PCR of Archived Newborn Blood Spots Reveals that Congenital Cytomegalovirus Infection is a Major Cause of Unexplained Hearing Loss in Minnesota Children

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http://www.cidmtr.umn.edu

Hearing Loss in Newborns in Minnesota

- Population ~5.3 million
- 70,000 deliveries/year
- Incidence of deafness: 1 in 1000 births
- Mild-to-moderate hearing loss: 3-5 in 1000 births
- Overall, ~200 infants born with hearing loss in Minnesota annually

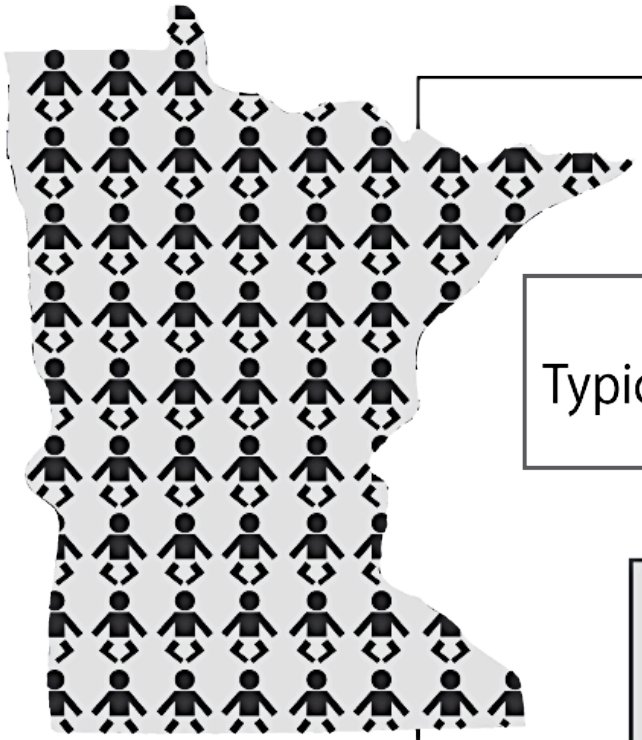
NHS in Minnesota

- 73,070 births
- 59657 (81.6%) screened
- 1363 (2.3%) failed
- 53 (3.9%) of failed had hearing loss
- .73/1000 of infants screened identified with etiology for hearing loss
- 1291 (95%) of failed NHS had no diagnosis

