EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Alethia CMV DNA Amplification Assay

DECISION SUMMARY

A. DEN Number:

DEN180040

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the Alethia CMV Assay Test System.

C. Measurands:

Cytomegalovirus (CMV) DNA

D. Type of Test:

Qualitative loop-mediated isothermal DNA amplification (LAMP) technology

E. Applicant:

Meridian Bioscience, Inc.

F. Proprietary and Established Names:

Alethia CMV Assay Test System **G. Regulatory Information:**

1. Regulation section:
   21 CFR 866.3181

2. Classification:
   Class II (Special Controls)

3. Product code:
   QDZ

4. Panel:
   83 - Microbiology

H. Indications For Use:

1. Indications for use:
   The Alethia CMV Assay Test System includes separately provided test kits for the Alethia CMV DNA Amplification Assay and the Alethia CMV External Control Reagents.

   The Alethia CMV DNA Amplification Assay, performed on the Alethia instrument, is a qualitative, in vitro diagnostic test system for the direct detection of Cytomegalovirus (CMV) DNA in saliva samples from neonates younger than 21 days of age. The test is used as an aid in the diagnosis of congenital CMV infection. The results of this test should be used in conjunction with the results of other clinical findings.

   Flocked swabs should be used to collect saliva from neonates. The swab can be collected dry, without viral transport media (VTM), or placed in no more than 1 mL VTM.

   The Alethia CMV External Control Reagents are used as part of a routine quality control program to aid the user in detection of unexpected conditions that may lead to test errors. The external controls are intended for use with the Alethia CMV DNA Amplification Assay; the controls are not intended for use with other assays or systems.

2. Special conditions for use statement(s):

For *in vitro* diagnostic use only

Prescription use only

3. Special instrument requirements:

   Alethia Automated Isothermal Amplification and Detection System

I. Device Description:

The Alethia CMV Assay Test System, including the Alethia CMV DNA Amplification Assay and the Alethia CMV External Controls, is based on loop-mediated amplification (LAMP) technology. The assay targets a region of the Cytomegalovirus genome that is conserved across multiple CMV strains. The Alethia CMV target is a 194 base pair (bp) sequence of the Human herpesvirus 5 genome.

LAMP uses specially designed primers to provide for specific and continuous isothermal DNA amplification. A by-product of amplification is magnesium pyrophosphate, which forms a white precipitate leading to a turbid solution. Reaction solution absorbance characteristics are monitored by the Alethia™ instrument.

Changes in reaction solution turbidity created by precipitation of magnesium pyrophosphate indicate the presence of target DNA. The absence of target DNA results in no significant change in sample absorbance.

Reagents/Materials Provided:

The maximum number of tests obtained from this test kit is listed on the outer box.

1. Alethia CMV Test Device: Two-chambered device containing lyophilized amplification reagents (DNA polymerase, deoxynucleotide triphosphates) with Cytomegalovirus specific primers (TEST Chamber) and human mitochondrial DNA-specific primers (CONTROL Chamber).
2. Alethia CMV Buffer I: Lysis solution containing 0.2N sodium hydroxide and 1% Triton-X 100.
3. Alethia CMV Buffer II: Tris-buffer solution containing 0.09% azide as a preservative.

Materials Required but Not Provided:

Alethia CMV External Control Reagents

J. Standard/Guidance Document Referenced (if applicable):

EP05-A3: Evaluation of Precsion Performance of Quantitative Measurement Procedures.

EP12-A2: User Protocol for Evaluation of Qualitative Test Performance.

EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

EP07-A3: Interference Testing in Clinical Chemistry. FDA Recognition Number 7-127.

MM03-3rd Edition: Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline.

K. Test Principle:

The Alethia CMV DNA Amplificaiton assay contains one lyophilized amplification reagent bead in each of two chambers: a TEST chamber with Cytomegalovirus specific primers and a CONTROL chamber with human mitochondrial DNA-specific primers. Human mitochondrial DNA in saliva samples, and the human mitochondrial DNA-specific primers in the Test Device CONTROL chamber, functions as the Internal Control for the assay. During specimen preparation, human mitochondrial DNA is liberated with the Cytomegalovirus DNA to allow for parallel processing of target DNA and Control DNA through amplification and detection. The Internal Control monitors DNA amplification inhibition, assay reagent performance, and sample processing effectiveness. The Control target must be amplified and detected in the final reaction or the test is considered invalid and results not reported.

The Alethia instrument monitors changes in absorbance characteristics by measuring transmission of light through the Test and Control reaction solutions. Light transmission is checked at the assay Run Start (Signalinitial, S_i) and at the assay Run End (Signalfinal, S_f). The Alethia instrument calculates the change in light transmission between Run End and Run Start (S_f:S_i) and compares the ratio to a fixed cut-off value.

Fixed cut-off values for the TEST chamber are used to report sample assay results. TEST chamber Sf:Si ratios less than 82% are reported as 'POSITIVE'. TEST chamber Sf:Si ratios greater than or equal to 82% are reported as 'NEGATIVE'. Numerical values are not reported.

