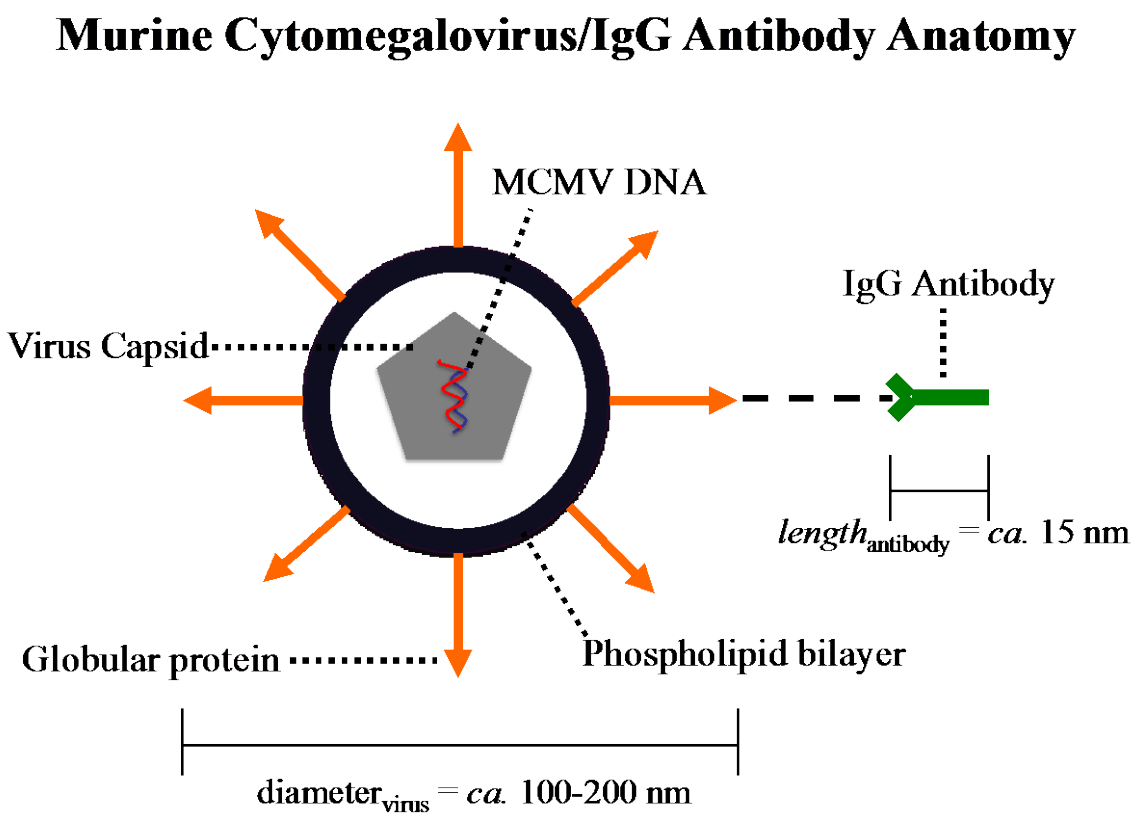
File Name:	013114 Pt NP with hydraz...	**Dispersant Name:**	Water
Record Number:	19	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Friday, October 31, 2014 9:00:...

