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Spot the difference: Minnesota's experience with spot-to-spot variability in the detection of Congenital Cytomegalovirus (cCMV) in dried blood spots using real-time PCR

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Newborn Screening Background

Newborn Screening

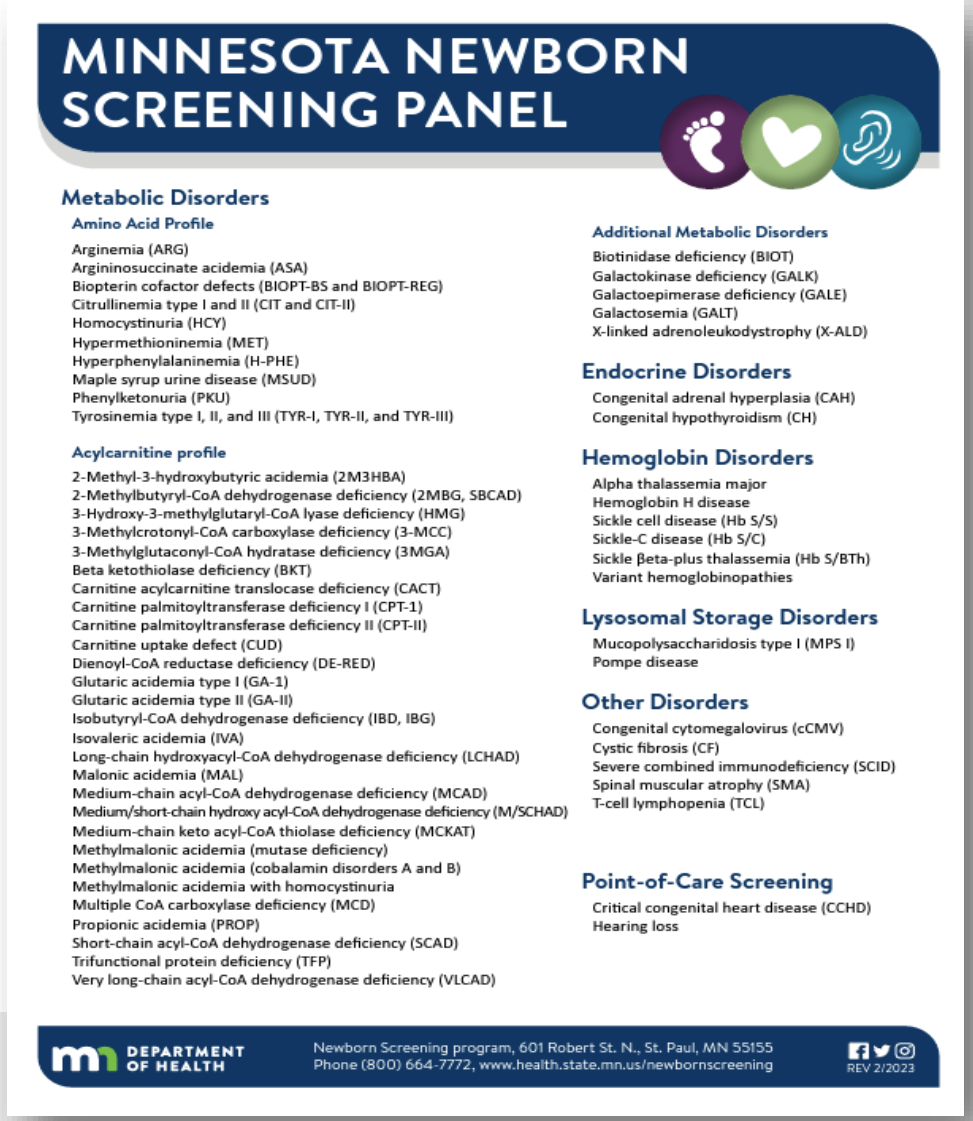
A public health program, mandated by law, which aims to reduce morbidity and mortality in newborns and children who have, or are at risk for, heritable or congenital disorders.