In 2007, NHS became mandatory in Minnesota

In 2012, Minnesota screened 66,784 (98.7%) newborns for hearing loss

*excludes infants that are deceased or whose parent(s) declined screening



884
Never Screened
(1.3%)

601
Did Not Pass
(0.9%)

But 39.9% did not complete diagnosis

199
Typical Hearing

162
Hearing Loss

240
No Diagnosis
(39.9%)

83
Enrolled Early
Intervention

31
Not Enrolled

48
Enrollment
Unknown

http://www.improveehdi.org/mn/

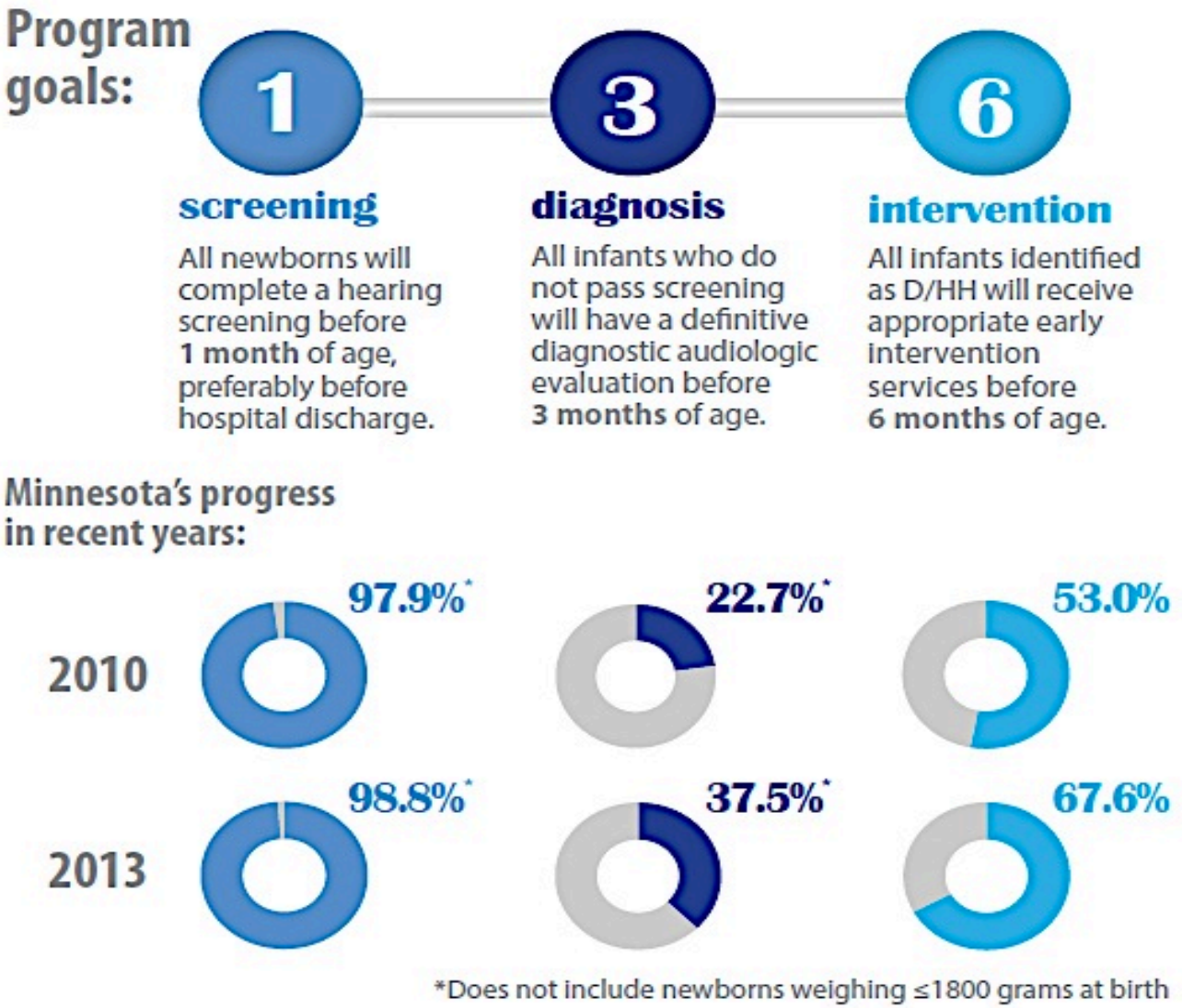
http://www.improveehdi.org/mn/

Minnesota's Early Hearing Detection and Intervention (EHDI) Program

"1-3-6" program

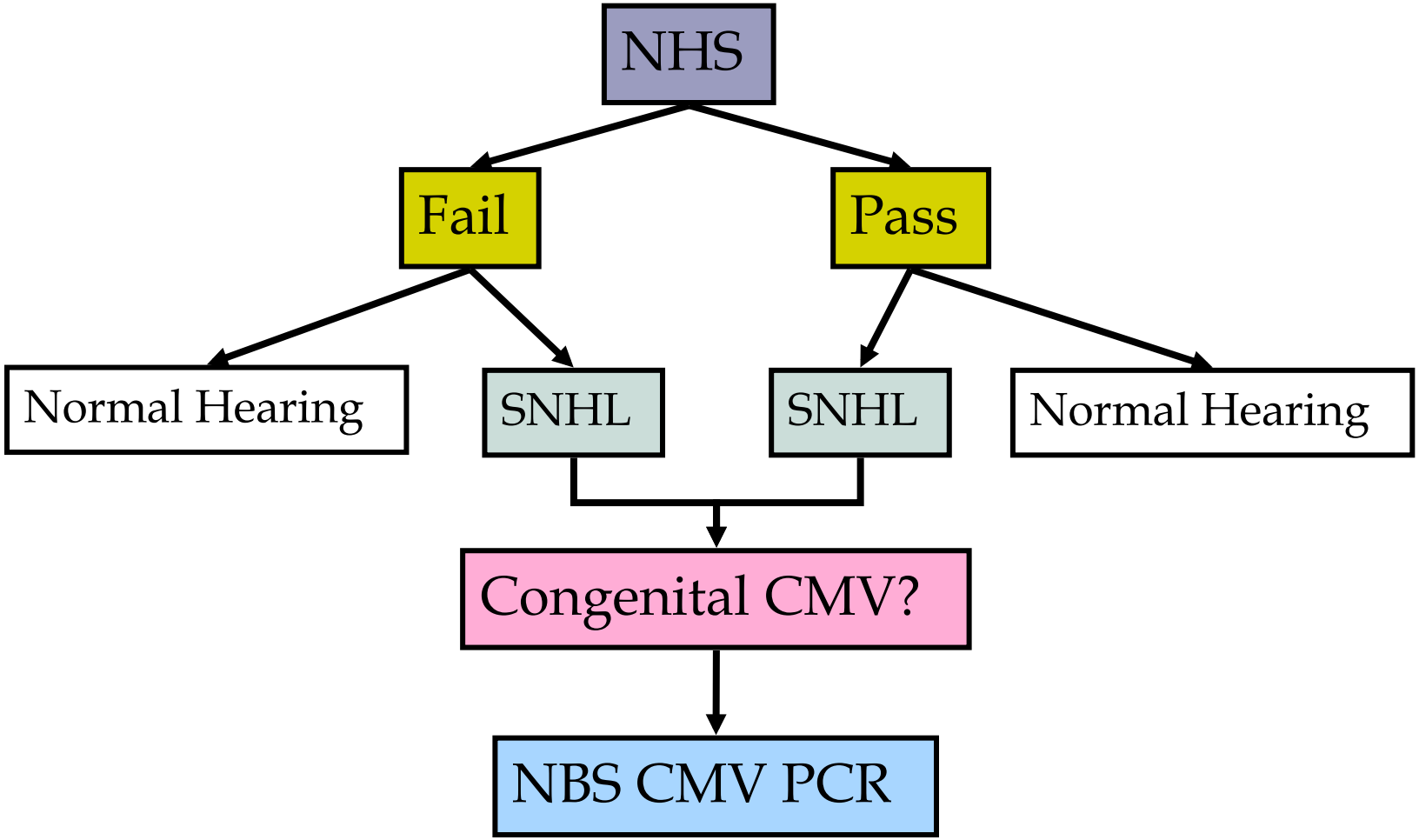
- Screen by 1 month
- Diagnose by 3 months
- Intervene by 6 months

McVicker, 2014 CMV Conference
Mandated Congenital Cytomegalovirus Testing: The Utah EHDI Experience



http://www.improveehdi.org/mn/

Can Testing the Newborn Blood Spot (NBS) for CMV DNA Improve Timely Etiologic Diagnosis for SNHL Following Failed NHS?