System

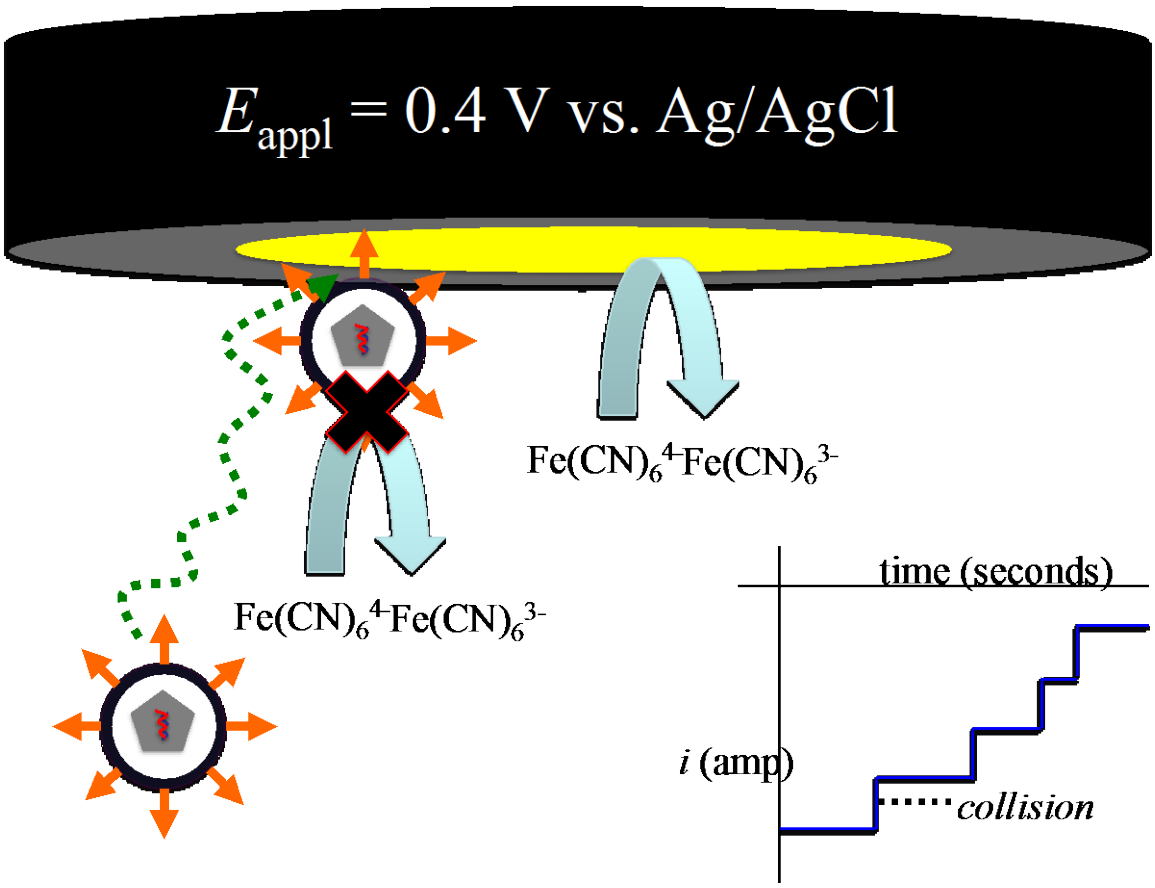
Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	185.4	**Measurement Position (mm):**	1.25
Cell Description:	Disposable sizing cuvette	**Attenuator:**	5

Here, we see a negative control with only virus and PSB.

Anatomy of MCMV:



Experimental Setup:



Nanosight:

Virus Nanosight analysis:
ANALYSIS REPORT (Size and Zeta Potential)

Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034
Zeta Potential / Concentration Particle Size / Concentration
Particle Size / Zeta Potential Mobility / Channel Depth
Operator: Sample: Date/Time of Capture: Video File: Analysis No: Date/Time of Report: Dispersant/Diluent: Concentration: Pre-treatment: Remarks:
jd
12 August 2014 12:00 'zeta-videos10.avi' 003 12/08/2014
None
14:46:31
RESULTS: Size Distribution: Zeta Distribution: Cumulative Data (nm): Cumult. Data Zeta (mV): User Lines: Total Concentration: Selected Concentration: Fitted Curve : Completed Tracks: Drift Velocity:
ANALYSIS SETTINGS: Frames Processed: Frames per Second: Calibration:
Blur: Detection Threshold: Min Track Length: Min Expected Size: Temperature: Viscosity:
Mean: 124 nm, Mode: 92 nm, SD: 81 nm Mean: -34.3 mV, Mode: -35.2 mV, SD: 19.8 mV D10: 33, D50: 100, D90: 247, D70: 154 D10: -50.8, D50: -35.2, D90: -14.0, D70: -29.4 596 nm, 0 nm 22.08 particles / frame, 4.50E8 particles / ml 0.00 particles / frame, 0.00E8 particles / ml Mean: 0 nm, SD: 0 507 15979 nm/s
749 of 749 24.97 145 nm/pixel Auto
21 Multi Auto Auto 24.00 ^{o}C 0.91 cP
ZETA SETTINGS: Average EP velocity: Dielectric Constant: Applied Voltage:
ZETA RESULTS: Mobility: Zeta Potential: Average Current:
-13563 nm/s 80.000000 24.000000
-2.68E-008 S/m -34.42 mV 2.0 uA