Fixed cut-off values for the CONTROL chamber are used to determine validity. CONTROL chamber S_f:S_i ratios less than 90% are considered valid and allow for reporting of TEST chamber results (POSITIVE or NEGATIVE). CONTROL chamber Sf:Si ratios greater than or equal to 90% are considered invalid and prevent reporting of TEST chamber results. Invalid CONTROL chamber reactions are reported as 'INVALID'. Numerical values are not reported.

More stringent cut-off criteria are applied to the CONTROL chamber reaction to ensure amplification is not inhibited, reagents are performing as intended, and that sample processing was performed appropriately.

Interpretation Of Results

Sample ID	Reported Result	Interpretation
Patient Specimen	POSITIVE	Cytomegalovirus target DNA was detected
	NEGATIVE	No Cytomegalovirus DNA detected
	INVALID*	No reportable result. The test should be repeated: Samples can be retested from the Buffer II preparation within 3 hours of sample preparation.
Positive Control	POSITIVE	Valid positive control result. Reagents active at time of use; Alethia instrument performing correctly.
	NEGATIVE	Incorrect control result. Repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services at 1-800-343-3858 (US) or your local distributor.
	INVALID	No reportable result. Run must be repeated. Improper sample preparation, reagent failure, instrument failure or internal control failure.
Negative Control	POSITIVE	Incorrect control result. Repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services at 1-800-343-3858 (US) or your local distributor.

	NEGATIVE	Valid negative control result. Reagents active at time of use, Alethia instrument performing correctly.
	INVALID	No reportable result. Run must be repeated. Improper sample preparation, reagent failure, instrument failure or internal control failure.
EMPTY WELL	NONE	No Alethia Test Device in the Alethia instrument well. OR The Alethia Test Device present is compromised due to sample preparation failure, dirty device or improperly seated device. Repeat testing on the sample.

*Interpretation Notes

- Invalid results may occur as a result of inhibitory specimens, improper sample preparation, reagent failure, no human DNA in the specimen, instrument failure, or internal control failure.
- For VTM specimens, retesting may be performed with the original specimen if sufficient volume remains (see Specimen Collection and Preparation section for additional guidance).

L. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Panels of two sample types, dry swab and VTM, were supplied to three laboratories for this reproducibility study. The panels included contrived CMV samples manufactured as moderate-positive, low-positive samples, and high-negative samples (30 replicates per site for each sample). The panel also included one true negative sample (10 replicates per site). Positive and Negative Controls were tested with each panel also (10 replicates per site). Testing was performed by different operators at each site on the same day for five days. Three lots of Alethia CMV kits and six Alethia instruments were used in this study. Mean values, repeatability, between-operator, between-day, and between-site components of variance for numeric values of S_f:S_i ratios, percent of positive and negative results are provided in the tables below for each sample type tested in the reproducibility study.

Reproducibility for Dry Swab Samples

Saliva Samples on Dry Swabs														
			Repeatability		Between-operators[1]		Between-day		Between-site[2]		Reproducibility		%Pos. results	%Neg results
	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV		
Moderate Positive	90	62.26	2.93	4.7%	1.69	2.7%	0.00	0.0%	0.91	1.5%	3.50	5.6%	100%	0%
Low Positive	90	62.38	4.44	7.1%	1.96	3.1%	1.11	1.8%	0.00	0.0%	4.98	8.0%	98.9%	1.1%
High Negative	90	99.05	6.68	6.7%	0.97	1.0%	0.00	0.0%	0.23	0.2%	6.76	6.8%	3.3%	96.7%
True	30	100.13	0.69	0.7%	n/a	n/a	0.31	0.3%	0.40	0.4%	0.86	0.9%	0%	100%

Negative[3]														
Positive Control[3]	30	61.84	2.72	4.4%	n/a	n/a	0.00	0.0%	1.11	1.8%	2.94	4.8%	100%	0%
Negative Control[3]	30	100.23	2.06	2.1%	n/a	n/a	0.17	0.2%	0.22	0.2%	2.08	2.1%	0%	100%

[1] Includes between-operator and between-instrument components

[2] Includes between-site and between-kit lot components

[3] Samples were run at each site for 5 days with 2 runs per day and 1 replicate per run.

Reproducibility for VTM samples

Saliva Samples in VTM														
			Repeatability		Between-operators[1]		Between-day		Between-site[2]		Reproducibility		%Pos results	%Neg results
	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV		
Moderate Positive	90	61.99	3.18	5.1%	1.02	1.6%	0.00	0.0%	1.20	1.9%	3.55	5.7%	100%	0%
Low Positive	90	61.71	2.37	3.8%	1.43	2.3%	0.00	0.0%	0.67	1.1%	2.85	4.6%	100%	0%
High Negative	90	100.11	4.82	4.8%	0.00	0.0%	0.74	0.7%	0.00	0.0%	4.88	4.9%	98.9%	1.1%
True Negative[3]	30	99.20	7.71	7.8%	N/A	N/A	0.00	0.0%	0.00	0.0%	7.71	7.8%	96.7%	3.3%
Positive Control[3]	30	62.22	2.39	3.8%	N/A	N/A	0.00	0.0%	0.49	0.8%	2.44	3.9%	100%	0%
Negative Control[3]	30	100.30	2.02	2.0%	N/A	N/A	0.00	0.0%	0.12	0.1%	2.02	2.0%	0%	100%

[1] Includes between-operator and between-instrument components
[2] Includes between-site and between-kit lot components
[3] Samples were run at each site for 5 days with 2 runs per day and 1 replicate per run.