Benefits of CMV NBS Screening

- Estimated cost/year of treating CMV infection and attendant complications in US is ~$2 billion
- NBS encourages more comprehensive evaluation, enables earlier physical therapy, therapy for SNHL
- Ganciclovir therapy lessens hearing loss and improves neurodevelopmental outcome
- Early identification allows monitoring of hearing loss
- Other disorders in NBS 1:5,000 to 1:100,000

Dollard, J Inherit Metab Dis. 2010, 33 (Suppl 2):S249–S254
Pass, Clin Infect Dis. 2011, 52:582-583
Khadambari, Acta Paediatr. 2013 Oct;102(10):928-33
Williams, Arch Dis Child Fetal Neonatal Ed. 2014 May;99(3):F230-6

Study Aims: Study 1

- Examine NBS from an anonymized group of infants who had failed NHS for the presence of CMV DNA by real time PCR
- Compare the incidence of DNAemia in this cohort to a cohort of matched, anonymized infants who successfully passed the newborn hearing screen
- Gather information about the range of viral loads in congenitally infected infants

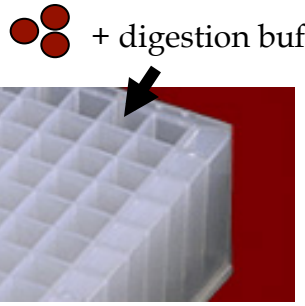
Study Design

- We collected three, 3 mm DBS punches from newborns obtained through the Neonatal Screening Program of the Minnesota Department of Health
- Spots were divided into two groups based on “pass” or “fail” status of NHS (bilateral failures)
- Real-time PCR (qPCR) was performed for detection of CMV, using UL54 (DNA polymerase) primers and FRET probes with the Roche Lightcycler®
- Viral load normalized to DNA recovery from sample using β–globin internal primers