Disorders screened for must meet certain criteria, such as:

1. Disorders **affect the neonate**, are **severe**, and **not obvious at birth**
2. Testing is **inexpensive**, **reliable**, and **able to screen 200+ babies per day**
3. Treatment is **available** and **effective**

What is Newborn Screening?

- Blood spot screening – more than 50 metabolic, endocrine, blood, genetic, infectious, and immune disorders
- Hearing screening – loss of hearing in the range speech is heard
- Heart (pulse oximetry) screening – critical congenital heart disease



MINNESOTA NEWBORN SCREENING PANEL

Metabolic Disorders

Amino Acid Profile

Arginemia (ARG)
Argininosuccinate acidemia (ASA)
Biopterin cofactor defects (BIOPT-BS and BIOPT-REG)
Citrullinemia type I and II (CIT and CIT-II)
Homocystinuria (HCY)
Hypermethioninemia (MET)
Hyperphenylalaninemia (H-PHE)
Maple syrup urine disease (MSUD)
Phenylketonuria (PKU)
Tyrosinemia type I, II, and III (TYR-I, TYR-II, and TYR-III)

Acylcarnitine profile

2-Methyl-3-hydroxybutyric acidemia (2M3HBA)
2-Methylbutyryl-CoA dehydrogenase deficiency (2MBG, SBCAD)
3-Hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG)
3-Methylcrotonyl-CoA carboxylase deficiency (3-MCC)
3-Methylglutaconyl-CoA hydratase deficiency (3MGA)
Beta ketothiolase deficiency (BKT)
Carnitine acylcarnitine translocase deficiency (CACT)
Carnitine palmitoyltransferase deficiency I (CPT-1)
Carnitine palmitoyltransferase deficiency II (CPT-II)
Carnitine uptake defect (CUD)
Dienoyl-CoA reductase deficiency (DE-RED)
Glutaric acidemia type I (GA-1)
Glutaric acidemia type II (GA-II)
Isobutyryl-CoA dehydrogenase deficiency (IBD, IBG)
Isovaleric acidemia (IVA)
Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)
Malonic acidemia (MAL)
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)
Medium/short-chain hydroxy acyl-CoA dehydrogenase deficiency (M/SCHAD)
Medium-chain keto acyl-CoA thiolase deficiency (MCKAT)
Methylmalonic acidemia (mutase deficiency)
Methylmalonic acidemia (cobalamin disorders A and B)
Methylmalonic acidemia with homocystinuria
Multiple CoA carboxylase deficiency (MCD)
Propionic acidemia (PROP)
Short-chain acyl-CoA dehydrogenase deficiency (SCAD)
Trifunctional protein deficiency (TFP)
Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)

Additional Metabolic Disorders

Biotinidase deficiency (BIOT)
Galactokinase deficiency (GALK)
Galactoepimerase deficiency (GALE)
Galactosemia (GALT)
X-linked adrenoleukodystrophy (X-ALD)

Endocrine Disorders

Congenital adrenal hyperplasia (CAH)
Congenital hypothyroidism (CH)

Hemoglobin Disorders

Alpha thalassemia major
Hemoglobin H disease
Sickle cell disease (Hb S/S)
Sickle-C disease (Hb S/C)
Sickle β-plus thalassemia (Hb S/BTh)
Variant hemoglobinopathies

Lysosomal Storage Disorders

Mucopolysaccharidosis type I (MPS I)
Pompe disease

Other Disorders

Congenital cytomegalovirus (cCMV)
Cystic fibrosis (CF)
Severe combined immunodeficiency (SCID)
Spinal muscular atrophy (SMA)
T-cell lymphopenia (TCL)

Point-of-Care Screening

Critical congenital heart disease (CCHD)
Hearing loss

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Newborn Screening program, 601 Robert St. N., St. Paul, MN 55155
Phone (800) 664-7772, www.health.state.mn.us/newbornscreening
REV 2/2023

Blood Spot Screening

Goal: Screen for >50 disorders affecting a variety of systems (e.g., blood, muscle, endocrine, pulmonary, metabolism, etc)