Within-Laboratory Precision Study

Panels of two sample types, dry swab and VTM, were tested at one site (internal) over 6 days. The panels included contrived CMV samples manufactured as moderate-positive, low-positive (around 2X LoD), and high-negative samples. The panel also included one true negative sample, Positive and Negative Controls. Three kit lots were used during the study, one lot per day of testing. Each kit lot was tested twice over the 6-day testing period. Mean values, repeatability, between-operator, between-day, and between-kit lot components of variance for numeric values of S_f:S_i ratios, percent of positive and negative results are provided in the tables below for each sample type tested in the precision study.

Within-Laboratory Precision for Dry Swab Samples

Saliva Samples on Dry Swabs														
			Repeatability[1]		Between-operators[1]		Between-day		Between-kit lot		Reproducibility		%Pos results	%Neg results
	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV		
Moderate Positive	48	60.29	1.99	3.3%	0.00	0.0%	0.00	0.0%	0.74	1.2%	2.12	3.5%	100%	0%
Low Positive	48	60.23	1.70	2.8%	0.62	1.0%	0.00	0.0%	0.74	1.2%	1.95	3.2%	100%	0%
High Negative	48	98.26	6.46	6.6%	4.36	4.4%	1.42	1.4%	0.80	0.8%	7.96	8.1%	4.2%	95.8%
True Negative	48	99.94	0.61	0.6%	0.00	0.0%	0.12	0.1%	0.14	0.1%	0.64	0.6%	0%	100%
Positive Control	24	60.57	1.96	3.2%	0.00	0.0%	1.47	2.4%	0.00	0.0%	2.45	4.0%	100%	0%
Negative Control	24	99.73	0.29	0.3%	0.00	0.0%	0.18	0.2%	0.16	0.2%	0.38	0.4%	0%	100%

[1] Includes between-operator and between-instrument components

Within-Laboratory Precision for VTM Samples

Saliva Samples in VTM														
			Repeatability		Between-operators[1]		Between-day		Between-kit lot		Reproducibility		%Pos results	%Neg results
	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV		
Moderate Positive	48	60.62	1.36	2.2%	0.91	1.5%	0.00	0.0%	0.77	1.3%	1.81	3.0%	100%	0%
Low Positive	48	60.55	1.67	2.8%	0.89	1.5%	0.72	1.2%	0.00	0.0%	2.02	3.3%	100%	0%
High Negative	48	99.91	0.64	0.6%	0.00	0.0%	0.22	0.2%	0.00	0.0%	0.68	0.7%	0%	100%
True Negative	48	100.00	0.84	0.8%	0.28	0.3%	0.00	0.0%	0.00	0.0%	0.89	0.9%	0%	100%
Positive Control	24	60.28	2.12	3.5%	0.00	0.0%	1.09	1.8%	0.00	0.0%	2.38	4.0%	100%	0%
Negative Control	24	100.29	1.53	1.5%	0.28	0.3%	0.00	0.0%	0.00	0.0%	1.56	1.6%	0%	100%

[1] Includes between-operator and between-instrument components

b. Linearity/assay reportable range:

Not Applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The stability of the Alethia CMV Assay Test System, including the Alethia CMV External Controls was evaluated in real-time studies, to support the proposed expiration dating period of 15 months for the Alethia CMV DNA Amplification Assay and the Alethia CMV External Controls. Three lots of Assay and External Control reagent components were used and the results obtained for the Alethia CMV DNA Amplification Assay and the Alethia CMV External Controls support expiration dating periods of 15 months for both the assay reagents and the external controls.

d. Detection limit:

The analytical sensitivity, described as Limit of Detection (LoD), is the concentration at which the Alethia CMV Assay Test System has positive results at least 95% of the time. The LoD of the Alethia CMV assay was determined for Cytomegalovirus strain Merlin in a negative sample matrix. Additionally saliva samples collected from CMV negative healthy adults were tested and confirmed negative by the the Alethia CMV assay. Three kit lots of Alethia CMV assay reagents and eight Alethia instruments were used. A minimum of 6 dilutions with 20 replicates were tested for each lot. LoD was determined using Probit analysis. LoD was determined separately for the two sample types, dry swab and swab in VTM. LoD concentrations for each sample type are summarized below.

Sample Type	LoD (Copies/mL)
Dry Swab[1]	1,025
Swab in VTM[2]	15,686

[1]The CMV cp/mL concentration in saliva on the swab for dry swab samples is calculated by multiplying the CMV concentration in Buffer I by a factor of 4.75 (the 4.75-fold dilution occurs when 0.080 mL of saliva on the swab is added to 0.3 mL of Buffer I [380÷80 = 4.75]; 100% transfer is assumed).

