Saliva is the Preferred Sample for Congenital Cytomegalovirus Diagnosis

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CMV Public Health & Policy Conference
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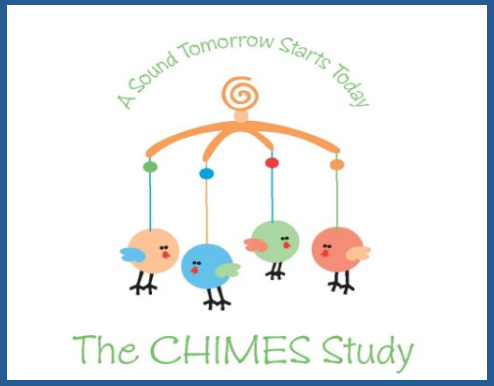


UAB MEDICINE
PEDIATRICS

Congenital CMV Infection

- Congenital Cytomegalovirus (cCMV) is the most common congenital infection
- The leading non-genetic cause of sensorineural hearing loss (SNHL)
- ~90% have no clinical findings at birth and ~10% symptomatic at birth
- 10-15% of asymptomatic and ~60% of symptomatic infants develop SNHL
  - Approximately 1/3 of SNHL is delayed-onset
- Most infants with CMV-associated SNHL missed on physical examination and newborn hearing screening

CMV Detection in Newborns

- Virus shed in urine and saliva in large quantities in congenital CMV
- Viral culture methods have traditionally been gold standard
- Standard culture techniques take 2-4 weeks
- Rapid culture methods using monoclonal antibodies to early viral antigens and centrifugation enhancement replaced traditional culture
  - Shell vial
  - Microtiter plate rapid culture testing of saliva and urine sensitive and specific

    Balcarek et al, JID, 1993; Boppana, JCM 1992

CMV Detection in Newborns
Dried Blood Spot testing

- Interest in DBS
  - Collected from all newborns
  - Easier to integrate into existing screening programs
  - Many initial studies showed high sensitivity
- Potential problems
  - Viral load in blood is low and variable
  - Chronic intrauterine infection
  - Negative blood PCR in some symptomatic infants
  - Amount of available DBS may be limited
  - Most initial studies included selected populations

Sensitivity of DBS PCR

Author	PCR method	Sensitivity
Barbi et al. 1996	Conventional/nested	100%
Johansson et al. 1997	Conventional	81%
Barbi et al. 1998	Conventional/nested	100%
Barbi et al. 2000	Conventional/nested	100%
Yamamoto et al. 2001	Multiplex, nested	71%
Scanga et al. 2006	Multiplex, TaqMan	100%
Soetens et al. 2008	Conventional and TaqMan	66% and 73%
Atkinson et al. 2009	TaqMan	74% and 72%

NIDCD CHIMES Study Project Design

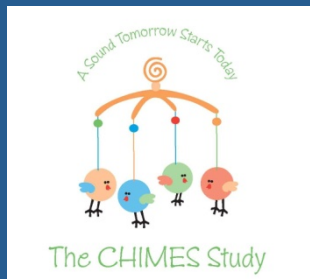
- Determine best diagnostic test for newborn screening (DBS?)
- Determine the relative contribution of CMV-related hearing loss to overall hearing loss in children

Study Population:

Between March 2007 and March 2012, 100,333 infants enrolled

Study hospital locations included:

- Birmingham, Alabama (n=12,194)
- Jackson, Mississippi (n=6,360)
- New Brunswick, New Jersey (n=15,093)
- Charlotte, North Carolina (n=10,715)
- Cincinnati, Ohio (n=14,126)
- Pittsburgh, Pennsylvania (n=19,200)
- Dallas, Texas (n=22,645)



DBS PCR for Newborn CMV Screening

Total positive: 92 (saliva, DBS or both)

Saliva Rapid Culture	DBS PCR		Total
	P	N	
P	28	64	91
N	0	20356	20357
Total	28	20420	20448