When: between 24-48 hours

Method used: multiple laboratory methods

Collected at hospital/birth site but tested at MDH Public Health Laboratory

Results: if nothing flags, results available within 5-7 days

Minnesota Timeline

1961: Dr. Guthrie develops first screening test for PKU

1964/5: MN began newborn screening – PKU only

1968: Congenital hypothyroidism

1974: Galactosemia

1988: Sickle cell disease/hemoglobinopathies

2001: Addition of MS/MS testing panel doubles (AA, FAO, OA)

2007: Hearing screening

2013: Severe combined immunodeficiency

2014: Critical congenital heart disease (CCHD)

2017: Lysosomal storage diseases (MPS I and Pompe) and X-ALD

2018: Spinal muscular atrophy

2023: Congenital cytomegalovirus (cCMV)

2024: Krabbe Disease

2025: Duchenne muscular dystrophy and Guanidinoacetate methyltransferase (GAMT) deficiency

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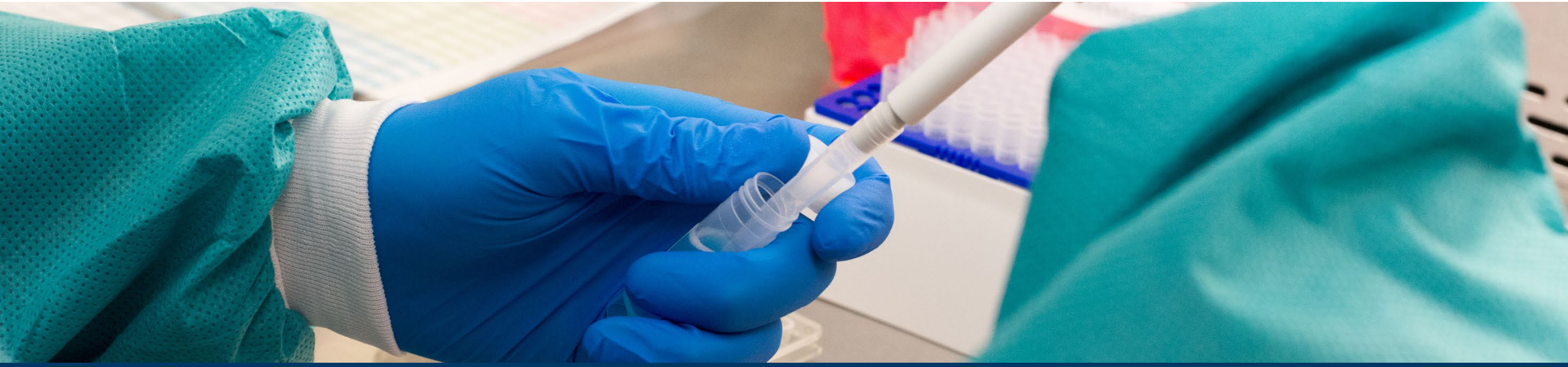
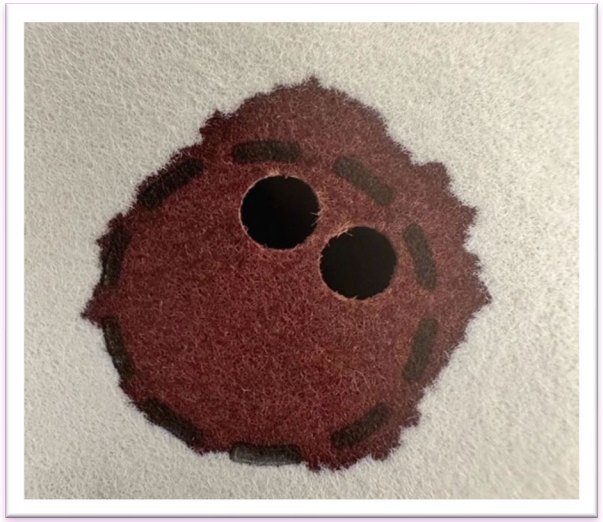