[2]The CMV cp/mL concentration in saliva on the swab for VTM samples is calculated by multiplying the CMV concentration in VTM by a factor of 13.5 (the 13.5-fold dilution occurs when 0.080 mL of saliva on the swab is placed in 1 mL of VTM [1080÷80 = 13.5]; 100% transfer is assumed).

Assay Inclusivity

The inclusivivty of the Alethia Alethia CMV Assay Test System was evaluated by testing samples containing 3 additional CMV strains other than CMV strain Merlin. These strains include Toledo, Towne, and AD-169. Quantified strains were diluted in simulated negative clinical matrix to approximately 2-3X LoD for both VTM and dry swab and

tested in triplicate. All strains tested for both sample types produced positive results with Alethia CMV.

CMV Strain Tested	Dry Swab Samples		VTM Swab Samples	
	Test concentration (copies/mL)	Test Results (n_{pos}/n_{total})	Test concentration (copies/mL)	Test Results (n_{pos}/n_{total})
AD-169	2,493	3/3	45,077	3/3
Toledo	2,493	3/3	45,077	3/3
Towne	2,493	3/3	45,077	3/3

e. Analytical specificity:

Cross Reactivity Studies

Cross-reactivity studies were performed with a panel of 40 microorganisms and human genomic DNA, each diluted in dry swab simulated negative clinical matrix. Microorganims selected include those with genetic similarity to CMV and those likely to be present in the oral cavity of neonates. Microorganims were diluted in dry swab simulated negative clinical matrix to the indicated concentration (see table below) and tested in triplicate. No cross-reactivity with the Alethia CMV assay was observed.

Microorganism	Test concentration	Microorganism	Test concentration
Acinetobacter baumannii	$1.2x10^7$ CFU/mL	Adenovirus	$3.80x10^5$ $TCID_{50}$/mL
Actinomyces odontolyticus	$1.2x10^7$ CFU/mL	Coronavirus	$2.19x10^5$ $TCID_{50}$/mL
Bordetella pertussis	$1.2x10^7$ CFU/mL	Coxsackievirus	$4.07x10^6$ $TCID_{50}$/mL
Candida albicans	$1.2x10^7$ CFU/mL	Enterovirus 71	$1.26x10^5$ $TCID_{50}$/mL
Escherichia coli ATCC 35218	$1.2x10^7$ CFU/mL	Epstein Barr Virus	$3.39x10^8$ cp/mL
Fusobacterium nucleatum	$1.2x10^7$ CFU/mL	Herpes Simplex Virus 1	$9.5x10^5$ $TCID_{50}$/mL
Haemophilus influenzae	$1.2x10^7$ CFU/mL	Herpes Simplex Virus 2	$1.3x10^5$ $TCID_{50}$/mL
Haemophilus parainfluenzae	$1.2x10^7$ CFU/mL	Human herpesvirus 6B	$6.16x10^7$ cp/mL
Moraxella catarrhalis	$1.2x10^7$ CFU/mL	Human herpesvirus 7	$3.80x10^5$ $TCID_{50}$/mL
Mycoplasma pneumoniae	$3.70x10^7$ CCU/mL	Human herpesvirus 8	$2.13x10^8$ cp/mL

Porphyromonas gingivalis	$1.2x10^7$ CFU/mL	Human metapneumovirus	$6.61x10^5$ $TCID_{50}$/mL
Pseudomonas aeruginosa	$1.2x10^7$ CFU/mL	Influenza A	$3.80x10^5$ $TCID_{50}$/mL
Staphylococcus aureus	$1.2x10^7$ CFU/mL	Influenza B	$4.57x10^5$ $TCID_{50}$/mL
Staphylococcus epidermidis	$1.2x10^7$ CFU/mL	Parainfluenza virus 1	$1.95x10^6$ $TCID_{50}$/mL
Streptococcus agalactiae (GBS)	$1.2x10^7$ CFU/mL	Parainfluenza virus 2	$5.89x10^6$ $TCID_{50}$/mL
Streptococcus anginosus (Group F)	$1.2x10^7$ CFU/mL	Parainfluenza virus 3	$2.19x10^5$ $TCID_{50}$/mL
Streptococcus mitis	$1.2x10^7$ CFU/mL	Respiratory syncytial virus A	$3.2x10^5$ $TCID_{50}$/mL
Streptococcus oralis	$1.2x10^7$ CFU/mL	Respiratory syncytial virus B	$4.6x10^5$ $TCID_{50}$/mL
Streptococcus salivarius	$1.2x10^7$ CFU/mL	Rhinovirus	$1.51x10^5$ $TCID_{50}$/mL
Streptococcus sanguinis	$1.2x10^7$ CFU/mL	Varicella Zoster Virus	$3.36x10^9$ cp/mL
Human Genomic DNA	$6.18x10^6$ cp/mL	n/a	n/a

Microbial Interference

Microbial interference studies were performed with a panel of 40 microorganisms and human genomic DNA, each diluted in dry swab simulated positive clinical matrix. Each sample tested contained CMV at a concentration of 3X LoD and microorganism or human genomic DNA at the test concentration indicated above (see table in Cross-reactivity section for microorganisms tested and final test concentrations). Once prepared, each sample was tested in triplicate. No microbial interference with the Alethia CMV DNA Amplification Assay was observed (i.e., all microorganisms diluted in dry swab simulated positive clinical matrix produced 3/3 positive test results with the Alethia CMV assay).