Sensitivity <40%

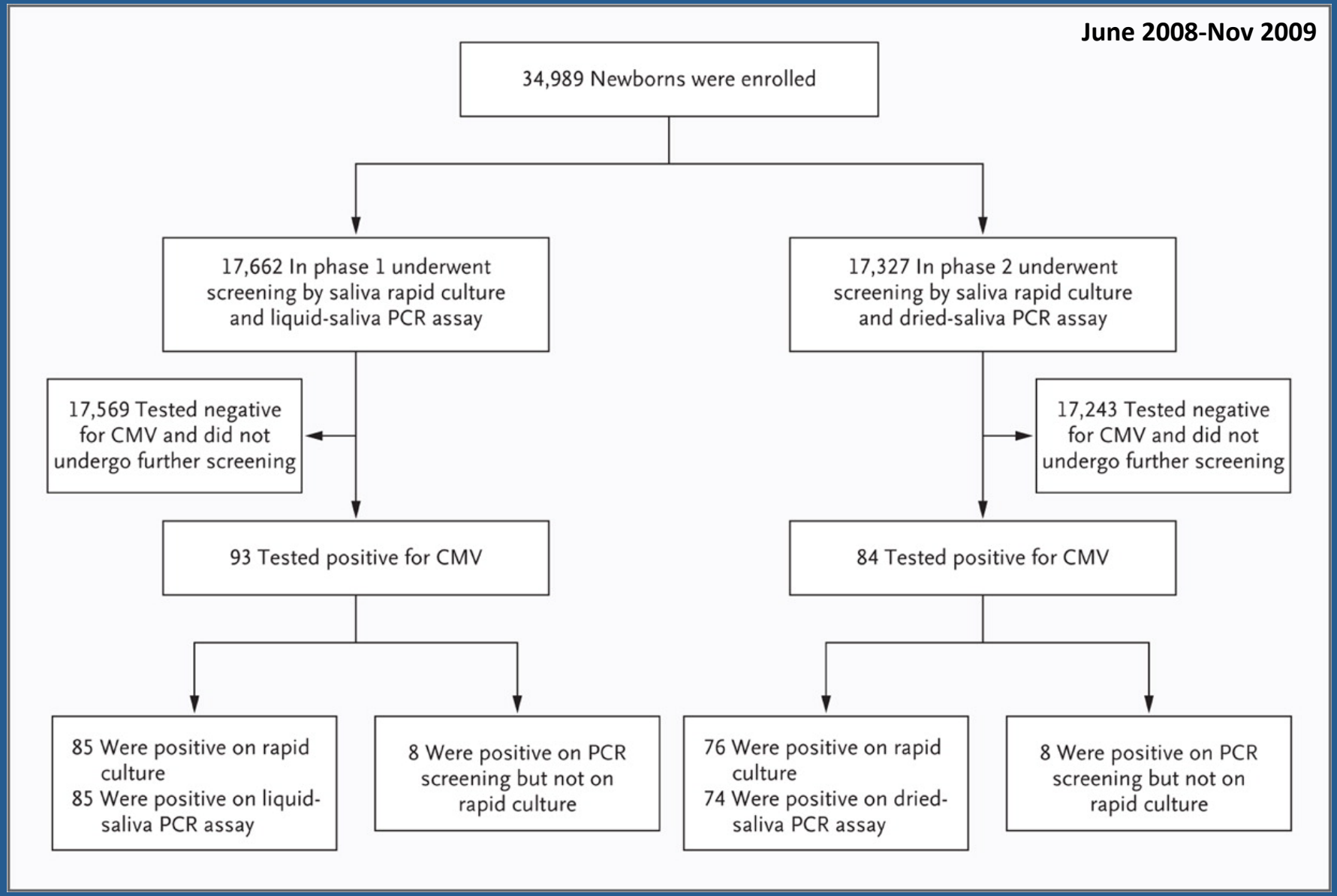
Boppana, et al. JAMA 2010;303:1375-1382

Saliva PCR for neonatal screening

- Warren et al, JCM, 1992
- Compared qualitative PCR to tissue culture and rapid culture (n=167)
- PCR sensitivity 89%
- Less sensitive PCR method, not all subjects congenitally infected