Laboratory Test

Cytomegalovirus Screening

- Started Screening on 2/6/2023
- Cytomegalovirus (CMV) screening involves performing real-time PCR, which measures the presence or absence of CMV DNA in the dried blood spot
- Laboratory developed assay using revvity NeoMDx™ cCMV real-time PCR reagents

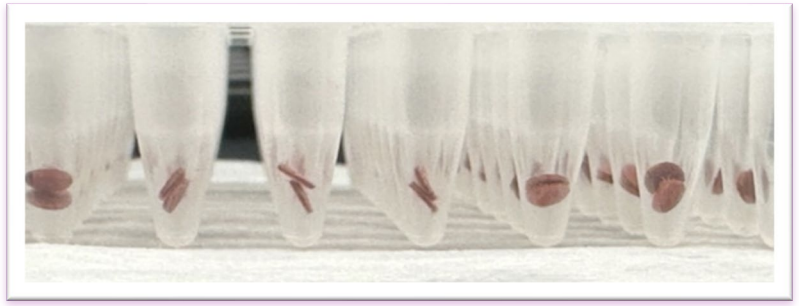


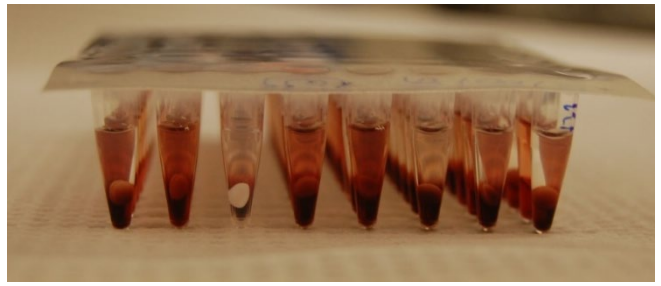
CMV Reagents

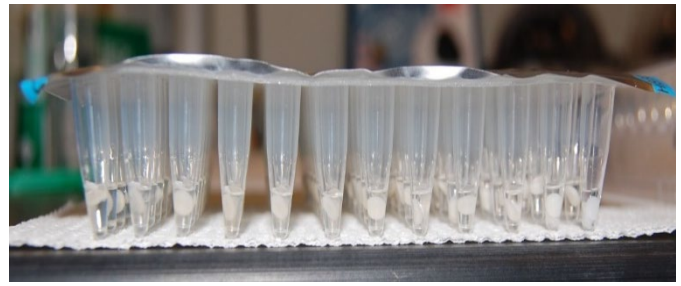
- revvity's CMV reagents
  - Quality control materials- 3 different levels of CMV in dried blood spots
  - Premade reagents with stability criteria and expiration dates

DNA Extraction

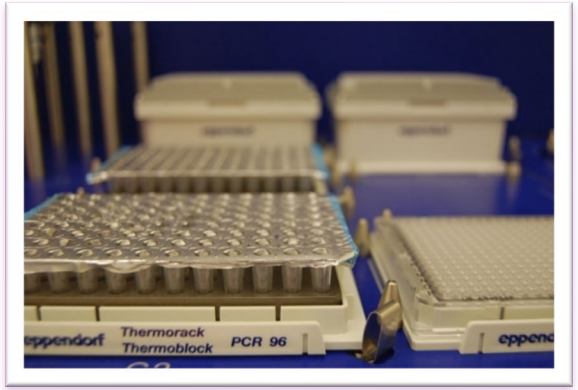
DNA extracted from dried blood spots using two punches for each specimen using Qiagen Solution 1 and 2







Eppendorf epMotion liquid handler transfers master mix and extracted DNA to 384-well PCR reaction plate