ANALYSIS REPORT (Diffusion Coefficient Data)

Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034
Zeta Potential / Concentration Diffusion Coefficient / Count
Particle Size / Zeta Potential Mobility / Channel Depth
Operator: Sample: Date/Time of Capture: Video File: Analysis No: Date/Time of Report: Dispersant/Diluent: Concentration: Pre-treatment: Remarks:
jd
12 August 2014 12:00 'zeta-videos10.avi' 002 12/08/2014
None
14:45:41
RESULTS: Diff .co. Dist.(E4 nm^2/s): Size Distribution: Zeta Distribution: Cumult. Dat.(E4 nm^2/s): Cumulative Data (nm): Cumult. Data Zeta (mV): User Lines: Total Concentration: Selected Concentration: Fitted Curve : Completed Tracks: Drift Velocity:
ANALYSIS SETTINGS: Frames Processed: Frames per Second: Calibration:
Blur: Detection Threshold: Min Track Length: Min Expected Size: Temperature: Viscosity:
Mean: 642, Mode: 233, SD: 524 Mean: 124 nm, Mode: 92 nm, SD: 81 nm Mean: -34.3 mV, Mode: -35.2 mV, SD: 19.8 mV D10: 190, D50: 462, D90: 1280, D70: 637 D10: 33, D50: 100, D90: 247, D70: 154 D10: -50.8, D50: -35.2, D90: -14.0, D70: -29.4 596 nm, 0 nm 22.08 particles / frame, 4.50E8 particles / ml 0.00 particles / frame, 0.00E8 particles / ml Mean: 0 nm, SD: 0 507 15979 nm/s
749 of 749 24.97 145 nm/pixel Auto
21 Multi Auto Auto 24.00 ^{o}C 0.91 cP
ZETA SETTINGS: Average EP velocity: Dielectric Constant: Applied Voltage:
ZETA RESULTS: Mobility: Zeta Potential: Average Current:
-13563 nm/s 80.000000 24.000000
-2.68E-008 S/m -34.42 mV 2.0 uA

Virus and antibody Nanosight analysis

ANALYSIS REPORT (Size and Zeta Potential)

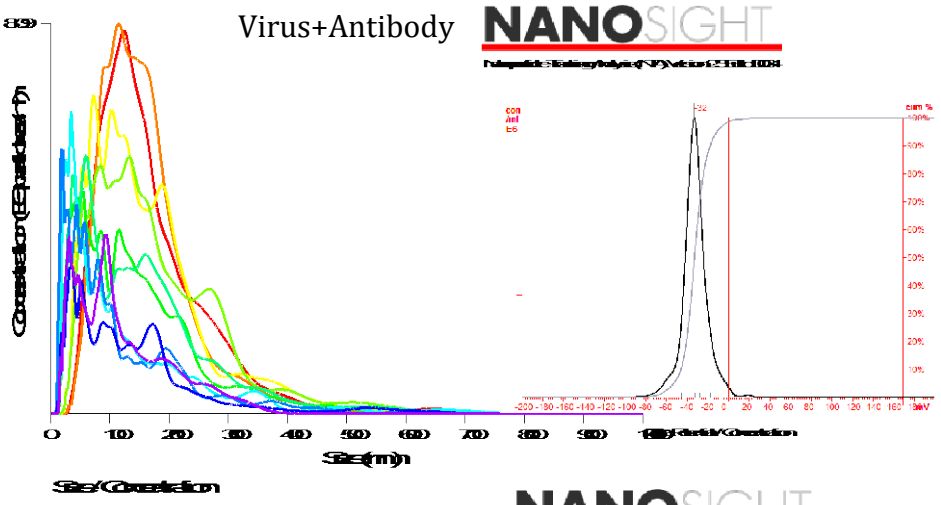
Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034
Zeta Potential / Concentration Particle Size / Concentration
Particle Size / Zeta Potential Mobility / Channel Depth
Operator: Sample: Date/Time of Capture: Video File: Analysis No: Date/Time of Report: Dispersant/Diluent: Concentration: Pre-treatment: Remarks:
jd
12 August 2014 15:25 'virus&antibody zeta potential10.avi' 003 12/08/2014
None
15:40:22
RESULTS: Size Distribution: Zeta Distribution: Cumulative Data (nm): Cumult. Data Zeta (mV): User Lines: Total Concentration: Selected Concentration: Fitted Curve : Completed Tracks: Drift Velocity:
ANALYSIS SETTINGS: Frames Processed: Frames per Second: Calibration:
Blur: Detection Threshold: Min Track Length: Min Expected Size: Temperature: Viscosity:
Mean: 184 nm, Mode: 134 nm, SD: 181 nm Mean: -32.2 mV, Mode: -32.2 mV, SD: 12.2 mV D10: 76, D50: 139, D90: 306, D70: 179 D10: -