Interfering Substances

Interference testing was performed in the presence of chemical and biological substances introduced directly into contrived CMV low positive (3X LoD) and negative samples. Two unique positive samples and one negative sample were tested in triplicate. The substances tested, the concentrations evaluated, and test results are shown in the following table. No interference was observed with the following substances with the Alethia CMV Assay Test System at the specified test concentrations (i.e., all positive replicates tested produced positive Alethia CMV test results and all negative replicates tested produced negative Alethia CMV test results).

Substance Tested	Test concentration	Test Results ($n_{detected}/n_{total}$)		
		CMV Negative Sample	CMV Positive Sample #1	CMV Positive Sample #2
Infants' Pain & Fever (Acetaminophen)	0.2 mg/mL	0/3	3/3	3/3
Acetylsalicylic acid	0.65 mg/mL	0/3	3/3	3/3
Caffeine	0.06 mg/mL	0/3	3/3	3/3
Enfamil™ Fer-In-Sol® (Ferrous Sulfate)	1.5 mg/mL	0/3	3/3	3/3
Enfamil Premium® Infant Formula Newborn	10% v/v	0/3	3/3	3/3
Infants' Mylicon® Gas Relief (Simethicone)	2 mg/0.3 mL	0/3	3/3	3/3
Gaviscon® infant (Sodium alginate)	1.2 mg/mL	0/3	3/3	3/3
Magnesium alginate	0.467 mg/mL	0/3	3/3	3/3
Infants' Ibuprofen	0.5 mg/mL	0/3	3/3	3/3
Enfamil™ Poly-Vi-Sol®	8% v/v	0/3	3/3	3/3
Little Remedies® Saline spray/drops	10% v/v	0/3	3/3	3/3
Methadone	0.002 mg/mL	0/3	3/3	3/3
Morphine sulphate	0.0005 mg/mL	0/3	3/3	3/3
Nystatin	1000 U/mL	0/3	3/3	3/3
Prednisone	0.0003	0/3	3/3	3/3

	mg/mL			
Casein	10 mg/mL	0/3	3/3	3/3
Mucin*	25 mg/mL	0/3	3/3	3/3
White blood cells	10% v/v	0/3	3/3	3/3
Whole blood	10% v/v	0/3	3/3	3/3

*When mucin was tested at a concentratin of 50 mg/mL, the negative sample tested negative in 3/3 replicates and CMV positive sample #1 tested positive in 3/3 replicates. CMV positive sample #2 produced two invalid test results, and one positive test result for the three replicates tested. The mucin concentration in the samples was reduced to 25 mg/mL and testing was repeated, producing the results shown in the table. No interference was observed with a mucin concentration of 25 mg/mL. A limitation was added to the device labeling to mitigate this finding.

f. Sample Stability:

Studies were performed to assess both sample stability and sample freeze-thaw stability.

For sample stability, a sample panel was prepared by spiking quantified CMV virus into simulated dry swab clinical matrix, BD UVT, and Puritan UniTranz-RT transport media. Negative samples and positive samples (3X LoD) of each type were prepared. Each (b)(4)

Results support that saliva swabs may be stored at room temperature (19-30 ºC), refrigerated (2-8 ºC), or frozen (≤-20 ºC) after collection and during transportation to the laboratory. Samples should be tested as soon as possible, but may be stored for up to 48 hours at 19-30 ºC , or 7 days refrigerated (2-8 ºC) prior to testing. Samples that will not be tested within 7 days should be frozen immediately at ≤-20 ºC .

An identical sample panel was stored frozen (b) (4) and subjected to multiple freeze-thaw cycles. Testing in triplicate s performed after each cycle. The data showed that all samples were stable afte (b) (4) reeze-thaw cycles. Results support that saliva samples on dry swabs or in VTM may be ozen and thawed up to 2 times as part of storage at ≤ -20 ºC prior to testing with the Alethia CMV assay.

e. Carryover Contamination:

Carryover Contamination Studies

A series of alternating true negative and high viral load samples (30X LoD) were processed using the Alethia CMV Assay Test System. A total of 30 high positive and 30 true negative samples were processed. Six runs were conducted with 10 samples each (5 high positive and 5 true negative samples). All samples tested produced expected results

with all positive samples producing positive Alethia CMV test results, and all negative samples producing negative Alethia CMV test results. No carryover was observed. Workflow studies revealed that foam present after vortexing samples presents an aerosol contamination risk (i.e., when bubbles break), and/or a transfer contamination risk if gloves come in contact with liquids. It is recommended that good laboratory practices be adhered to when performing the Alethia CMV assay; gloves should be changed frequently.

f. Assay cut-off:

Assay cutoff for numerical values of S_f:S_i ratios is 82%.

