



Comparison of specimen types for CMV screening

1. Minnesota Study testing saliva and DBS
2. Pilot study testing urine on filter paper

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Minnesota Study to Establish Clinical Sensitivity of DBS for CMV

Partners in Study

1. Univ MN – PI **Mark R. Schleiss**, MD
2. CDC (main funding source) – PI **Sheila Dollard**, PhD
3. MN Department of Health Newborn Screening Laboratory
 - **Mark McCann** (Director)
 - **Maggie Dreon** (project manager)



Rationale for Study

- Sensitivity of DBS for CMV varies widely across studies:
- Most important variable is DNA extraction

Highest sensitivity: **80%** Johansson 1997; **70%** Soetens 2008 (unsuitable methods)

Lowest sensitivity: **28%** CHIMES (M48 high throughput robot)

CDC NBS Branch determined low sensitivity in CHIMES due to **M48 robot** used:

Koontz et al., Evaluation of DNA extraction methods for CMV. JMM. 2015

- Public Health emphasizes best use of limited health care dollars, using existing infrastructures when possible (NBS program)

Hypothesis: 70-80% DBS analytical sensitivity may identify all children with symptoms and sequelae (100% clinical sensitivity)

Study Design

Babies born at Minnesota area hospitals offered enrollment

- 30,000 infants over 5 years (by 2021)
- Exclude parents who refuse newborn screening
- Exclude critically ill or extremely premature infants

Specimens and testing

- Saliva swab collected for study tested by UMN only
- DBS already collected for NBS tested by CDC and UMN
- Infants CMV + on any test (out of 3) receive urine confirmation testing

Clinical follow-up

- CMV+ children reported to parents and PCP, examined at birth for symptoms
- Annual review of medical records and follow-up by primary care physician until age 4 years

DBS Processing with Quanta Extraction Buffer

- CDC receives DBS in 96-well plates (three 3 mm punches per specimen)
 - Add 50 μ L of Quanta buffer to wells of 96-well plate
 - Thermomixer incubation 25 minutes 95°C
 - Use eluate directly for PCR
- Cost per specimen: \$0.50 for Quanta DBS buffer
- Quanta DBS buffer used by U.S. NBS programs for DNA testing (SCID, CF, SMA)

Thermomixer
\$3,500



Quanta + CMV PCR throughput
(1 technologist)

# 96-well plates	# DBS specimens	Time
2	80	2 hrs
8	320	7 hrs

MN NBS Study Results through July, 2018

Total infants enrolled = 8,085

Confirmed CMV positive: **30** (0.37% birth prevalence)

Saliva testing

- 26/30 positive = 87% sensitivity
- 4/29 saliva positives were false-positive = 14% FP





DBS testing

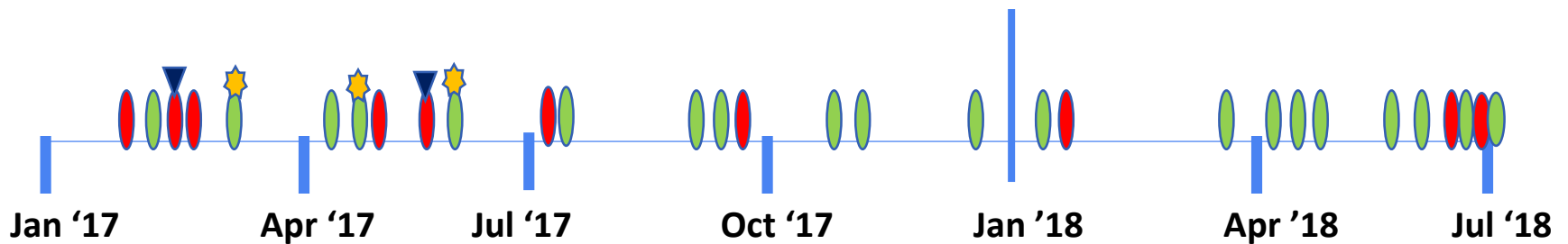
- 21/28 positive = 75% sensitivity
- 2/23 DBS positives were false-positive = 8.7% FP

Race / Ethnicity	Percentage of Births
White	59%
Black	15%
Hispanic	6%
Somali	6%
American Indian	2%
Other (w/ mixed race)	12%