Methods Study 1

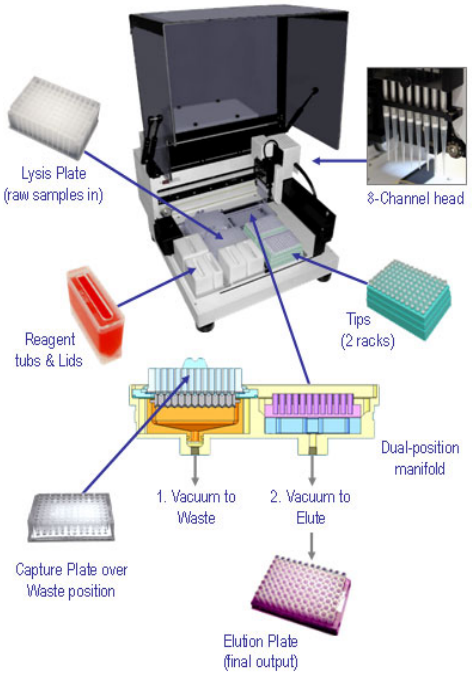
1. Collect blood

2. Digest 3mm spots



3. Automated NA extraction

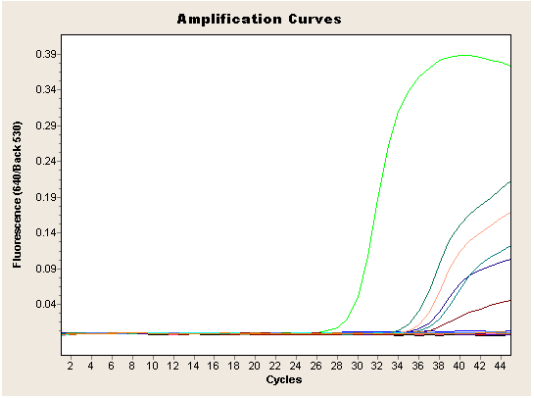
Corbett X-tractor



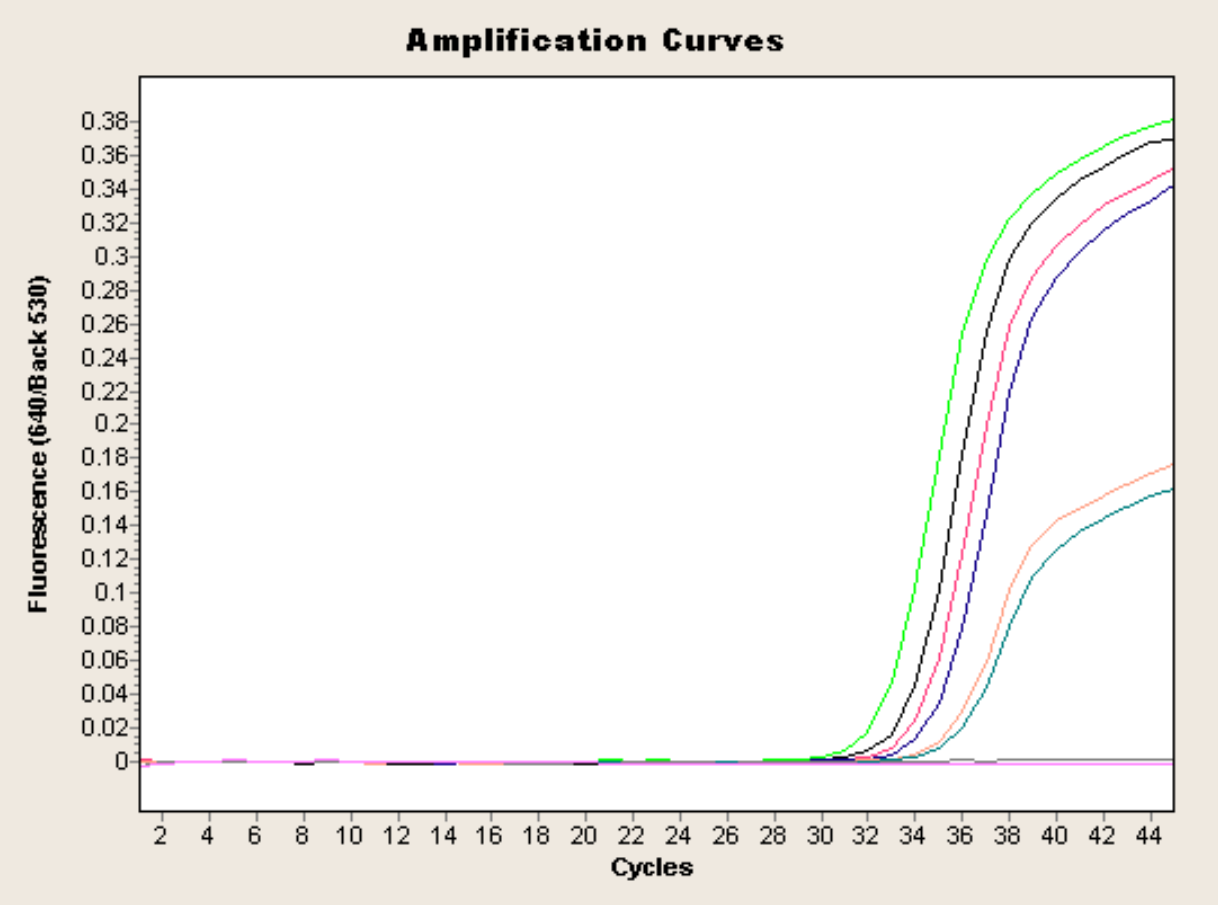
4. Real time PCR

Lightcycler®

5. Report



Assay Sensitivity



	Cp	Concentration copies/rxn
	30.87	100
	31.86	50
	33.15	25
	33.07	12.5
	33.49	5
	33.81	2.5
	none	1
	none	NTC

Assay sensitivity is 5 copies/reaction. This converts to ~50 copies/µg of DNA or ~2500 (3.4 $\log_{10}$) copies/ml of blood.

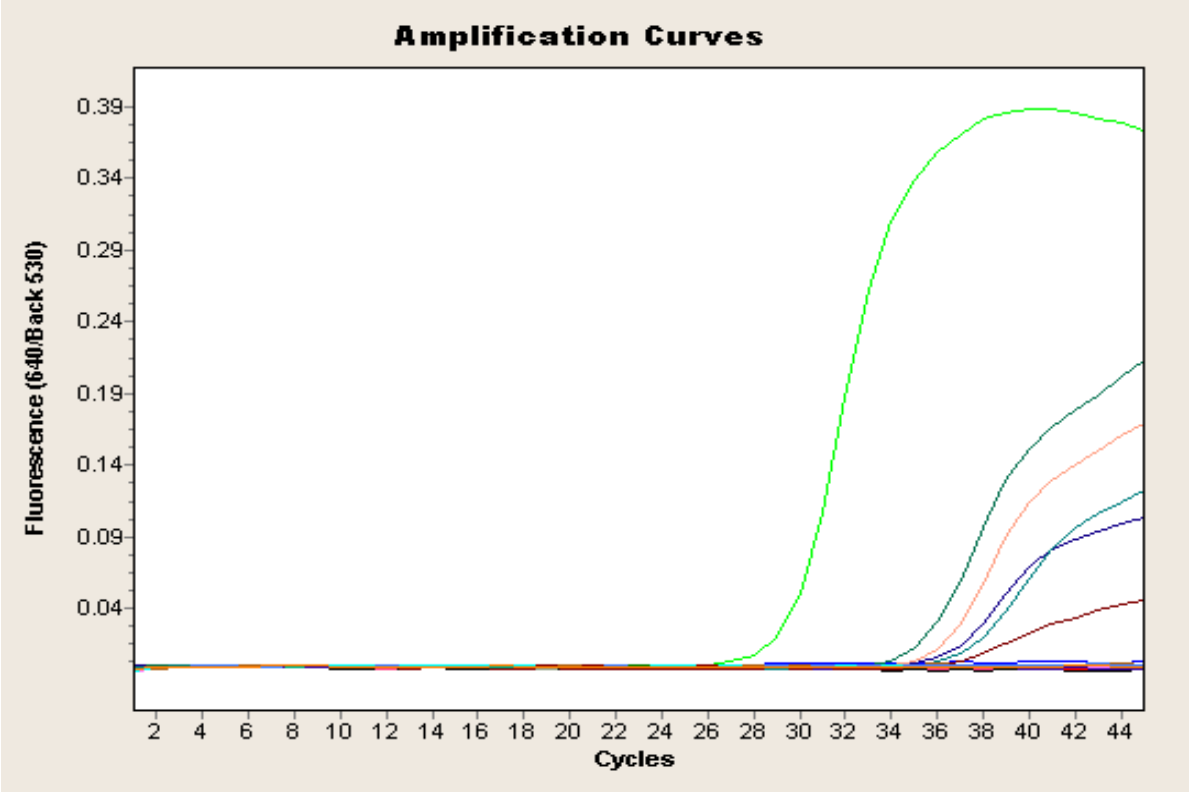
Sample Breakdown

Infant Gender	Pass (N=479)		Refer (N=479)	
	Total (%)	CMV positive	Total (%)	CMV positive
Female	240 (50.1%)	0	181 (37.8%)	3
Male	239 (49.9%)	2	298 (62.2%)	10
Race				
Asian	13 (2.7%)	0	25 (5.2%)	2
Black	27 (5.6%)	0	37 (7.7%)	1
Hispanic/Latino	30 (6.3%)	0	46 (9.6%)	0
Native American	8 (1.7%)	0	7 (1.5%)	0
Other	23 (4.8%)	0	26 (5.4%)	0
Unreported	26 (5.4%)	0	36 (7.5%)	0
White	352 (73.5%)	2	302 (63.0%)	10
Total CMV-Positive	**2/479 (0.4%)***		**13/479 (2.7%)***	
*P=0.02 (Fisher's exact test)				