2. Comparison studies:

a. Method comparison with predicate device:
Not Applicable

b. Matrix comparison:
The viral transport media (VTM) for saliva samples include BD UVT and Puritan UniTranz-RT transport medium. To demonstrate equivalency between the different types of transport media, a matrix equivalency study was performed. Negative and contrived CMV positive (approximately 4X LoD) samples were prepared in each media type and tested in triplicate. For negative samples, all Alethia CMV test results in each media type were negative; and for spiked positive CMV samples, all Alethia CMV test results in each media type were .Results support comparable performance of BD UVT and Puritan UniTranz-RT transport medium.

3. Clinical studies:

a. Clinical Sensitivity:
Not Applicable

b. Clinical specificity:
Not Applicable

c. Other clinical supportive data (when a. and b. are not applicable):

The Alethia CMV Assay Test System was evaluated from August 2017 to March 2018 at seven clinical study sites representing geographically distinct regions throughout the United States, Canada, Europe, and Australia. One-thousand five-hundred and fourteen (1,514) specimens were prospectively collected and tested with Alethia CMV assay (forty-seven of these specimens were frozen at ≤ -20ºC after collection and tested later). The saliva swabs were collected at least one hour after breastfeeding.

For estimation of composite reference method (CRM) positive percent agreement, thirty-four (34) archived specimens were also tested by Alethia CMV assay. The archived samples were de-identified samples previously evaluated from prospective clinical studies and found to have CMV infection. These samples were collected from infants less than 21 days of age and stored at -80 ºC after the completion of the initial testing. The status of breastfeeding time in relation to time of saliva samples collected was not available.

All samples were tested with Alethia CMV Assay Test System at the study sites, then shipped to Meridian Bioscience, Inc. for CRM testing. The CRM consisted of two manufacturer-developed and validated PCR assays. Samples positive by either PCR assay were further tested by bidirectional sequencing (BDS). Samples were considered positive when bi-directional sequencing results from either comparator PCR assay confirmed the presence of CMV DNA. Samples were considered negative when neither of the comparator PCR assays produced amplicon at the end of the 40-cycle amplification or BDS was negative.

Composite Reference Method

PCR #1	PCR #2	Bidirectional Sequencing	Composite Reference Method
+	+	+	+
+	+	-	-
+	-	+	+
+	-	-	-
-	+	+	+
-	+	-	-
-	-	n/a	-

Performance of the Alethia CMV Assay Test System was based on evaluation of 1,514 prospectively collected samples. From the prospectively collected samples; five samples were positive by CRM, 1,475 samples were negative by CRM (prevalence was 0.3% (5/1,514)), and 34 samples were invalid by the CRM and removed from the analysis (one of the samples was invalid by the Alethis CMV Assay). Among archived preselected positive samples, there were 34 samples positive by CRM.

Alethia CMV DNA Amplification Assay Performance

1. Prospective Study results:

	Composite Reference Method		
Alethia CMV	Positive	Negative	Total
positive	5	3	8
negative	0	1472	1472
Total	5	1475	1480

	Estimate	95% CI
Positive Percent Agreement	100% (5/5)	56.7%; 100%
Negative Percent Agreement	99.8% (1,472/1,475)	99.4%; 99.9%

2. Preselected Positive archived sample study:

	Composite Reference Method		
Alethia CMV	Positive	Negative	Total
positive	34	0	34
negative	0	0	0
Total	34	0	34

	Estimate	95% CI
Positive Percent Agreement	100% (34/34)	89.9%; 100%
Negative Percent Agreement	Not Applicable	

3. Combined Positive and Negative percent agreements:
   The positive samples from the prospective study (5) and archived pre-selected positive sample study (34) both demonstrated 100% agreement with the composite reference method and were thus presented combined in the table below.

	Estimate	95% CI
Positive Percent Agreement	100% (39/39)	91.0%; 100%
Negative Percent Agreement	99.8% (1,472/1,475)	99.4%; 99.9%

Invalid Results by Alethia CMV Assay Test System

Twenty-seven (27) samples produced invalid results during initial testing with Alethia CMV assay. The rate of initial invalid results was 1.7% (27/1,548) and ranged from 0.7% to 4.1% at different clinical sites. After Alethia CMV assay re-testing, 26 samples had valid results. The rate of final invalid results was 0.06% (1/1,548) with 95% CI: 0.01%; 0.37%.

Additional Clinical Study Description

Alethia CMV Assay Performance by Age (prospective and preselected positive sample types combined)

Age Group	Samples Positive by CRM			Samples Negative by CRM		
	Positive Percent Agreement	Alethia/ CRM	95% CI	Negative Percent Agreement	Alethia/CRM	95% CI
0-3 days	100%	15/15	79.6%; 100%	99.9%	1,351/1,353	99.5%; 100%
4-7 days	n/a	n/a	n/a	99.1%	108/109	95.0%; 99.8%
8-10 days	n/a	n/a	n/a	100%	4/4	51.0%; 100%
11-13 days	n/a	n/a	n/a	100%	2/2	34.2%; 100%
14-17 days	100%	1/1	20.7%; 100%	100%	6/6	61.0%; 100%
18-20 days	n/a	n/a	n/a	100%	1/1	20.7%; 100%
Known <21 days	100%	23/23	85.7%; 100%	n/a	n/a	n/a
Clinical Site Totals	100%	39/39	91.0%; 100%	99.8%	1,472/1,475	99.4%; 99.9%