- Yamamoto et al, J Clin Virol 2006
- 4439 infants prospectively screened
- Urine PCR compared with saliva PCR
- 99.7% agreement between urine and saliva PCR
- No comparison with “gold standard” tissue culture

Saliva PCR for neonatal screening- NIDCD CHIMES Study



Boppana SB et al. N Engl J Med 2011;364:2111-2118.

Saliva PCR for neonatal screening- NIDCD CHIMES Study

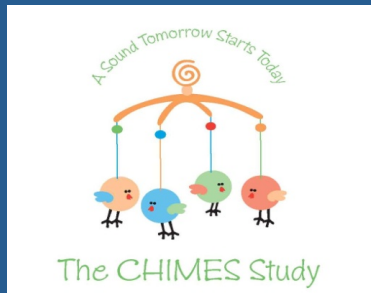
PCR is as good as rapid culture

Table 2. Real-Time Polymerase-Chain-Reaction (PCR) Assays of Liquid- and Dried-Saliva Specimens, vs. Rapid Culture, Used to Screen for Congenital Cytomegalovirus Infection.

Rapid Culture	Liquid-Saliva PCR Assay			Dried-Saliva PCR Assay		
	Positive	Negative	Total	Positive	Negative	Total
Positive	85	0	85	74	2	76
Negative	8	17,569	17,577	8	17,243	17,251
Total	93	17,569	17,662	82	17,245	17,327
Sensitivity (95% CI) — %		100 (95.8–100)			97.4 (90.8–99.7)	
Specificity (95% CI) — %		99.9 (99.9–100)			99.9 (99.9–100)	
Positive likelihood ratio (95% CI)		2197 (1099–4393)			2100 (1049–4202)	
Negative likelihood ratio (95% CI)		0 (0.0–0.1)			0.03 (0.0–0.1)	
Positive predictive value (95% CI) — %		91.4 (83.8–96.2)			90.2 (81.7–95.7)	
Negative predictive value (95% CI) — %		100 (99.9–100)			99.9 (99.9–100)	

Boppana SB et al. N Engl J Med 2011;364:2111-2118.

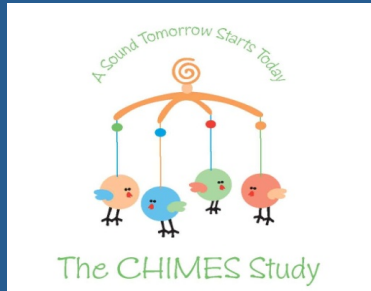
Is saliva PCR a better newborn screening tool for congenital CMV infection than culture based techniques?