Timeline of Results

each oval = one CMV+ infant confirmed with urine testing

-  CMV infection no symptoms or sequelae to date (n=20)
-  CMV infection with symptoms or sequelae (n=9)
-  CMV infection with negative saliva swab (n=4) (later positive w/more testing)
-  CMV infection with symptoms or sequelae with negative DBS (n=2)



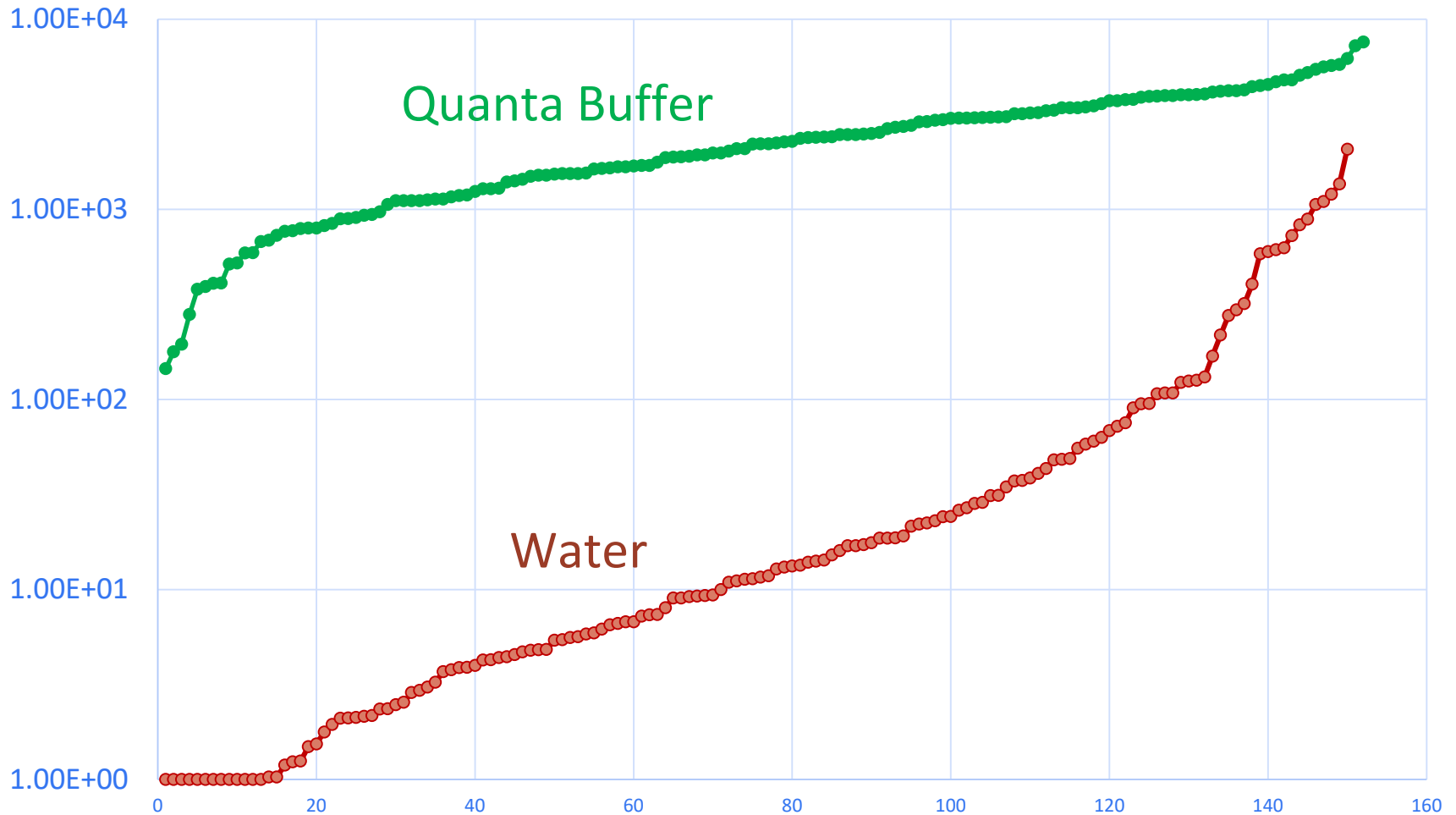
1. Jan-Jun 2017 Saliva collection changes from water to Quanta Saliva buffer
2. Jul 2017 DBS modification from Quanta

MN Study Summary So Far

- **Saliva:** collection worked fairly well with challenges
 - Wide range in saliva specimen quality (cell counts had 3 log range)
 - PCR inhibition (excessive viral DNA, inhibitors)
- **DBS**
 - Relatively high analytical sensitivity (75%)
 - Analyses ongoing to correlate viral load with symptoms and sequelae
 - Does DBS positivity predict sequelae/symptoms?

