Retrospective testing of newborn screening dried blood spots (DBS) in symptomatic children to confirm diagnosis of congenital CMV infection (cCMV)

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Introduction

Children with disabilities resulting from congenital CMV (cCMV) infection may not be tested at birth, leading to a delay in diagnosis and treatment. Dried blood spot (DBS) testing for presence of CMV DNA by PCR may aid in the retrospective diagnosis of cCMV of these children.
The aim of this study is to evaluate DBS testing for diagnosis of cCMV in two groups of children:
* infants who were tested for cCMV by urine or saliva-based testing during the first 21 days of life
* infants with clinical symptoms consistent with possible cCMV and evidence of CMV infection at time of evaluation but no CMV testing performed in the first 21 days of life.

Definitions

CcCMV Confirmed congenital CMV infection - positive detection of CMV by culture in at least one urine or saliva specimen collected in first 21 days of life
PcCMV Possible congenital CMV infection - clinical symptoms consistent with possible cCMV but no CMV testing performed in the first 21 days of life
NcCMV No congenital CMV infection - negative detection of CMV by culture in urine or saliva specimen collected in first 21 days of life
DBS Dried Blood Spot from Texas Department of State Health Services (DSHS) Newborn Screening Lab-retrieved with signed maternal permission; DBS routinely collected for newborn screening days 1 and 3 of life and saved in DSHS Lab
Sens Sensitivity is the probability of testing positive if the disease is truly present as determined by the "gold standard"
Spec Specificity is the probability of testing negative if the patient is truly not diseased as measured by the "gold standard"
PVP Predictive Value - Positive - the probability of actually having the disease if the test is positive
PVN Predictive Value-Negative - the probability of actually not having the disease if the test is negative

Methods and Materials

DBS Retrieval
Retrieved from TX DSHS after written consent from mother (DSHS Specimen Decision form and DBS Consent form)
DBS processing
DBS stored at -20C in plastic bag with desiccant until processing
DNA Extraction
Thermal shock method for nucleic acid extraction from dried blood spots (DBS).
One 6 mm punch of DBS (equiv. to four 3 mm punches) used for DNA elution
6 mm punch in 2 ml microfuge tube with 60 µl of MEM
Vortex to immerse DBS in MEM
Soak at room temperature 2 hours, slow continuous shaking (300 rpm)
Incubate at 55ºC 1 hour, continue slow shaking (300 rpm).
Transfer to 99ºC thermomixer for 7 minutes, then to ice block (precooled) to -20 ºC or wet ice for rapid cooling for 2 min up to one hour
Cfg 2 minutes to bring condensation down
Transfer DBS with fluid to Swab Extraction Tube System
Cfg 14000 rpm for 5 minutes to elute all liquid from DBS
Eluent stored at -80ºC before PCR.
PCR method
Taqman-based targeting the IE region of CMV (Boppana, 2010)
Statistical Analysis: Sensitivity, Specificity, Predictive Value-Positive and Predictive Value-Negative as measured against "gold standard" tests for cCMV.

Results

Table 1. Demographics of patients whose DBS were tested for CMV.

	Total	Females	Males
Total DBS Tested	48	20	28
DBS Unavailable for testing	10	7	3
Total DBS Requested	58		

Table 2. DBS performance for detection of CcCMV.

		CMV►		
		Positive	Negative	Row Totals
DBS	Positive	17	0	*17*
	Negative	1*	3	*4*
	Column Totals>	*18*	*3*	*Total 21*

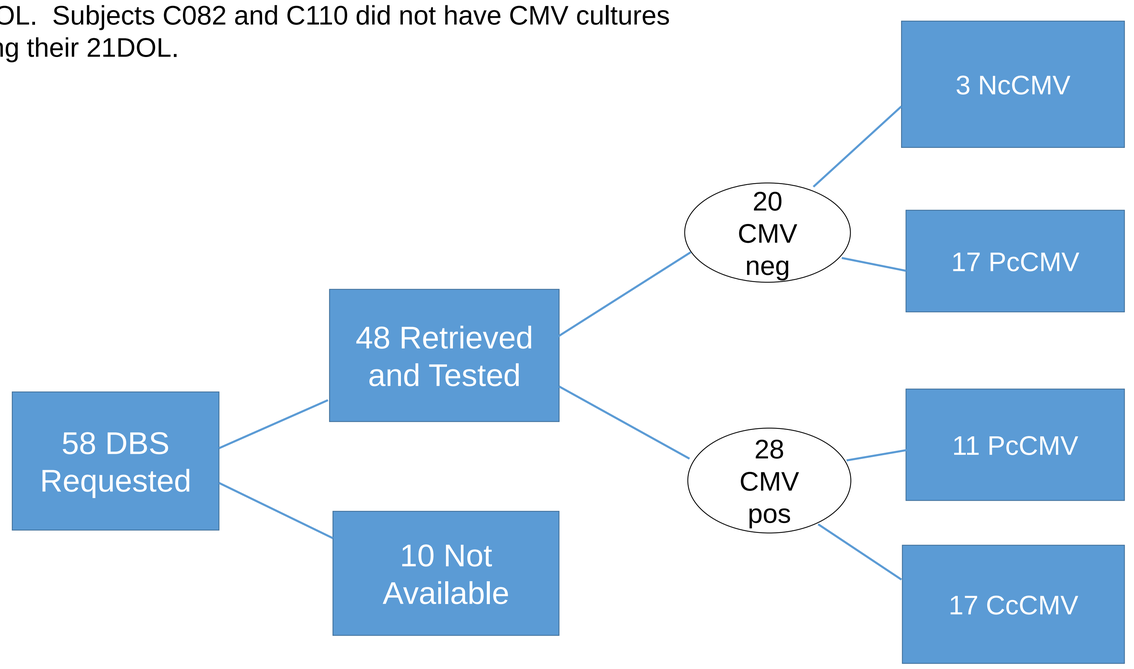
Sens: 94.4 %
Spec: 100 %
PVP: 100 %
PVN: 75.0 %

Note:
* Low reaction positive in one DBS sample.
►Viral cultures performed <21 DOL

Table 3. DBS results discrepancies.

PID	No. Subjects w/Inconsistent DBS Results	DBS Result Variations
C002,C016,C C082,C080	4	Positive/Equivocal
C084,C110	2	Positive/Negative
C106	1	Negative/Equivocal/Positive
C059	1	Negative (low reactivity detected)
C011	1	Positive (1 spot available for testing)
TOTAL	9	

Note: Table shows 7 of 9 subjects whose DBS results were inconsistent but show positive results for CMV CX during 21DOL. Subjects C082 and C110 did not have CMV cultures during their 21DOL.



Conclusions

- CMV DNA PCR results from retrieved DBS samples agree with "gold standard" CMV urine culture obtained in first 21 days of life.
- DBS samples appear useful for diagnosis of cCMV.