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

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.
- Dr. Boppana reports receiving research funding and consulting fees from Merck and GSK.
- The other authors report no relevant disclosures.
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

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
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 -20°C
  – 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
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 were 1552 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

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<th>Alethia™</th>
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<th>Total</th>
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<td><strong>1428</strong></td>
<td><strong>34</strong></td>
<td><strong>1467</strong></td>
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</table>

### Percent Agreement

- **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

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<tr>
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<td>Total</td>
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<td>51</td>
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**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

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<td>1479</td>
<td>34</td>
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#### Percent Agreement

- **Positive**: 100% (39/39)  
  95% CI (91.0% – 100.0%)
- **Negative**: 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