Should we also consider **urine**?

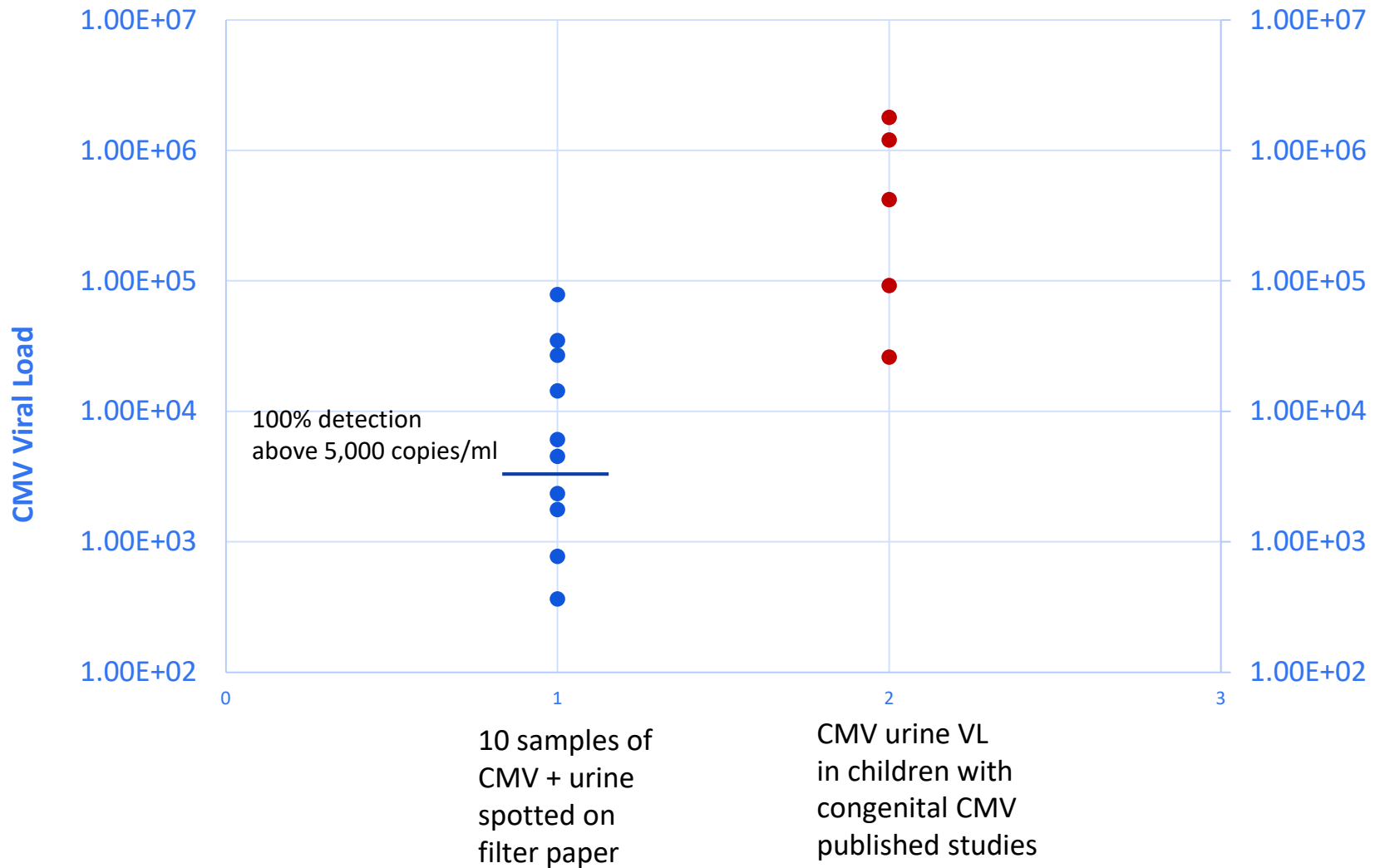
Human Cell Count in Saliva Swabs According to Elution Method