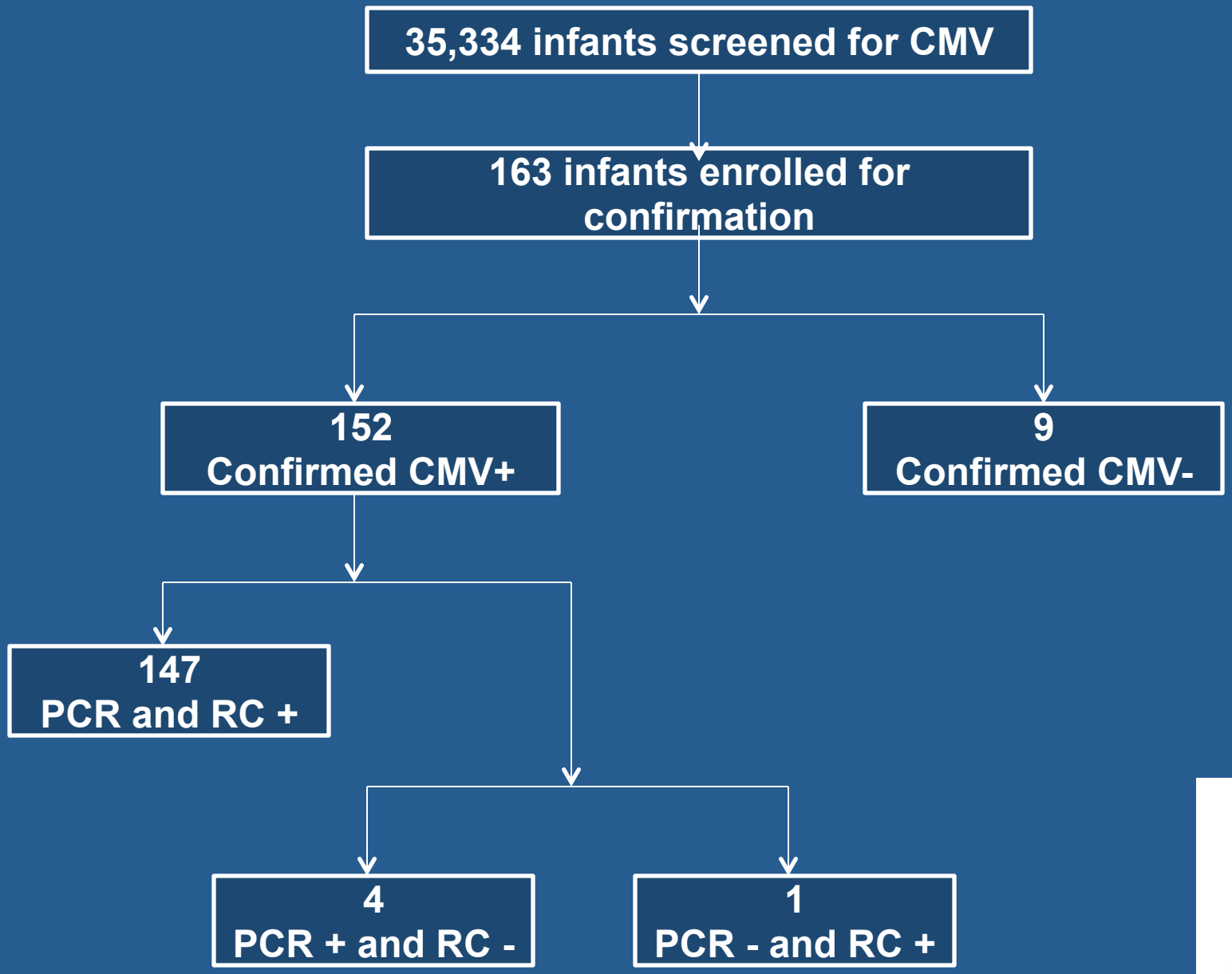
Newborn CMV screening

- 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

Infants positive by PCR/RC on initial screening enrolled for confirmation of cCMV