CMV Viral Load/DNAemia

Subject	NHS Group	HCMV concentration (copies/μg total DNA)
1	Pass	9.45E+02
2	Pass	1.74E+03
3	Refer	5.91E+02
4	Refer	2.18E+03
5	Refer	1.79E+03
6	Refer	4.00E+03
7	Refer	6.70E+03
8	Refer	8.43E+02
9	Refer	5.41E+02
10	Refer	1.65E+03
11	Refer	1.48E+03
12	Refer	5.83E+02
13	Refer	1.21E+03
14	Refer	2.66E+03
15	Refer	3.17E+02

Examples of Positive DBS by qPCR



	Cp	Concentration copies/rxn
std	27.89	1000
S173	33.68	23.2
S174	34.75	11.6
S236	34.49	13.7
S333	35.60	6.66
S335	35.03	9.69
NTC	none	none

Conclusions

- 13/479 (**2.7%)** of infants who **failed NHS** were identified by qPCR to be **CMV positive**
- 2/479 (**0.4%**) of infants who **passed NHS** were **CMV positive** by qPCR
- Linking CMV DNA detection to NHS screening programs may be useful in determination of etiology of SNHL in newborns

Schornagel 2014 CMV Conference Poster P11
Williams 2014, Arch Dis Child Fetal Neonatal Ed 99(3):F230-6

Study 2 – Contribution of CMV Infection in the Setting of Established Hearing Loss in Minnesota Children

- The majority of children who have CMV-related hearing loss do not have it at birth
- Many ‘failed’ newborn hearing screenings are found in children with normal hearing
- Diagnostic evaluations for nonsyndromic SNHL are often unsuccessful in older children
- Congenital CMV infection cannot be reliably diagnosed beyond the first 2–3 weeks of life

(Dahle et al., *J Am Acad Audiol*; 11:283, 2000)

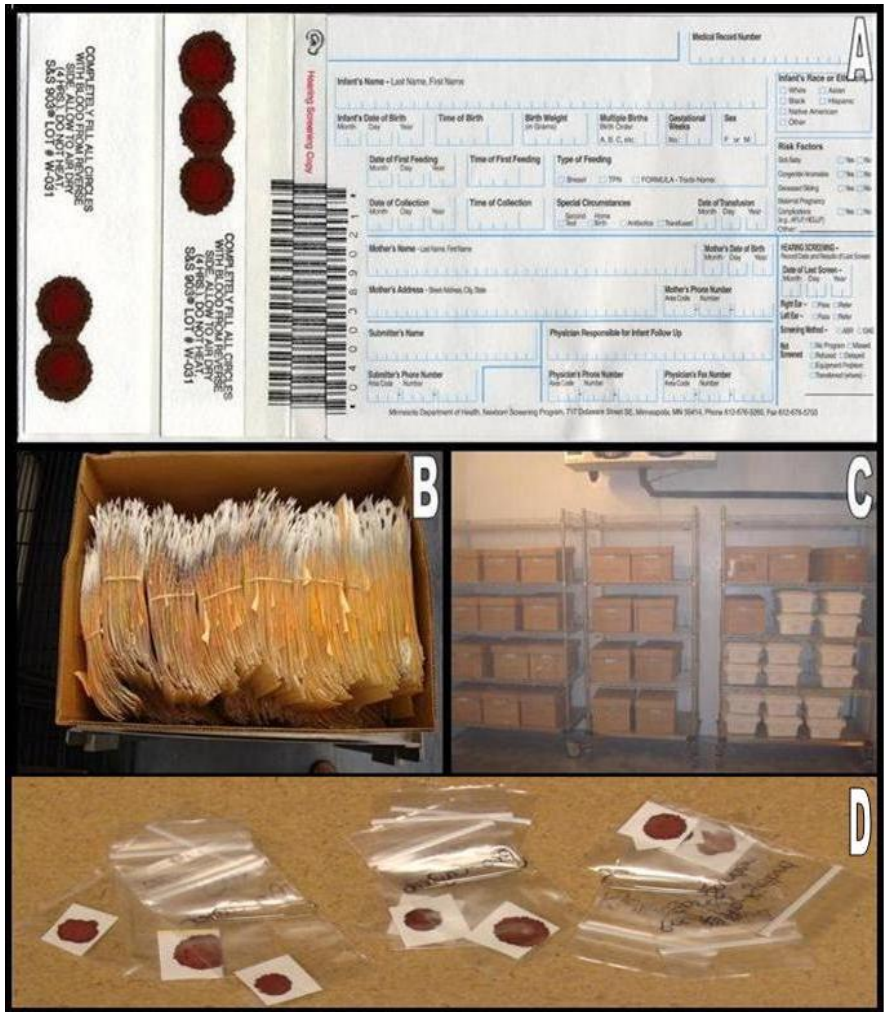
Goals of Study #2

- To examine archived newborn blood spots for the presence of CMV DNA by real-time PCR in children 6 months – ten years of age in a referral clinic population
- Collaboration with Minnesota Department of Health Newborn Screening Program
- Archived, stored blood spots are available from State Health Department dating back to 2001
- 'Lions Clinic' in Otolaryngology at University of Minnesota: a multidisciplinary clinic for evaluation and therapy of hearing loss in children