Pilot Study Testing Urine on Filter Paper

- Several studies successfully collected urine on filter paper strips inserted into diapers of newborns for diagnostic testing
 - Naoki Inoue (CMV), [McCann](#), Tuchman, Auray-Blais (NBS for amino acid and urea metabolic disorders)
- **What urine viral load is detectable with filter paper:** CDC liquid urine specimens from children 1-4 years old shedding CMV
 - Whole urines specimens quantitated for CMV and spotted onto Whatman 903 filter paper used in the U.S. newborn screening program
 - Air dry filter paper, two 3 mm punches tested by Quanta extraction method used for DBS
 - Performed quadruplicate testing of 10 samples on 3 different days (12 replicates per sample total)

CMV viral loads in whole urine that are detectable by filter paper testing



Universal Screening Specimen Comparison

DBS

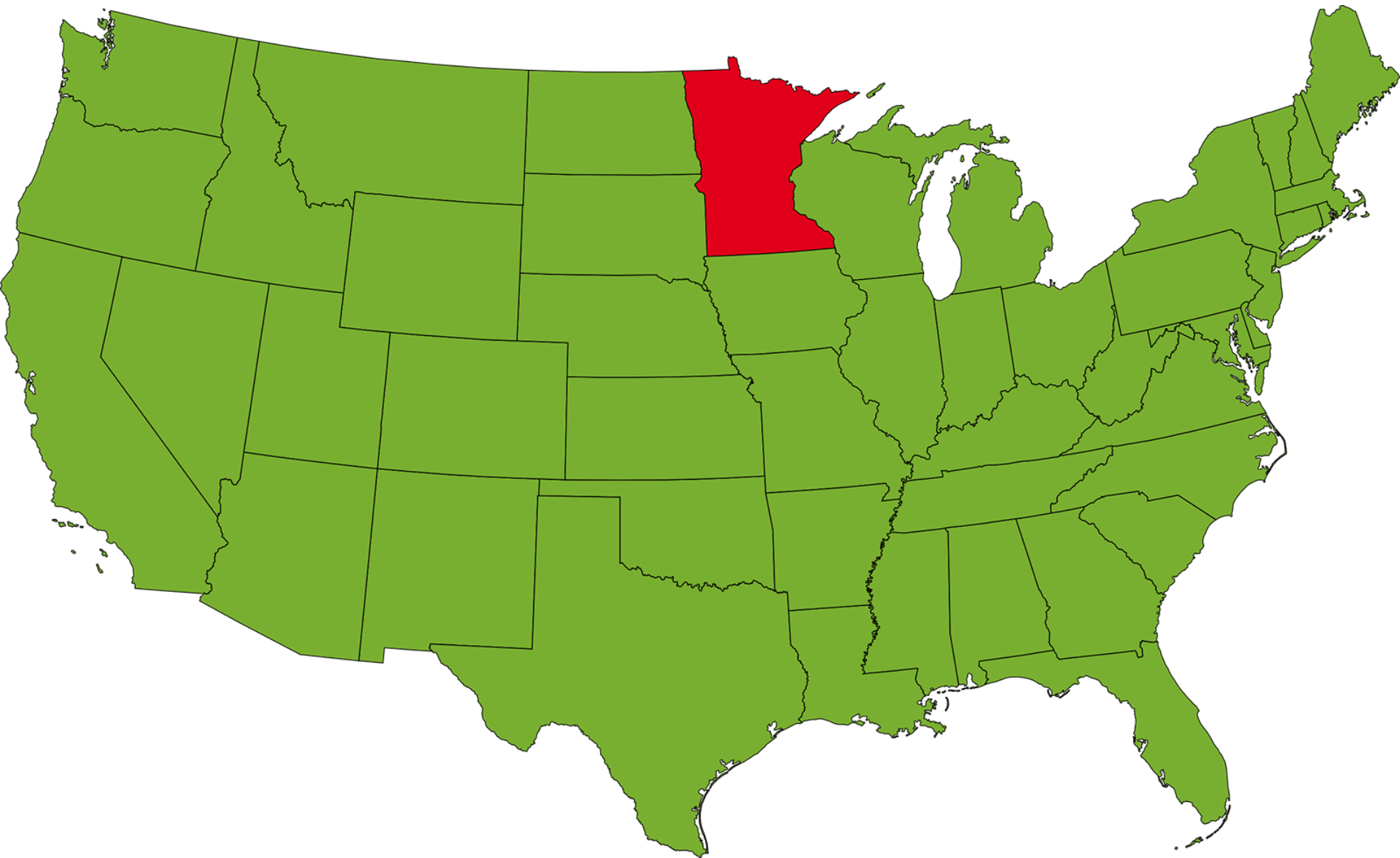
- **Pros:** already collected, NBS infrastructure provides low cost, >99% clinical follow-up
- **Cons:** Low sensitivity; 70% analytical with Quanta, clinical unknown