Jun 2008 – Dec 2009



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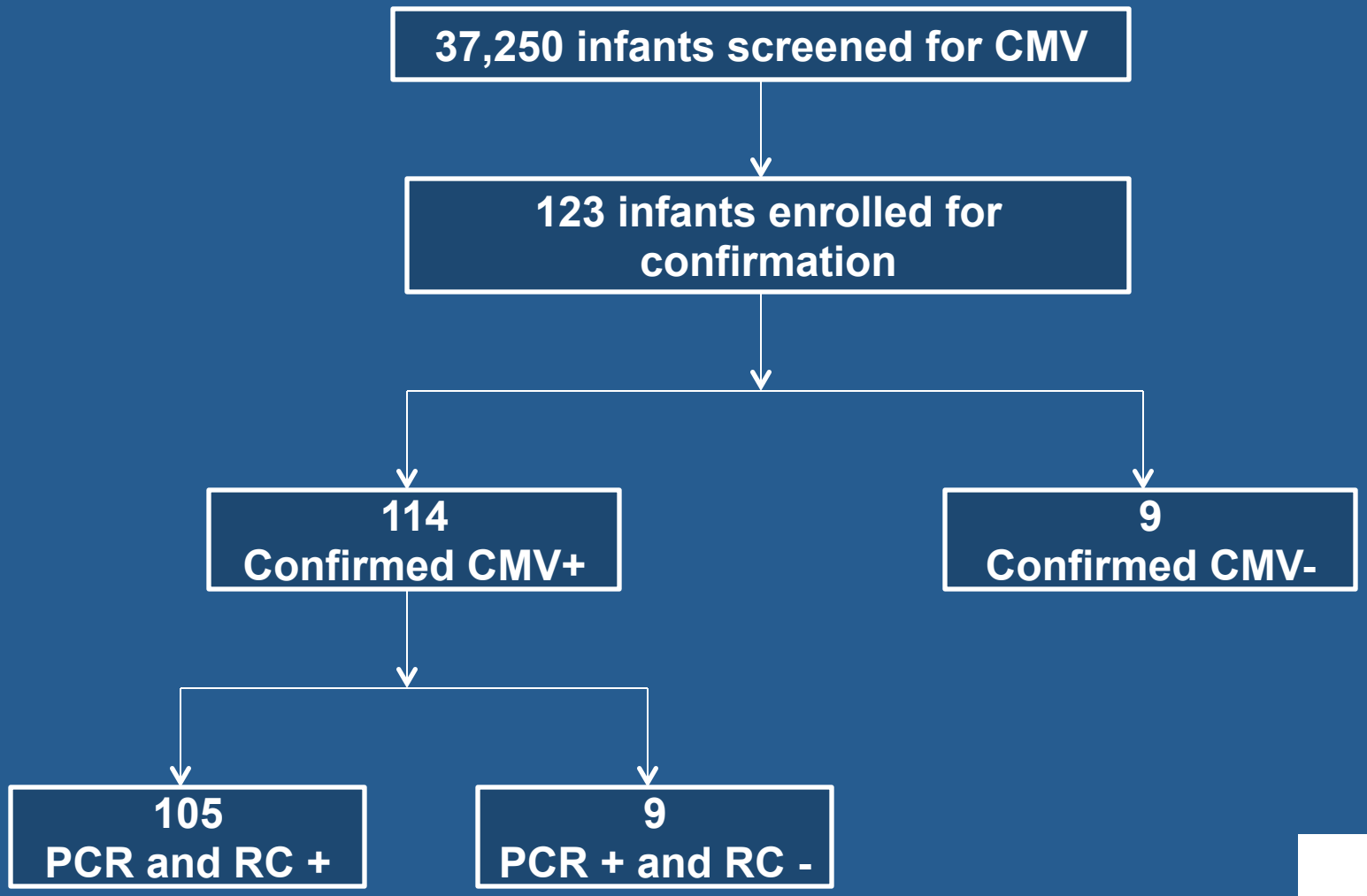
Jan 2010 – Nov 2011



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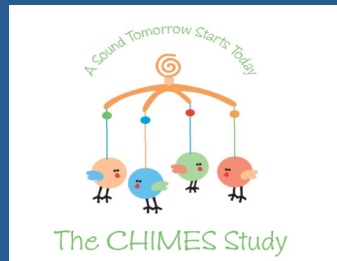
PCR and RC Discordant samples