Alethia CMV Assay Sex-Specific Performance (prospective and preselected positive sample types combined)

Sex	Samples Positive by CRM	Samples Negative by CRM

	Positive Percent Agreement	Alethia/CRM	95% CI	Negative Percent Agreement	Alethia/CRM	95% CI
Female	100%	5/5	56.6%; 100%	99.7%	708/710	99.0%; 99.9%
Male	100%	11/11	74.1%; 100%	99.9%	763/764	99.3%; 100%
Unknown	100%	23/23	85.7%; 100%	100%	1/1	20.7%; 100%
Clinical Site Totals	100%	39/39	91.0%; 100%	99.8%	1,472/1,475	99.4%; 99.9%

4. Clinical cut-off:

   Not Applicable

5. Expected values/Reference range:

   The observed prevalence of CMV from prospectively collected saliva specimens during this study was 0.3% (5/1,514). The results stratified by age and sex is provided below.

Sex/Age Group	Samples positive by CRM	Prevalence in Overall Prospectively Tested Population
Female		
14-17 days	1	0.1%
Male		
0-3 days	4	0.3%
Total	5	0.3%

M. Instrument Name:

Alethia Automated Isothermal Amplification and Detection System

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No _____X___

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X_____ or No _____

The current assay and associated instrument are being (b) (4) with a name change from ***illumipro-10*** to Alethia. Therefore, the name of the CMV test kit will be Alethia CMV Assay Test System performed on the Alethia Automated Isothermal Amplification and Detection System. According to Meridian Bioscience Inc., the (b) (4)

In the Alethia CMV Assay Test System submission, software updates and hardware changes for the Alethia instrument were reported as compared to the previously cleared ***illumipro-10*** instrument in K160829. Changes to the test system included the following:

- Instrument and Assay Tradenames
- External Barcode scanner
- LIS Module
- Bug fixes

Meridian reported that no new hazards were identified since the software and hardware changes were made to the test system. FDA has previously reviewed the applicant's Hazard Analysis and software development processes in K123423.

The software revision for the Alethia instrument will be (b) (4) (b)(4) to (b) (4) . A new assay packet, (b) (4) which adds the ability to run the Alethia CMV Assay (addition to the existing cleared assays), will be installed on all new Alethia instruments. In addition, Meridian stated that the new CMV assay packet will also be made available to existing ***illumigene illumipro-10*** instrument users.

3. Specimen Identification:

Sample Identification information can be entered directly using the keypad, the onboard barcode scanner, optional external keyboard, or optional handheld barcode scanner. Please refer to K160829 for additional details related to sample identification.

4. Specimen Sampling and Handling:

The Alethia CMV Assay Test System includes two separate CMV Buffers for sample processing and preparation. Saliva swab specimens are first treated with CMV Buffer I (lysis buffer); viral capsids are disrupted and nucleic acids are released. The lysate is then

added to CMV Buffer II (reaction buffer). Incubation, loop-mediated isothermal amplification, and detection are automated using the Alethia instrument.

5. Calibration:

   Please refer to K160829 for details.

6. Quality Control:

   The Alethia CMV External Control Reagents contains Positive and Negative Control Reagents for use with the Alethia CMV DNA Amplification Assay. External controls are used as part of a routine quality control program to aid the user in detection of unexpected conditions that may lead to test errors. When unacceptable quality control test results are obtained, all test results should be considered invalid. QC test failures are an indication that either reagents, test environment or operator performance have changed

   The Alethia CMV Positive Control: Tris-buffered solution with plasmid containing DNA inserts (Cytomegalovirus and human mitochondrial DNA inserts) and azide (0.09%) as a preservative.The Alethia CMV Negative Control: Tris-buffered solution with plasmid containing human mitochondrial DNA insert and azide (0.09%) as a preservative.

   The External Positive Control is processed using the same DNA extraction methodology as the clinical samples. The performance of External Positive and Negative Controls were validated as part of the clinical study.

   The Positive Control is manufactured in an aqueous solution matrix. Although specimen matrix interference has not been observed with this assay, the aqueous matrix of the controls may not adequately control for specimen matrix effects. The user is referred to the Clinical and Laboratory Standards Institute guideline EP14-A3 (Evaluation of Commutability of Processed Samples: Approved Guideline – Third Edition, August 2014) in the package insert if the user wishes to supply controls in the sample matrix.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None

P. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

Q. Identified Risks to Health and Mitigation Measures:

Identified Risks to Health	Mitigation Measures

Identified Risks to Health	Mitigation Measures
Risk of false results	General controls and Special Controls (1) and (2)
Failure to correctly interpret test results	General Controls and Special Controls 1(i), (iv), (v), (vi)
Failure to correctly operate the device	General Controls and Special Controls (1) and (2)

R. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

There are no other FDA cleared or approved assays currently on the market for the detection of CMV from the saliva of infants. With this assay, infants can be diagnosed with congenital CMV, and appropriate management can be started to mitigate the sequelae of congenital CMV disease, including potentially improving long-term audiologic and neurodevelopmental outcomes. While the clinical performance of the Alethia CMV assay suggest that patients will benefit from the assay, the low prevalence of congenital CMV in the prospective study and incomplete clinical data in the retrospective trial are sources of potential uncertainty. However, the likely benefits from use of this assay as an aid in diagnosing congenital CMV outweigh the potential uncertainty associated with the clinical trial.