Saliva

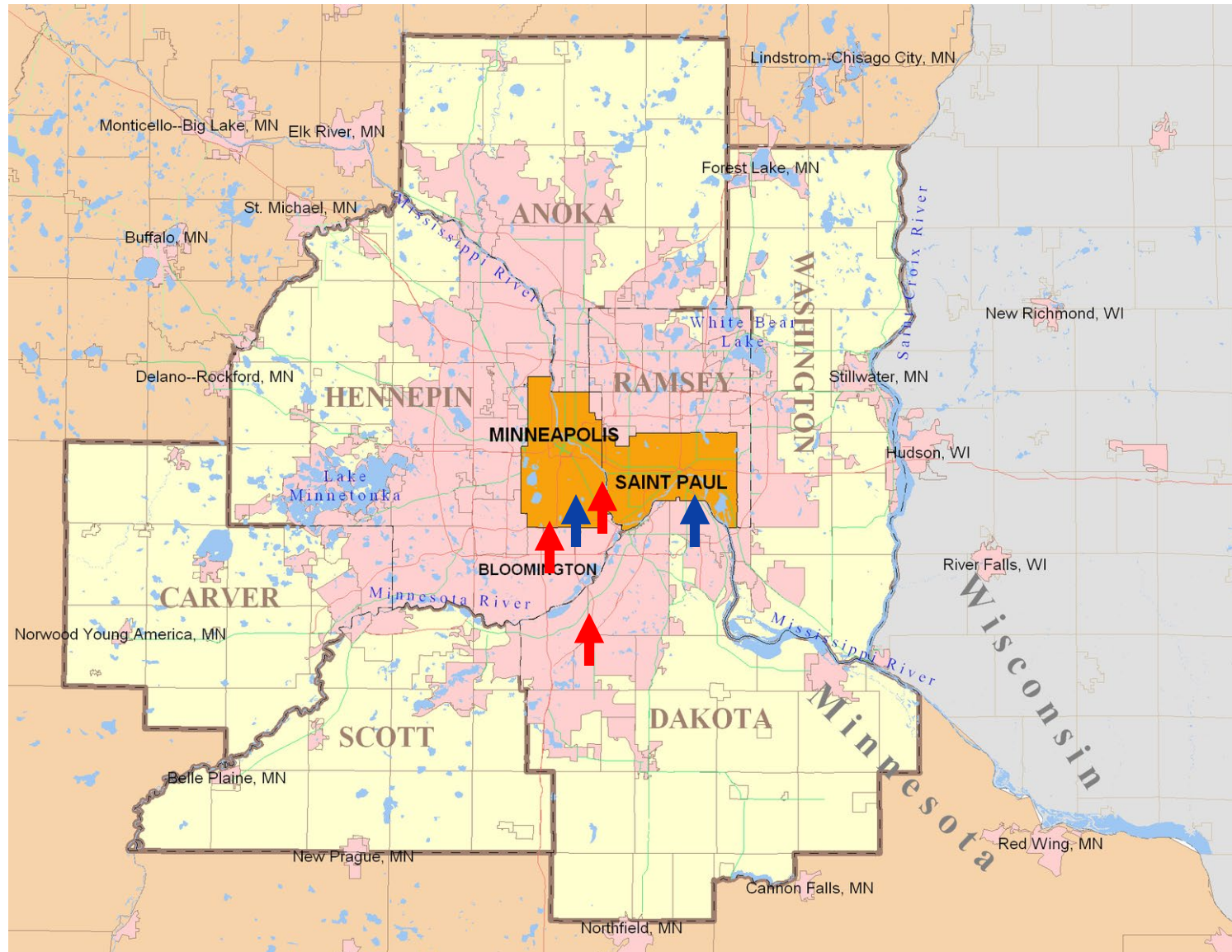
- **Pros:** high sensitivity, tested on large scale (CHIMES) and appears feasible
- **Cons:** collection and testing need new infrastructure, requires confirmation with urine, variable sample quality, possible false negatives