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

Newborn CMV screening-Summary

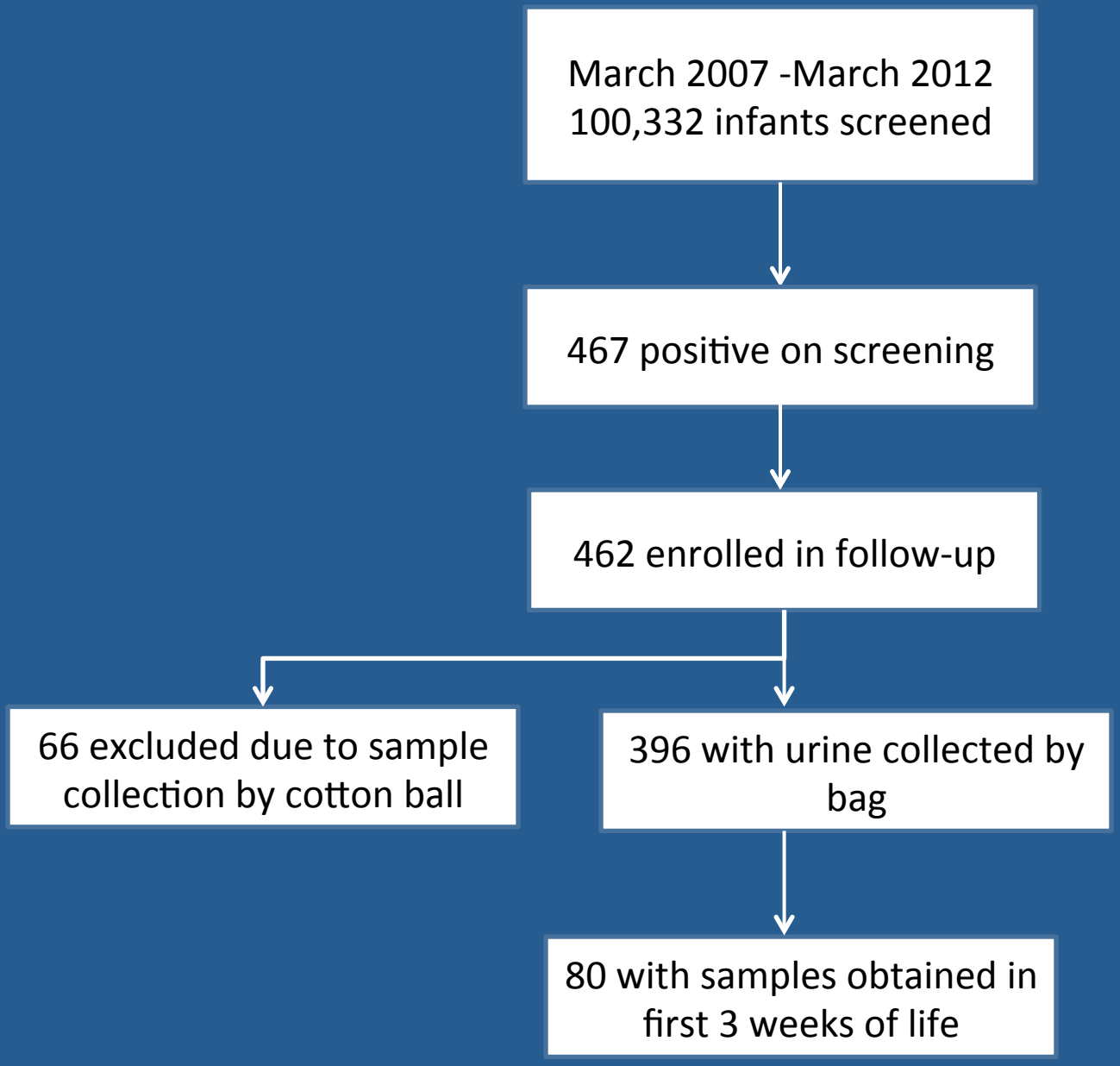
- DBS PCR lacks sensitivity for newborn screening
- Saliva real-time PCR identifies more infants with cCMV than RC
- Saliva real-time PCR is a better newborn screening tool
  - Convenient and non-invasive sample collection
  - Elimination of DNA extraction step
  - High throughput method
  - Less expensive
- 400-1200 additional infants with cCMV could be identified annually using real-time PCR of saliva for screening



Saliva PCR for diagnosis of congenital CMV

- Can our real-time assay can be used for the clinical diagnosis of congenital CMV infection?
- Does this PCR assay works equally well in both urine and saliva samples?