Summary of the Assessment of Risk

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results and failure to correctly operate the instrument. Risks of false positives include unnecessary additional evaluation, such as neurological imaging, ophthalmological exam or other diagnostic testing, and unnecessary antiviral therapy. Prescribed treatment can be associated with the development of adverse events, particularly bone marrow suppression. Other potential risks could include treatment of mildly symptomatic or asymptomatic patients where it is unknown if the probable benefits outweigh the potential risks of antiviral therapy. The performance of the assay in the clinical trial suggests that false positive results will be uncommon, although several states are currently considering universal screening programs for congenital CMV, and there may be meaningful absolute numbers of false positives when large numbers of infants undergo routine screening. However, clinical practice for congenital CMV, including other diagnostic tests to diagnose congenital CMV, will further mitigate the potential risks of false positives. False negatives did not occur in prospective or retrospective specimens, and percent positive agreement was 100% for prospective and retrospective specimens, although the numbers of positive results in the prospective trial was very small, reflecting the low prevalence of congenital CMV. It is unknown if there are patient subgroups or clinical cofactors which may be more likely to be associated with false negative results. The theoretical risk of a false negative result would be a missed opportunity to treat symptomatic patients, which has been associated with improved neurodevelopmental outcomes and improved audiologic outcomes, although the sensitivity of the assay suggests that false negatives will be uncommon.

Summary of the Assessment of Benefit-Risk

The probable clinical benefits outweigh the potential risks of the proposed assay in light of the special controls established for this device and general controls. The Alethia CMV Assay Test System is the first assay authorized for marketing by FDA for the qualitative detection of CMV DNA directly from the saliva of infants younger than 21 days of age. The required special controls will ensure that errors will be uncommon, and the proposed assay labeling will facilitate accurate assay implementation and interpretation of results. The clinical performance observed in the prospective and retrospective clinical trials suggests that errors will be uncommon and that the Alethia CMV assay may provide substantial benefits to infants as an accurate and sensitive aid in the diagnosis of congenital CMV.

S. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

T. Conclusion:

The information provided in this de novo submission is sufficient to classify this device into class II under regulation 21 CFR 866.3181. FDA believes that thespecial controls, in combination with the general controls, provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: QDZ

Device Type: Cytomegalovirus nucleic acid detection device for congenital cytomegalovirus infection

Class: II (special controls)

Regulation: 21 CFR 866.3181

(a) Identification. A cytomegalovirus nucleic acid detection device for congenital cytomegalovirus infection is an in vitro diagnostic device intended for the qualitative detection of cytomegalovirus DNA in clinical samples from newborn babies to aid in the diagnosis of congenital cytomegalovirus infection. Negative results do not preclude infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive results should be interpreted with consideration of other clinical information and laboratory findings and should not be used as the sole basis for treatment or other patient management decisions.

(b) Classification: Class II (special controls). The special controls for this device are:

(1) The 21 CFR 809.10 labeling must include:

(i) An intended use with a detailed description of what the device detects, the type of results provided to the user, the clinical indications appropriate for test use, and the specific population(s) to be tested.

(ii) A detailed device description, including all device components, instrument requirements, ancillary reagents required but not provided, and an explanation of the methodology, including all pre-analytical methods for specimen processing.

(iii) Performance characteristics from analytical and clinical studies required under paragraphs (b)(2)(ii) and (b)(2)(iii) of this section.

(iv) A detailed explanation of the interpretation of results and criteria for validity of results.

(v) A limiting statement that device results are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions.

(vi) As applicable, a limiting statement and specific sample collection recommendations to indicate that breast milk can result in false positive results for saliva samples if samples are collected less than one hour after breastfeeding. Sample collection a minimum of one hour from breastfeeding must be recommended.

(vii) Detailed instructions for use that minimize the risk of generating a false result.

(2) Design verification and validation must include:

(i) Detailed device description documentation, including but not limited to, methodology from obtaining sample to result, design of primer/probe sequences, rationale for sequence selection, and computational path from collected raw data to reported result (e.g., how collected raw signals are converted into a reported result).

(ii) Detailed documentation of analytical studies including but not limited to, characterization of the cut-off, analytical sensitivity (limit of detection), inclusivity, reproducibility, interference, cross reactivity, instrument and method carryover/cross contamination, sample stability and handling.

(iii) Detailed documentation from a clinical study documenting sensitivity and specificity of the device; if the number of positive samples in the clinical study is

insufficient to properly estimate device sensitivity, additional pre-selected positive samples must be evaluated to supplement the study. Clinical study subjects must be consistent with the intended use population (i.e., infants younger than 21 days of age), and device results must be compared to FDA-accepted comparator methods. Documentation from the clinical study must include the clinical study protocol, the clinical study report, testing results, and results of all statistical analyses.

(iv) Detailed documentation for device software, including, but not limited to, software applications and hardware-based devices that incorporate software.