Materials and Methods

- Study approval was obtained from the University of Minnesota Institutional Review Board
- Hearing impaired children underwent Lion's Clinic evaluation by a geneticist, an otolaryngologist and infectious diseases physician
- Data on identified genetic and anatomic causes of hearing loss was compiled from clinic records
- Signed parental consent given to obtain the newborn blood spots of enrolled children, retrieved from the Minnesota Department of Health for PCR analysis

Materials and Methods



- Retrieval of newborn blood spots from the Minnesota Department of Health archival storage facility
- Newborn blood spots are boxed and stored in a walk-in refrigerated facility (4 ^{o}C)
- Blood spots retrieved following signed consent from Newborn Screening Program staff member

Materials and Methods

- Six 3 mm punches were obtained from each NBS
- DNA was extracted using the QIAcube system for improved recovery
- qPCR was performed in the Roche 480 using Roche CMV UL83 hybridization probe set
- NRAS gene used as internal control for DNA recovery/quantification of viral load

Soetens J Clin Microbiol. 2008;46:943-6
Göhring, J Clin Virol. 2010;48:278-81
de Vries, J Clin Virol. 2009;46 Suppl 4:S37-42
Koontz, 2012 CMV Abstract F2

Assessment of Blood Spot PCR in Children Known to Have Congenital CMV

Subject	DOB	CMV PCR on NBS (co/ml)	CMV Cx Urine	CMV PCR from Blood (co/ml)
BB-M	9/06	N/A	Pos (<21d)	22300 (4m)
DC-M	11/06	5973000	Pos (<21d)	476000 (5m)
IG-F	10/08	1379400	Pos (<21d)	5800 (1m)
MH-F	4/08	37950	Pos (<21d)	2800 (1m)
JJ-M	8/07	160710	Pos (<21d)	4200 (2m)
WK-M	2/08	333300	Pos (<21d)	Neg (2m)
JK-M	7/07	363000	Pos (<21d)	78400 (4m)
SK-F	7/07	300630	Pos (<21d)	4500 (4m)
AS-F	6/08	207240	Pos (<21d)	55200 (1wk)
ET-M	4/08	38600	Pos (<21d)	4600 (1wk)

- Ten newborn blood spots were tested by real-time PCR in children who were evaluated for congenital CMV infection by conventional methods
- All had clinical evidence of congenital infection
- 9/10 were positive with mean viral load of 72,600 copies/ml

Results from Lion's Clinic Cohort

- 70 families were approached
- 68 (97%) gave consent
- CMV DNA was found on 19 of these newborn blood spots (28%)
- An additional 5 children (7%) had post-natal explanation for acquired hearing loss
- 23 children (34%) were identified as having a genetic or anatomic etiology for hearing loss
  - Connexin (GJB2) mutations (n=4)
  - Mondini malformation (n=3)
  - Other anatomic or presumed genetic causes – see table
- Etiology remained unclear in 21 children (31%)

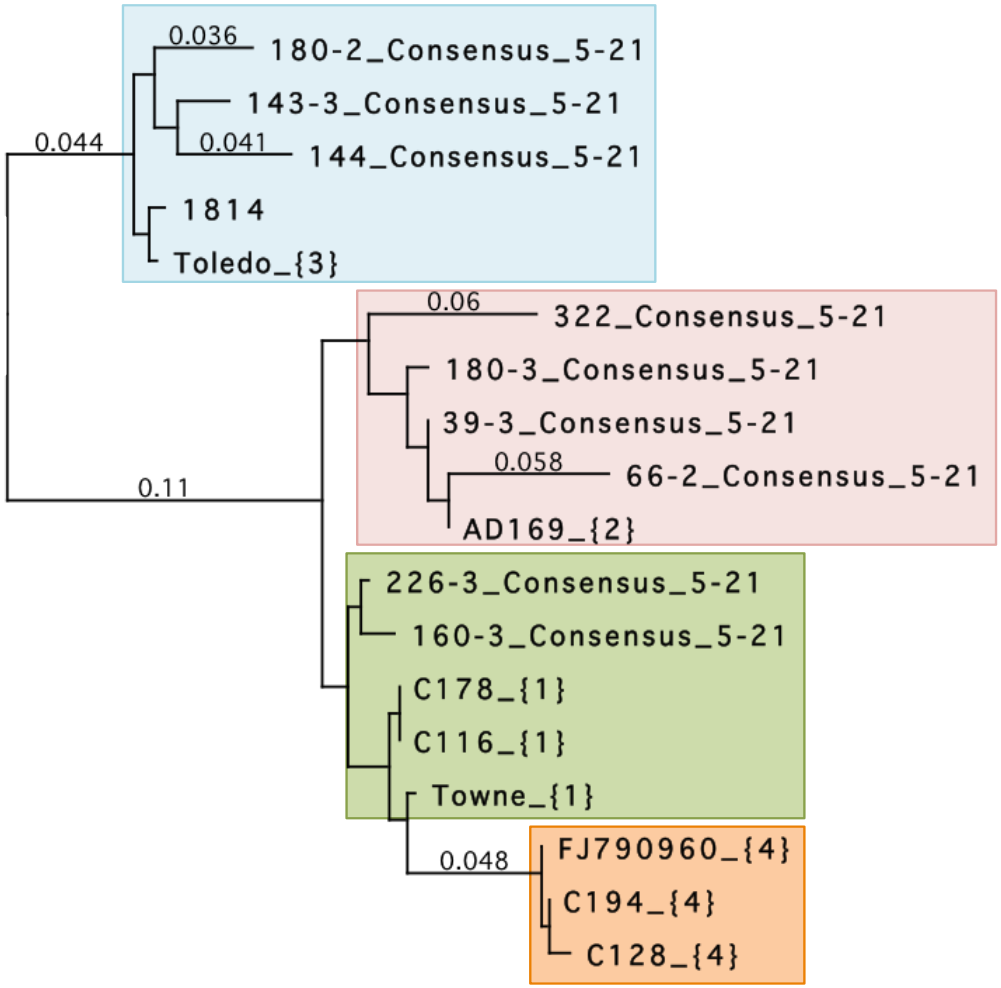
Clinical Course and Outcomes of the Hearing-Impaired Children Retrospectively Identified with Congenital CMV Infection

