

Impact of breastfeeding on saliva PCR results for newborn CMV screening

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DISCLOSURES

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Congenital CMV Infection

- Congenital Cytomegalovirus (cCMV) is the most common congenital infection
- cCMV is leading non-genetic cause of sensorineural hearing loss (SNHL) and developmental disabilities
- Most babies (~90%) with cCMV are not identified in the nursery because of absence of clinical findings at birth
- Most CMV-associated SNHL missed on physical examination and newborn hearing screening
- Rapid, reliable and inexpensive screening test required to identify at-risk children

CMV Detection in newborns

- Gold Standard used to be the detection of virus in urine or saliva by culture-based methods
- PCR of saliva shown to be highly sensitive and specific and comparable to a culture-based method for screening newborns for CMV (NEJM, 2011)
- Most CMV seropositive mothers shed CMV in breast milk which could lead to false positive PCR results

Objective

To determine whether the presence of CMV in breast milk leads to false positive saliva PCR results in newborns

Methods

Analyzed data from two studies:

- Breast milk transmission study:
 - Colostrum and newborn saliva samples analyzed for the presence of CMV
- CHIMES study:
 - False positive results of saliva PCR

Newborn CMV screening

- Specimens collected with a sterile polyester-fiber-tipped-applicator by swabbing infant's oral cavity
- All samples shipped to the central laboratory at UAB
- Rapid culture (RC) performed on all saliva samples for detection of early antigen fluorescent foci (DEAFF)
- Detection of CMV DNA on all saliva samples performed by real time PCR
- PCR performed without a DNA extraction step

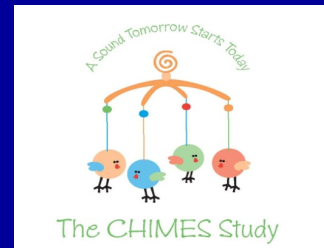
CMV positivity in breast milk

Characteristic	CMV Positive (n=31)	CMV Negative (180)
Race & Ethnicity		
Black	15 (48%)	101 (56%)
White, Hispanic	1 (3%)	12 (7%)
White, Non-Hispanic	15 (48%)	63 (35%)
Asian	0	4 (2%)
Insurance		
Private	15 (48%)	63 (35%)
Public or No Insurance	16 (52%)	117 (65%)
Maternal Age (Mean \pm SD)	27.9 \pm 5.8	26.9 \pm 5.2
Sample collection (days)	1.9 \pm 0.81	1.8 \pm 0.8
Newborn saliva	0	1 (0.6%)

Breast milk study

- 15% (31/211) colostrum samples were CMV positive
- None of the infant saliva samples were CMV positive
- Median viral load in colostrum – 2.1×10^3 IU/mL
- CMV positivity in breast milk was not different based on race and ethnicity

Screening Saliva False Positives by Race & Ethnicity



	# False Pos.	N	False Pos %
Black	6	23,857	0.03%
White, Hispanic	5	32,189	0.02%
White, Non-Hispanic	10	36,962	0.03%
Asian	1	4,150	0.02%
Multiracial	1	2,408	0.04%
TOTAL	23	99,622	0.02%

From CDC breastfeeding webpage: 2008 racial and ethnic disparities in breastfeeding in the U.S.

Breastfeeding rates: 58.9% for blacks, 75.2% for whites, 80.0% for Hispanics

Accessed April 10, 2015 <http://www.cdc.gov/breastfeeding/resources/breastfeeding-trends.htm>

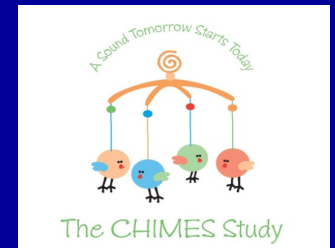
False Positive: confirmed cases **cCMV- 1:17**

False Positive: confirmed cases **metabolic disorders 56:1**

Kwon et al. Arch Pediatr Adolesc Med/Vol 154 July 2000

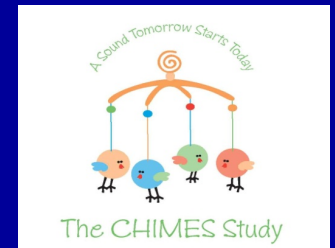
Is PCR of newborn saliva superior to culture-based methods in identifying babies with cCMV?