Results



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Urine culture vs PCR

	Urine	
	Culture +	Culture -
PCR+	76	3
PCR -	0	1

P=0.688

Saliva culture vs PCR

	Saliva	
	Culture +	Culture -
PCR+	78	2
PCR -	0	0

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Culture- Saliva vs Urine

	Urine Culture +	**Urine Culture -**
Saliva Culture+	74	4
Saliva Culture -	2	0

PCR- Saliva vs Urine

	Urine PCR +	Urine PCR -
Saliva PCR +	79	1
Saliva PCR -	0	0

P=0.688

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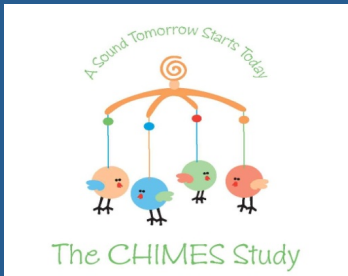
Summary-Diagnosis of congenital CMV

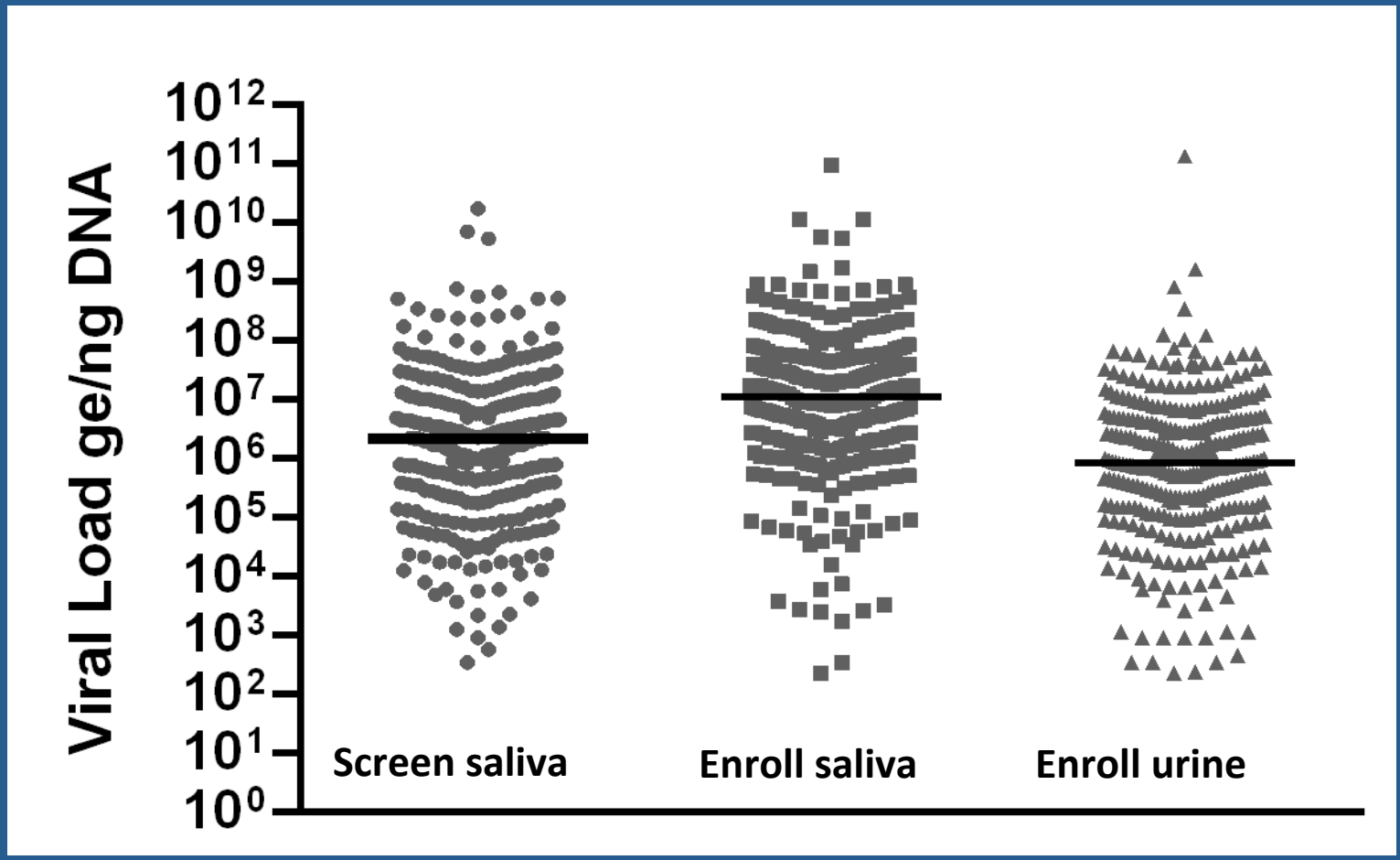
- PCR amplification performs as well as rapid culture to detect virus in both urine and saliva

