Evaluation of the Alethia™ assay for newborn cytomegalovirus testing using oral swab samples

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Disclosures

- The Alethia™ CMV Assay is for investigational-use only and not yet cleared for clinical use
- This work was funded by Meridian Bioscience, Inc.
- Dr. Gantt receives research funding and consulting fees from Merck, GSK and VBI Vaccines Inc.
- Dr. Rawlinson serves on advisory boards for Merck, Genetic Signatures and receives research funding from Abbott
- Dr. Park is PI on the NIH U01 CMV ValEAR trial
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Introduction

- cCMV is a major cause of childhood disability but frequently goes undiagnosed
  - Definitive diagnosis requires detection of CMV in a sample collected within 3 weeks of birth
- Early diagnosis of cCMV may improve neurodevelopmental outcomes
  - Antiviral therapy for selected infants
  - Close monitoring, early intervention for hearing loss
- Saliva is a preferred sample type for detection of CMV in congenitally-infected newborns
- Altheia™ (formerly Illumigene) is a simple, investigational-use only assay suitable for rapid CMV saliva testing outside reference labs

Rawlinson Lancet Infect Dis 2017; Gantt Paeds & Child Health 2017

Saliva testing for cCMV

- Saliva and urine are recommended sample types for newborn CMV testing
  - Very high viral loads
  - Equivalent sensitivity
- Saliva collection using oral swabs is more convenient than urine
- Detection of CMV in saliva of breastfed newborns requires confirmation by urine testing

ScienceDaily

Boppana NEJM 2011; Pinninti PIDJ 2015

Alethia™ CMV Assay

- An investigational-use only LAMP (loop-mediated isothermal amplification) assay developed by Meridian Bioscience, Inc. and performed on the Alethia™ instrument
- Detects CMV DNA in saliva swab samples
- Simple sample preparation
  - Less than 2 minutes of hands-on time
  - Place the sample into the instrument and press “run”
  - No thermal cycling
- 1-10 samples per run, with results in <1 hr
  - 2 independent, 5-sample chambers
- Footprint similar to 8.5” x 11” sheet of paper
  - Does not require an auxiliary computer

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Alethia™ Instrument

alethia™
by meridian BIOSCIENCE

Alethia™ procedures

- Flocked saliva swabs are collected dry without transport media or placed in ≤1ml of transport medium
  - Sampling should be collected >1 hour after breastfeeding
- Testing ASAP recommended but may be stored at room temp for 72 hrs or refrigerated for ≤7 days; samples not tested within 7 days should be frozen immediately at -20C
  - Sample is added to the “Buffer 1” tube, vortexed for 10 sec and incubated for 2 min
  - 50uL of ”Buffer 1” is then added to the “Buffer 2” tube and vortexed for 10 sec
  - 50 µL of “Buffer 2” is then transferred to both the “Test” and “Control” chambers of the Alethia™ test device
    - Internal control assay detects human mitochondrial DNA
    - External positive and negative controls should be performed per applicable federal, state, and local regulations or accrediting agencies
- The test device is then inserted into the Alethia™ instrument, and the ”run” button is pressed to start
- Qualitative positive or negative results are displayed at the end of the run

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Alethia™ CMV clinical trial

- Objective: to determine the performance of Alethia™ for detection of CMV DNA in saliva swabs from newborns
- Study population: Newborns up to 21 days of age, at study sites in Vancouver, Sydney, Salt Lake City, Birmingham, and Bologna
  - Minimum 1000 infants and 5 positive results from prospectively enrolled cohort
  - Supplemented with banked saliva samples collected from newborns previously tested for CMV clinically
- All aspects of the trial were approved by the ethics boards at each site

Study methods

- Swabs were collected prospectively, placed in a dry transport tube or 1mL of transport medium and tested within 7 days using the Alethia™ instrument at each study site
- Confirmatory testing was performed by 2 validated PCR tests followed by bi-directional sequencing on positive PCR samples ("composite reference method", CRM)
- Archived frozen swabs from newborns tested for congenital CMV were also tested by Alethia™ and confirmed by CRM

Samples

- There were1552 eligible samples tested
  - 1467 collected and tested prospectively
  - 85 frozen samples tested retrospectively
    - Including 38 that were positive by local assays
- There were 63 ineligible samples
  - 46 were tested ≥7 days after collection
  - 11 had invalid Alethia™ external control results
  - 4 were collected ≤1 hour after breastfeeding
  - 2 had inadequate/missing assay data recorded

Alethia™ results

- 1476 eligible prospective samples:
  - 8 (0.6%) were positive by Alethia™ testing
  - 1458 samples (99.4%) were negative by Alethia™ testing
  - 1 (0.1%) sample was invalid by Alethia™ testing
- 85 retrospectively tested samples:
  - 37 (43.5%) were positive by Alethia™ testing;
  - 48 were negative by Alethia™ testing
  - 0 were invalid by Alethia™ testing

Alethia™ vs. CRM: prospective samples

	Composite Reference Method			
Alethia™	**Positive**	**Negative**	**Invalid**	**Total**
Positive	5	3	0	8
Negative	0	1425	33	1458
Invalid	0	0	1	1
Total	5	1428	34	1467

Positive Percent Agreement	100% (5/5)	95% CI (47.8% – 100.0%)
Negative Percent Agreement	99.8% (1425/1428)	95% CI (99.4% – 100.0%)

Alethia™ vs. CRM: retrospective samples

	Composite Reference Method			
Alethia™	**Positive**	**Negative**	**Invalid**	**Total**
Positive	34	3	0	37
Negative	0	48	0	48
Invalid	0	0	0	0
Total	34	51	0	85

Positive Percent Agreement	100% (34/34)	95% CI (89.7% – 100.0%)
Negative Percent Agreement	94.4% (48/51)	95% CI (84.6% – 98.8%)

Alethia™ vs. CRM: all samples

	Composite Reference Method			
Alethia™	**Positive**	**Negative**	**Invalid**	**Total**
Positive	39	6	0	45
Negative	0	1473	33	1506
Invalid	0	0	1	1
Total	39	1479	34	1552

Positive Percent Agreement	100% (39/39)	95% CI (91.0% – 100.0%)
Negative Percent Agreement	99.6% (1473/1479)	95% CI (99.1% – 99.9%)

Summary

- Alethia™ CMV results were comparable to CRM
  - High PPA, with no false negatives observed
  - High NPA, with few false positives
- Alethia™ CMV DNA Amplification Assay is a qualitative diagnostic test for direct detection of CMV in saliva of neonates <21 days of age
- Alethia™ CMV results should be used in conjunction with the results of other diagnostic tests and clinical findings

Conclusions

- Alethia™ CMV aims to be the first FDA-cleared assay for detection of CMV in saliva of neonates
  - Additional plans to obtain CE Mark in Europe and clearance in Canada and Australia
- Upon clearance, Alethia™ CMV will aid in the diagnosis of cCMV infection
  - Confirmatory testing recommended for all positive results
- Alethia™ is suitable for any setting in which moderately complex testing can be performed
  - Allows simple, rapid, decentralized cCMV testing
  - E.g., birthing hospitals, physician office labs, as well as reference labs