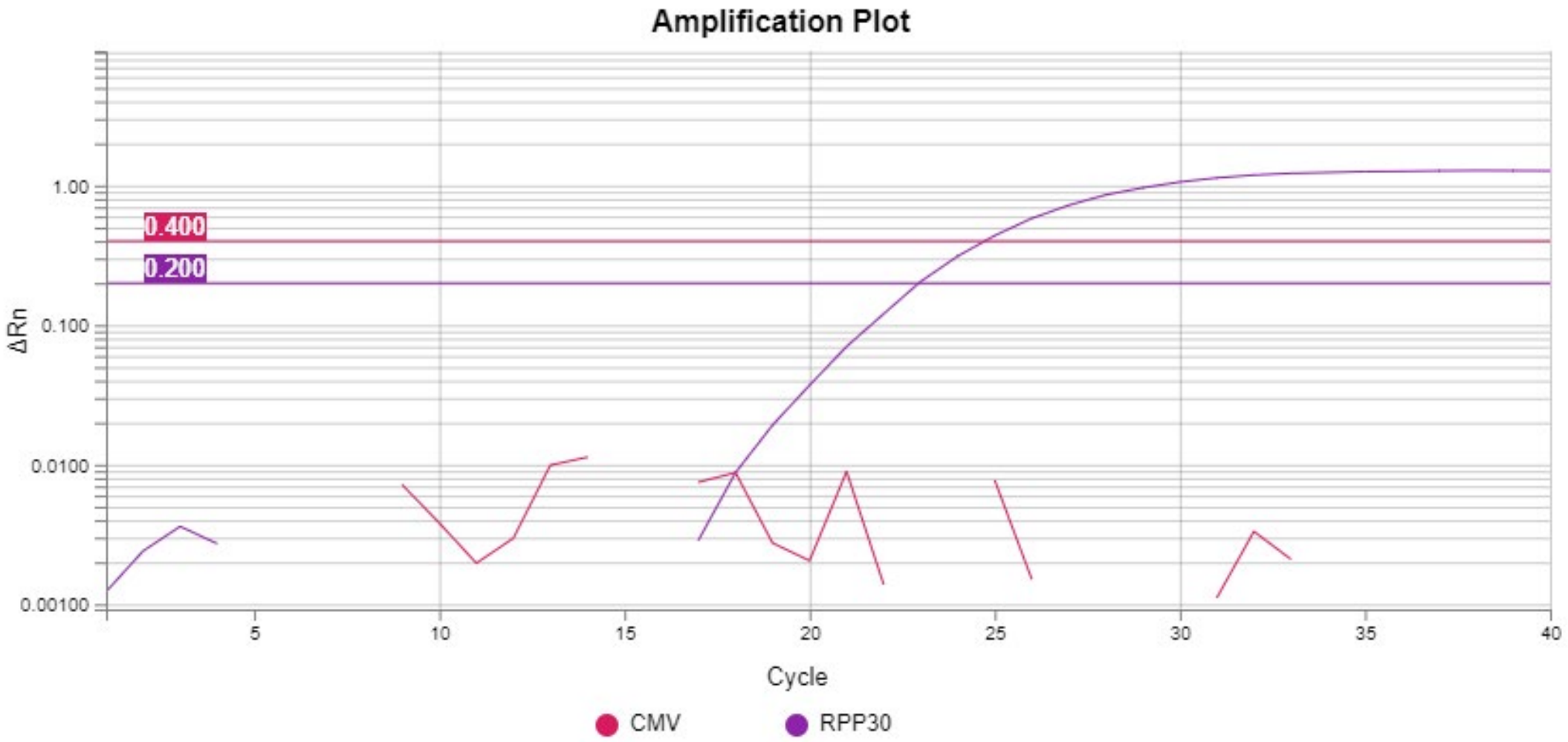


Real-Time PCR

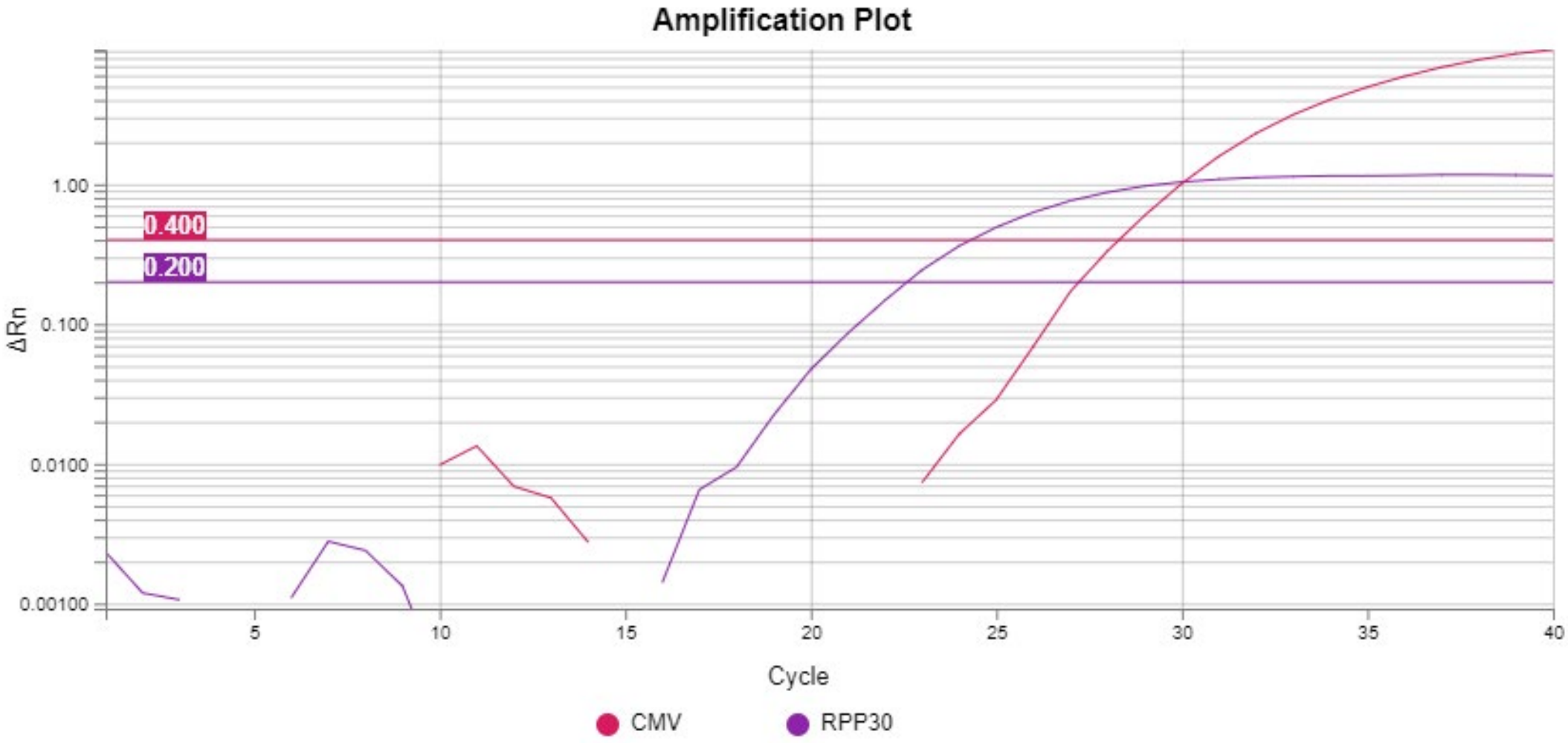
QuantStudio 7 Pro Real-Time PCR System 384-well plate

1 Cycle	1 Cycle	40 Cycles	End
(Ramp 1.6°C/sec) **37°C – 2:00 min**	(Ramp 1.6°C/sec) **94°C – 10:00 min**	(Ramp 1.6°C/sec) **93°C – 10 sec** (Ramp 1.6°C/sec) **60°C – 30 sec** (Ramp 1.6°C/sec) **69°C – 40 sec**	