- PCR of the saliva appears superior and detected CMV in samples that were negative by culture, no samples were negative by PCR.

- Saliva samples- ease of collection, transportation and storage



Viral Load



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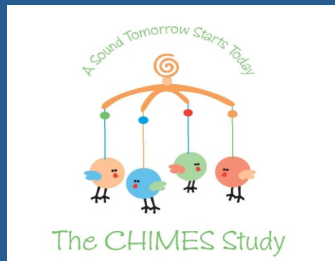
Breast Feeding and excretion of CMV

Method of Detection	Time of detection in days
PCR, Culture	≤ 56
PCR, Culture	8-119
PCR, Culture	10
PCR, Culture	3.5
PCR	≤ 14
PCR, Culture	12.3 ± 9.4
PCR, Culture	≤ 14

Adapted from Kurath et al, Clin Microbiol Infect 2010 Aug;16(8):1172-8

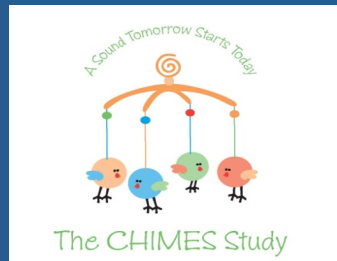
Newborn CMV Saliva-Feasibility and Acceptability

- Ireland (Waters et al., JCV, 59 ;2014)
  - 12 month pilot screening study (n=1044)
  - Urine collected initially in the study-recruitment low and issues with sample contamination
  - Switched to saliva and improved subject recruitment
  - Samples pooled
  - Incidence 0.19%
- England (Williams et al., Arch Dis Child Fetal Neonatal Ed, 99 ;2014)
  - Infants who failed NBHS screened for CMV (n=411)
  - Maternal anxiety assessed, acceptability of sample collection
  - 50% urine samples returned compared with 99% saliva
  - Screening supported by most mothers and anxiety not increased

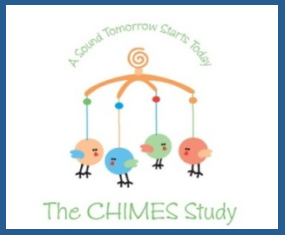


Summary

- DBS PCR lacks sensitivity for newborn screening
- Saliva real-time PCR identifies more infants with cCMV than RC for newborn screening
- Saliva real-time PCR is a as good as urine for diagnosing cCMV
- High viral load in saliva
- Use of saliva for screening generally acceptable and feasible
- Saliva overall is preferred due to
  - Convenient and non-invasive sample collection
  - Elimination of DNA extraction step
  - High throughput method
  - Less expensive



The CHIMES study Investigators and Personnel
Sponsor: NIDCD



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