Newborn CMV screening



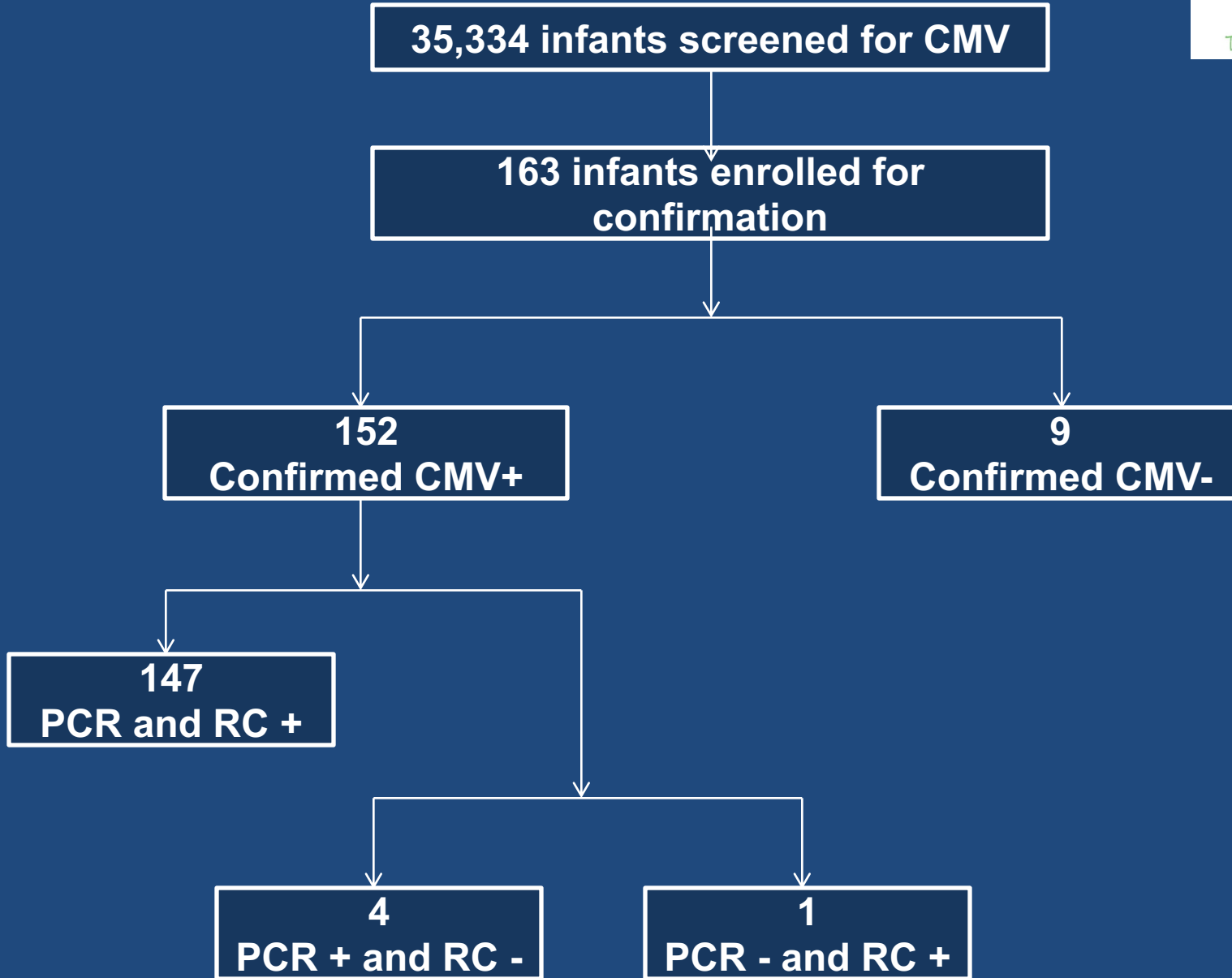
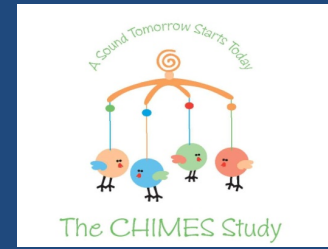
- Between June 2008 and December 2009
 - 35,334 infants screened
 - Saliva PCR and RC
- Between January 2010 and November 2011
 - 37,250 infants screened
 - Saliva PCR
 - RC performed on all PCR + saliva samples