- 7/19 children had passed the newborn hearing screen at birth (37%)
- 15/19 children had bilateral hearing loss (79%)
- Cochlear implantation was performed in 10 children (53%)
- The diagnosis of congenital CMV had not been suspected in any of these subjects

Subject	DOB	NBHS	Current/last documented Status	Cochlear Implant
1. MA-F	2/05	Passed	Severe-to-profound L>R	L (8/08); R (7/06)
2. JB-J	1/08	Failed	Mild-to-mod bilateral (?)	
3. JB-M	7/01	Passed	L: Normal; R: profound	
4. DC-M	11/06	Passed	Severe -to-profound - Bilat	B (12/08)
5. TF-M	9/06	Failed	R: Profound; L: Moderate	
6. MF-F	9/06	Failed	Profound - Bilat	R (11/08)
7. NF-F	10/06	Failed	L: Severe-to-profound; R: Profound	R (7/09)
8. IG-F	10/08	Failed	Profound - Bilat	B (12/09)
9. CH-F	2/08	Failed	N/A	
10. JJ-M	8/07	Passed	L: Mod-to-severe; R: profound	
11. SK-F	10/04	Passed	Profound - Bilat	L (7/06)
12. JL-M	2/07	Failed	Profound - Bilat	B (5/09)
13. MM-F	12/99	N/A	R: Mod; L: Normal	
14. AO-M	11/01	Failed	L: Profound; R: Normal	
15. AR-M	11/08	Failed	R: Severe-to-profound; L: Mod to profound	
16. AS-M	6/08	Passed	R: Mod-to-severe; L: Mild	
17. ET-M	4/08	Failed	Profound - Bilat	L (5/09)
18. GT-F	12/06	Failed	L: Mod-to-severe; R: Profound	R (2/09)
19. TW-M	9/05	Passed	Profound - B, neuropathy	L (9/07)

Etiology	Patients	%
Genetic/Anatomic	**23**	**34**
GJB2	4	6
Pendred Syndrome	1	1.5
3P deletion	1	1.5
Syndromic (NOS)	1	1.5
EVA	2	3
Cochlear Dysplasia	7	10
Family Hx	7	10
Acquired	**24**	**35**
Congenital CMV Infection	19	27
Meningitis	1	1.5
NICU	2	3
Cerebral Infarct	1	1.5
Multiple AOM	1	1.5
Unknown	**21**	**31**

DBS Isolates: gB genotyping



Isolate No.	gB Genotype Group
39	2
66	2
322	2
143	3
144	3
160	1
226	1
194	4
128	4

Chou and Dennison, J Infect Dis 1991;163:1229-34

Comparisons to Other Studies…

- A UK study (median age: 7) indicated 8/35 (23%) children with unexplained SNHL tested positive for CMV DNA on DBS
- A study from the Netherlands (DECIBEL study) reported rates of congenital CMV in children with permanent hearing important of 8% (14/179) overall and 23% (9/39) in the profoundly affected subset
- A study in North Carolina identified cCMV in 11/112 (10%) of children age 1-4 with varying degrees of SNHL
- A study reported at this meeting

Demmler-Harrison, 2014 CMV Conference Poster P14
Kimani Arch Otolaryngol Head Neck Surg. 2010;136(10):999-1004
Walter, 2008 Arch Dis Child Fetal Neonatal Ed 2008 Jul;93(4):F280-5
Korver, J Clin Virol. 2009 Dec;46 Suppl 4:S27-31

The Rapidly Changing Landscape of Newborn Screening in Minnesota…

- Citizens' Council for Health Freedom (CCHF), St. Paul, Minnesota
- A 501(c)3 non-profit organization founded in 1998
- Intense lobbying effort contributed to events in Minnesota Senate session in 2011 which saw introduction of SFB1017 (SF760) by David Hann (R-Eden Prairie)
- Bill changes newborn screening to "opt-in" system
- Bill mandated destroying blood spots after assays completed and eliminated long-term storage
- Bill would put MDH labs out of compliance with CLIA laws and would have ended newborn screening registry

Bearder vs. State of Minnesota

- *Bearder v. State of Minnesota* was brought by nine families who wanted the state to obtain written informed consent to collect, store or use infants' blood samples
- The lawsuit was spearheaded by the Citizens' Council for Health Freedom
- The opinion centered on the meaning and interplay of two Minnesota laws: the newborn screening statutes, Minn. Stat 144.125 – 144.128 (2010), and the Minnesota statute entitled "Treatment of Genetic Information Held by Government Entities And Other Persons," Minn. Stat. 13.386 (2010; also known as the "Genetic Privacy Act"
- The Act prohibits the collection, use, storage or dissemination of a person's genetic information without written informed consent

Bearder vs. State of Minnesota

- The Minnesota Supreme Court Ruled:
  - (1) the blood samples collected and stored by the Department were genetic information subject to the restrictions of the Genetic Privacy Act; and
  - (2) the newborn screening statutes provided an express exception to the Genetic Privacy Act only to the extent that the Department was authorized to administer newborn screening by testing the samples for disorders and to store the test results, and the newborn screening statutes did not expressly authorize the Department to collect, use, store, or disseminate the blood samples for any other use without written consent.