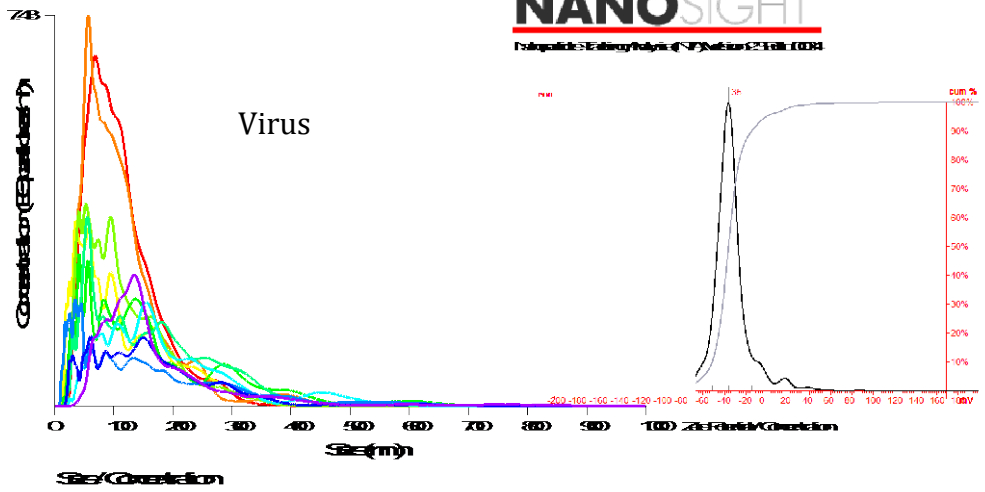
45.4, D50: -31.8, D90: -17.0, D70: -27.0 55 nm, 0 nm 15.78 particles / frame, 3.06E8 particles / ml 0.00 particles / frame, 0.00E8 particles / ml Mean: 0 nm, SD: 0 370 14521 nm/s
749 of 749 24.98 145 nm/pixel Auto
21 Multi Auto Auto 24.00 oC 0.91 cP
ZETA SETTINGS: Average EP velocity: Dielectric Constant: Applied Voltage:
ZETA RESULTS: Mobility: Zeta Potential: Average Current:
-12895 nm/s 80.000000 24.000000
-2.55E-008 S/m -32.72 mV 5.0 uA

ANALYSIS REPORT (Diffusion Coefficient Data)

Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034
Zeta Potential / Concentration Diffusion Coefficient / Count
Particle Size / Zeta Potential Mobility / Channel Depth
Operator: Sample: Date/Time of Capture: Video File: Analysis No: Date/Time of Report: Dispersant/Diluent: Concentration: Pre-treatment: Remarks:
jd
12 August 2014 15:25 'virus&antibody zeta potential10.avi' 002 12/08/2014
None
15:39:35
RESULTS: Diff .co. Dist.(E4 nm2/s): Size Distribution: Zeta Distribution: Cumult. Dat.(E4 nm2/s): Cumulative Data (nm): Cumult. Data Zeta (mV): User Lines: Total Concentration: Selected Concentration: Fitted Curve : Completed Tracks: Drift Velocity:
ANALYSIS SETTINGS: Frames Processed: Frames per Second: Calibration:
Blur: Detection Threshold: Min Track Length: Min Expected Size: Temperature: Viscosity:
Mean: 368, Mode: 338, SD: 184 Mean: 184 nm, Mode: 134 nm, SD: 181 nm Mean: -32.2 mV, Mode: -32.2 mV, SD: 12.2 mV D10: 155, D50: 342, D90: 621, D70: 424 D10: 76, D50: 139, D90: 306, D70: 179 D10: -45.4, D50: -31.8, D90: -17.0, D70: -27.0 596 nm, 0 nm 15.78 particles / frame, 3.06E8 particles / ml 0.00 particles / frame, 0.00E8 particles / ml Mean: 0 nm, SD: 0 370 14521 nm/s
749 of 749 24.98 145 nm/pixel Auto
21 Multi Auto Auto 24.00 oC 0.91 cP
ZETA SETTINGS: Average EP velocity: Dielectric Constant: Applied Voltage:
ZETA RESULTS: Mobility: Zeta Potential: Average Current:
-12895 nm/s 80.000000 24.000000
-2.55E-008 S/m -32.72 mV 5.0 uA