Confirmation of cCMV

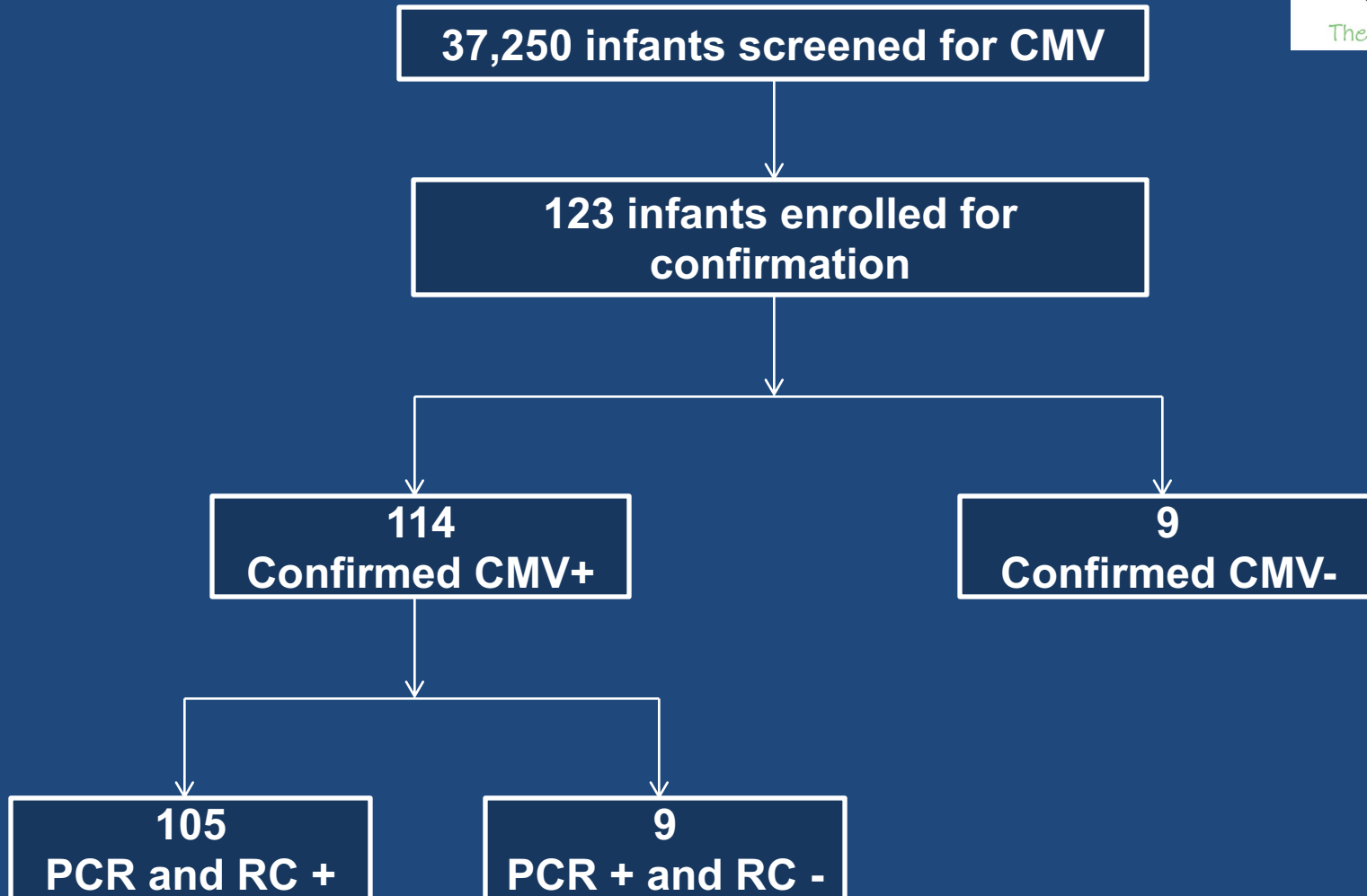
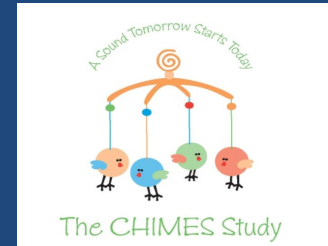


- Infants positive by PCR/RC on initial screening enrolled for confirmation of cCMV
- Saliva and urine samples collected at enrollment visit were tested by PCR and RC for confirmation
- Infants confirmed to have congenital CMV infection enrolled in follow up to monitor hearing outcome

Jun 2008 – Dec 2009



Jan 2010 – Nov 2011



PCR and RC Discordant samples

Sample	PCR (copies/ml)	RC (cells/slide)
1	5.5	0
2	6.1	0
3	11.5	0
4	14	0
5	14.9	0
6	43.4	0
7	1600	0
8	4300	0
9	5000	0
10	21000	0
11	41200	0
12	56000	0
13	415000	0
14	0	4

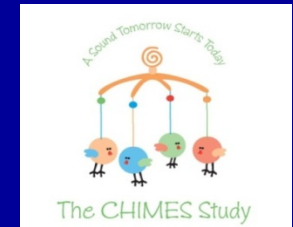
Conclusions

- Saliva PCR has low false positive rates
- Saliva real-time PCR identifies Saliva PCR has low false positive rates
- Saliva real-time PCR is a better newborn screening tool
 - Convenient and non-invasive sample collection
 - Elimination of DNA extraction step
 - High throughput method
 - Less expensive
- 400-1200 additional infants with cCMV could be identified annually using real-time PCR for screening

The CHIMES study Investigators and Personnel

Sponsor: NIDCD

Children and their families



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