Urine on filter paper

- **Pros:** high sensitivity and specificity, testing uses NBS infrastructure, possible use for other disorders
- **Cons:** collection needs new infrastructure, not tested on large scale for CMV



Congenital CMV Infections in Minnesota Newborn Screening Study



Congenital CMV Infections in Minnesota Newborn Screening Study: Site of Enrollment

Facility	Enrolled	Percentage
Allina/Abbot NW	2108	26%
United (St. Paul)	872	10.6%
Fairview/UMMC	1644	20%
Fairview/Burnsville	1386	16.9%
Fairview/Edina	2171	26.5%
TOTAL	8181	

Total cCMV Infections: 30 (0.37%)

Congenital CMV Infections in Minnesota Newborn Screening Study: Diagnostic Evaluation

Positive Saliva/Blood Spot Screen



MDH Notification to Primary Care Provider



Infectious Diseases Referral

Laboratory Evaluation

- IgM/IgG
- Hepatic Panel
- CBC/diff
- Urine PCR
- Blood PCR



- Ophthalmology
- Audiology
- Head Ultrasound

Congenital CMV Infections in Minnesota Newborn Screening Study: Disease Classification

Review

Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy



William D Rawlinson, Suresh B Boppana, Karen B Fowler, David W Kimberlin, Tiziana Lazzarotto, Sophie Alain, Kate Daly, Sara Doutré, Laura Gibson, Michelle L Giles, Janelle Greenlee, Stuart T Hamilton, Gail J Harrison, Lisa Hui, Cheryl A Jones, Pamela Palasanthiran, Mark R Schleiss, Antonia W Shand, Wendy J van Zuylen

*Lancet Infect Dis 2017;
17: e177-88*

Congenital CMV Infections in Minnesota Newborn Screening Study: Disease Classification

Category	Asymptomatic	Asymptomatic with Isolated SNHL	Mildly Symptomatic	Moderately-to-Severely Symptomatic	Total
NICU	0	1	1	2	4
Term	21	1	4	0	26
Total	21	2	5	2	30

Mildly Symptomatic Disease in Term Newborns: 15% (4/26)

Congenital CMV Infections in Minnesota Newborn Screening Study: Hearing Outcomes to Date

Classification	Hearing Screen Refer/Total	Hearing Loss Any SNHL/Total
NICU	2/4 (50%)	2/4 (50%)
Term	1/26 (4%)	2/26 (8%)*
Total	3/30 (10%)	4/30 (13%)

*One infant developed unilateral moderate SNHL at 4 months

Congenital CMV Infections in Minnesota Newborn Screening Study: CNS Imaging Findings

Head Ultrasound Findings

- Normal (n=20)
- Abnormal CMV-associated (n=4)
 - Periventricular calcifications/WM disease
 - Cystic changes
- Mineralizing vasculopathy (n=2)
- LSV (n=1)
- No imaging (n=3)

Congenital CMV Infections in Minnesota Newborn Screening Study: Antiviral Therapy

Category	Asymptomatic	Asymptomatic with Isolated SNHL	Mildly Symptomatic	Moderately-to-Severely Symptomatic	Total
NICU	0	1/1	0/1	2/2	3/4
Term	1/21*	1/1	3/4	0	5/26
Total	1/21	2/2	3/5	2/2	8/30

* Valcyte was commenced at 4 months of age in one asymptomatic infant who developed SNHL beyond the newborn period

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