NATURE | NEWS

Minnesota starts to destroy stored blood spots

Court ruling that the state must get consent to store samples from newborn screening could hinder biomedical research.

Meredith Wadman

03 February 2012

Minnesota's state health department has this week begun to destroy blood samples that are routinely collected to diagnose serious inherited and congenital diseases in newborn babies. It was compelled to do so by a state Supreme Court decision that such samples cannot not be stored or used for anything except diagnosis without the informed consent of the parents.

Heel-prick tests in Minnesota can be used to screen newborn babies for 53 serious diseases, but the samples are also crucial for biomedical research.

ISTOCKPHOTO

"We're going to begin destroying a valuable public health resource, the residual blood spots from about 200 babies born in Minnesota each day," said Edward Ehlinger, Minnesota's health commissioner, in a press release on 31 January. He warned that the new policy "will compromise our ability to assure the quality and accuracy of the newborn screening program". The Minnesota Department of Health (MDH) will now actively ask parents for consent to store blood spots collected from infants who have been diagnosed with one of the 53 diseases tested for, and automatically destroy samples from children who have been given the all-clear.

Minnesota Senate Bill SF2047

- April, 2014 the Newborn Screening Program Modifications bill (SF 2047) passed on a 41 to 22 vote and was signed into law by Governor Dayton
- The new bill enables MDH to retain these spots indefinitely again, and to use them and test results in research related to newborn screening
- Parents are able to choose if they do not want their child's samples and results stored
- The bill would require more education for parents about newborn screening, and would allow the samples and results to be used in public health studies not related to newborn testing if the parents or guardians provide their written consent
- If a parent chooses against storage, or has a change of mind and revokes consent they had given previously, the test results must be destroyed within one week of such a request

Reactions to SF2047

- The ACLU of Minnesota called the bill “a dangerous effort to enable unlimited retention and minimize parental consent,” and noted “obtaining informed consent for the retention and later use after newborn screening for all purposes is not only possible but practical.”
- The Citizen's Council for Health Freedom in a statement reacting to the vote said the Senate "just awarded state government ownership of the genetic information of every newborn baby in Minnesota…it just voted to repeal genetic privacy rights at birth"
- The Council for Responsible Genetics' President Jeremy Gruber stated that the bill was “…a strike against privacy rights”, that the “opt-out model the bill proposes is faulty”, and that there is “little transparency in how newborn samples and data are handled and managed”

http://www.genomeweb.com/clinical-genomics/minn-senate-passes-bill-restore-newborn-screening-bloodspot-data-retention-polic

NATURE | NEWS

Minnesota to resume storing newborn blood samples

Law allows researchers to retain blood spots indefinitely, overriding a court ruling.

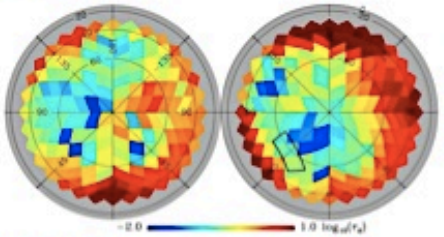
Sara Reardon

09 May 2014

Minnesota will once again allow blood spots to be kept and used for further research following routine newborn screening of genetic and congenital diseases, thanks to a bill signed into law by the state's governor, Mark Dayton, this week. But privacy advocates opposing the law say that they will keep fighting to prevent newborns' DNA from being stored indefinitely.

The bill effectively reverses a 2011 ruling by the state's Supreme Court, which said that the practice violated the state's genetic-privacy laws. Under court orders, health officials destroyed more than 1 million blood spots dating back to 1997. The new law, which goes into effect on 1 August, will allow officials to keep the blood spots indefinitely.

Top Story



Full-galaxy dust map muddles search for gravitational waves

Planck probe's survey of polarized light casts further doubt on BICEP2 discovery claims and could complicate Planck's own plans.

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TESTING UPDATE

NEWBORN BLOODSPOT RETENTION REINSTATED IN MINNESOTA

Practice expected to benefit larger newborn screening studies, public health, disease research

Minnesota's law prohibits the sale of bloodspots but allows sharing deidentified blood samples and test results for research conducted outside of the Minnesota Department of Health.

CCHF and the Minnesota Chapter of the American Civil Liberties Union questioned whether deidentified genetic information made available for medical research could be kept truly anonymous.

Newborn bloodspot retention reinstated in Minnesota
Am. J. Med. Genet., 2014, 164: viii–ix. doi: 10.1002/ajmg.a.36733

Summary and Discussion

- Congenital CMV infection is found in approximately one in four children with SNHL referred to a university-based referral clinic
- The relatively low incidence of GJB2 mutation in our study population might be due to selection bias in a tertiary/referral clinic
- These results confirm the tremendous impact this common congenital infection has on hearing loss in childhood

Summary and Discussion

- Thirty-seven percent of children with SNHL who were born with congenital CMV infection passed their newborn hearing screen
- Cochlear implantation was performed in 10 children
- Hearing loss was typically severe and bilateral
- The diagnosis of congenital CMV had not been considered in our cohort of children
- Retrospective blood spot testing was readily accepted by parents – public policy implications?
- Could a prospective CMV screening approach in all newborns have changed the outcome?

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