CMV Not Detected

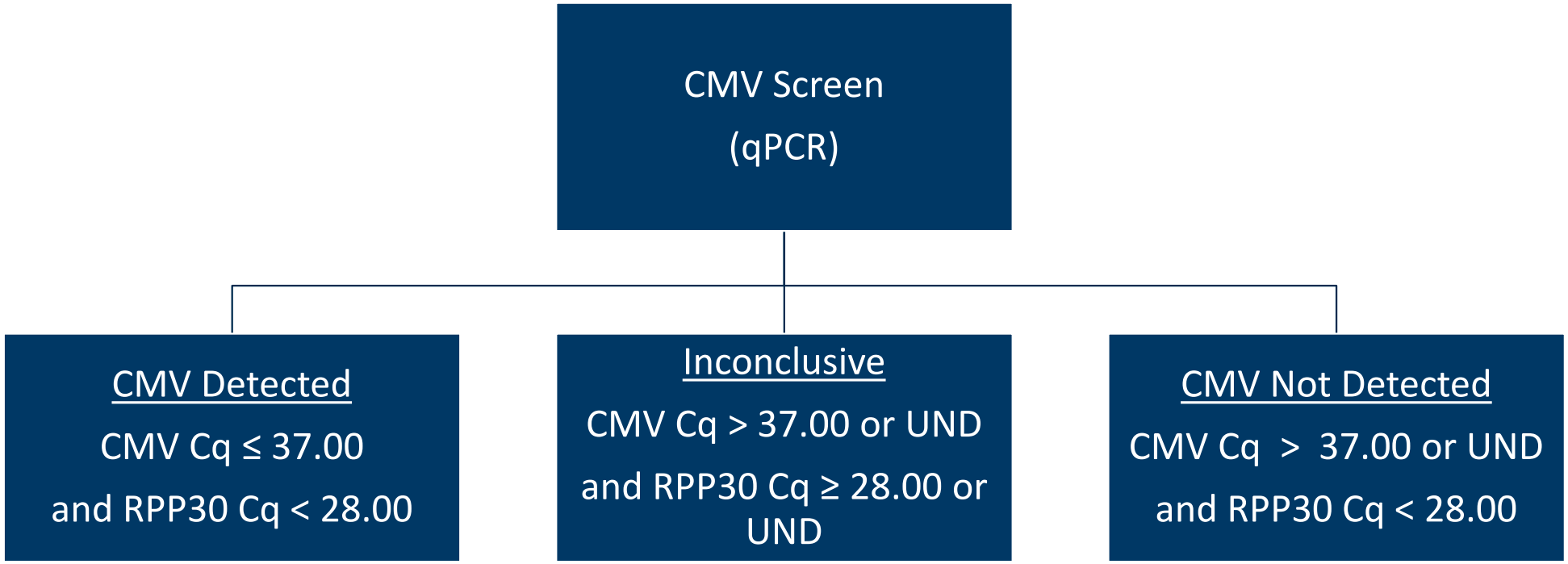


CMV Detected



Cut-offs

Retest: CMV Cq ≤ 40.00 and/or RPP30 Cq ≥ 28.00



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Experiences with Blood Spot Variability

Specimen 1

	CMV Cq	RPP30 Cq
Initial Result	UND	24.07

Reported Result: CMV Not Detected

Specimen 2

	CMV Cq	RPP30 Cq
Initial Result	39.92	24.99
Retest 1	38.63	25.40
Retest 2	38.06	24.84

Reported Result: CMV Not Detected

Specimen 3

	CMV Cq	RPP30 Cq
Initial Result	**34.59**	24.81
Retest 1	**33.81**	23.98
Retest 2	**30.49**	23.27

Reported Result: CMV Detected

Confirmed clinically to have CMV

Specimen 4

	CMV Cq	RPP30 Cq
Initial Result	**36.38**	22.84
Retest 1	UND	25.55
Retest 2	38.43	24.09
Retest 3	UND	25.35
Retest 4	**34.26**	25.32

Reported Result: CMV Detected

Confirmed clinically to have CMV

Specimen 5

	CMV Cq	RPP30 Cq
Initial Result	38.84	23.24
Retest 1	38.76	23.76
Retest 2	**34.21**	23.60
Retest 3	**36.11**	24.18
Retest 4	UND	24.22

Reported Result: CMV Detected

Confirmed clinically to have CMV

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Reported False Negatives

False Negatives in Newborn Screening

- Newborn screening is a population-based screening
  - Cut-offs are set to detect the disorder while minimizing false positive results
  - Babies missed by screening are considered false negatives
- False negatives can occur in Newborn Screening
- False negatives reported to the program require immediate investigations

Reportable Condition

- In September 2024, congenital cytomegalovirus became a reportable condition.
- Positive clinical CMV results collected from infants ≤ 90 days of age or from amniotic fluid must be reported to MDH within one working day.
- The Newborn Screening Program performs an investigation on all babies with clinical positive CMV results at ≤ 21 days life that were not identified by screening.