MCMV Size Distribution

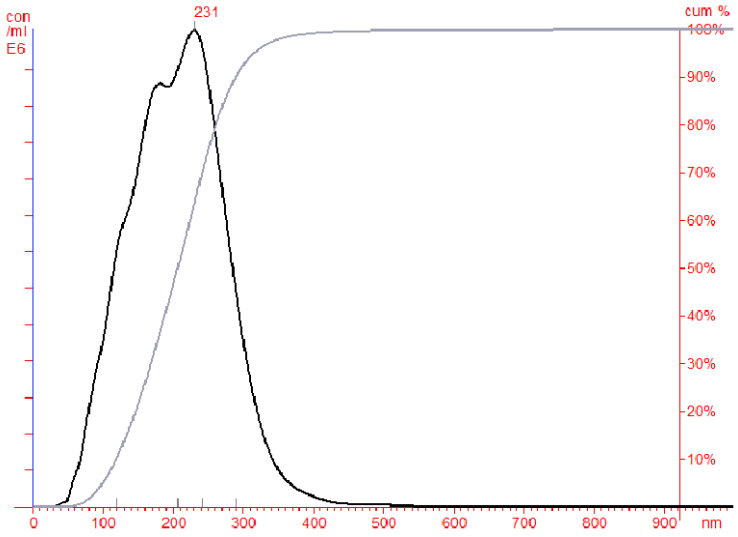




MHV68 Size Distribution

NANOSIGHT

Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034

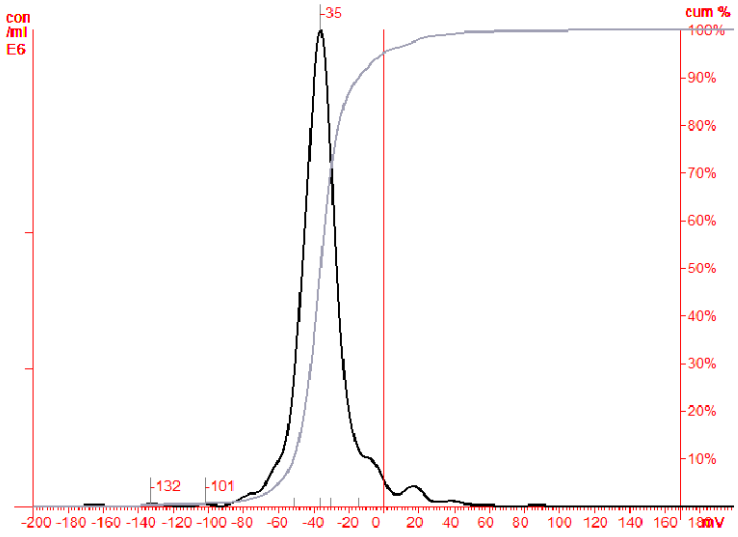


Particle Size / Concentration

MCMV Zeta Potential

NANOSIGHT

Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034

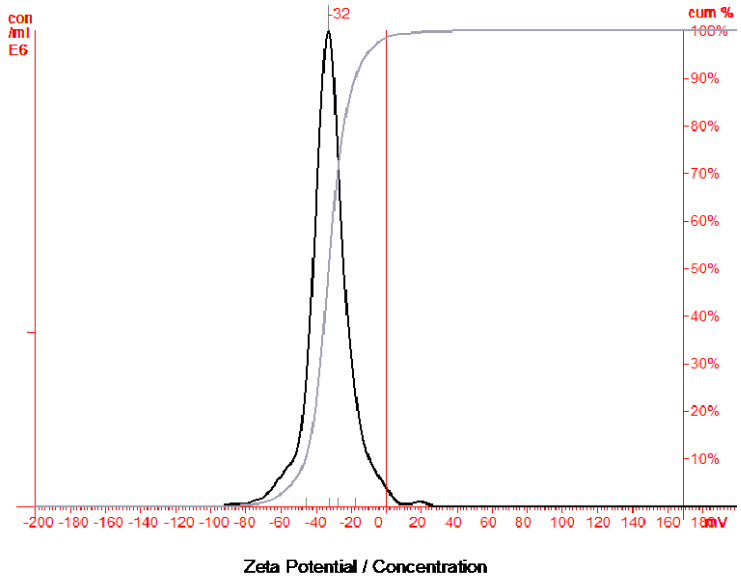


Zeta Potential / Concentration

MCMV with primary antibody (Ab 97.3) Zeta Potential

NANOSIGHT

Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034

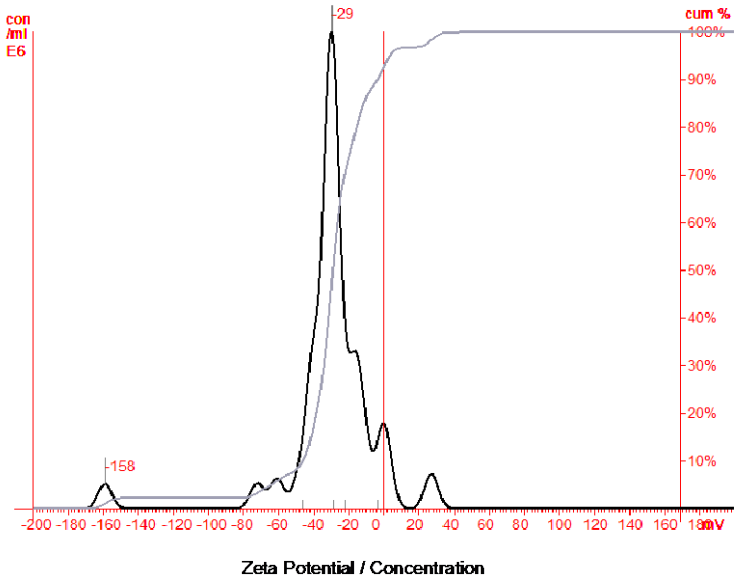


Zeta Potential / Concentration

MHV68 Zeta Potential

NANOSIGHT

Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 0034



Zeta Potential / Concentration

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[i] Binnicker, M. J.; Espy, M. E. (2013) Comparison of six real-time PCR assays for qualitative detection of cytomegalovirus in clinical specimens. J. Clin. Microbiol. 125(5): 3749-3752

[ii] de Vries, J. J. et al. (2012) Real-time PCR versus viral culture on urine as a gold standard in the diagnosis of congenital cytomegalovirus infection. J. Clin. Virol. 52(2): 167-170.

[iii] Monplaisir, S. B.; Leduc, N. P.; Onji, P. A.; Martineu, B.; Kurstak, E. (1972) Electron microscopy in the rapid diagnosis of cytomegalovirus: Ultrastructural observation and comparison of methods of diagnosis. J. Infect. Dis. 125(5): 533-538.