False Negative Investigation – Baby A

Provider reported CMV positive urine sample

Initial Result	CMV Cq	RPP30 Cq
Reported Result	UND	24.10

Investigation	CMV Cq	RPP30 Cq
Spot 1	UND	23.72
Spot 2	UND	23.87
Spot 3	UND	23.72
Spot 4	UND	23.93
Spot 5	UND	24.00
Spot 6	UND	23.71

Investigation Conclusion: No CMV detected in the blood spot

False Negative Investigation – Baby B

Provider reported CMV positive saliva on day 3 of life, positive urine on day 4 of life

	CMV Cq	RPP30 Cq
Reported Result	39.85	24.56

Investigation	CMV Cq	RPP30 Cq
Spot 1	UND	24.99
Spot 2	UND	24.67
Spot 3	UND	24.72
Spot 4	UND	24.71
Spot 5	UND	24.94
Spot 6	UND	24.77

Investigation Conclusion: No CMV detected in the blood spot

False Negative Investigation – Baby C

First specimen collected at 29 hours (No CMV Detected)

	CMV Cq	RPP30 Cq
Reported Result	39.53	23.90

Provider reported CMV positive urine sample day 7 of life. Second specimen received and was being tested.

False Negative Investigation – Baby C

Second specimen collected at 13 days **(CMV Detected)**

	CMV Cq	RPP30 Cq
Initial Result	**34.93**	25.66
Retest 1	**32.95**	24.31
Retest 2	**33.91**	24.91

Third specimen collected at 22 days **(CMV Detected)**

	CMV Cq	RPP30 Cq
Initial Result	**28.82**	23.42
Retest 1	**28.78**	23.57
Retest 2	**29.06**	24.19

Twin Case Investigation- Twin A

	CMV Cq	RPP30 Cq
Reported Result	UND	24.03

Investigation	CMV Cq	RPP30 Cq
Spot 1	UND	23.78
Spot 2	UND	24.10
Spot 3	UND	23.89
Spot 4	UND	23.99
Spot 5	UND	23.70

Reported DBS Result: CMV Not detected

Provider reported false negative: CMV detected via urine

Twin Case Investigation- Twin B

	CMV Cq	RPP30 Cq
Reported Result	**35.58**	24.06

Investigation	CMV Cq	RPP30 Cq
Spot 1	UND	23.90
Spot 2	**36.56**	24.00
Spot 3	UND	24.49
Spot 4	**34.55**	24.18
Spot 5	**34.29**	24.18

Reported DBS Result: CMV detected

Provider reported false positive: CMV Not detected via urine

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Data

Specimen Data

February 6, 2023 – August 1, 2025

- 160,406 specimens screened
- 1,304 specimens retested (anything with a CMV Cq value ≤ 40.00 or RPP30 ≥ 28.00)
  - Approximately a 0.81% retest rate
    - Severe Combined Immunodeficiency 1.04% retest rate
    - Congenital Adrenal Hyperplasia 0.73% retest rate
    - Galactosemia 0.08% retest rate
- 512 specimens reported out as CMV detected (CMV Cq value ≤37.00)
  - Approximately 1 in 313 specimens screened reported as CMV detected

Summary

- The Minnesota Newborn Screening Laboratory has been able to successfully screen for congenital CMV
- There is a high amount of variability of CMV DNA in dried blood spots
- DNA extraction methods used with dried blood spots could reduce viral load due to the loss of DNA material during wash steps
- Congenital CMV being a reported condition will allow the program to identify potential false negatives
- As additional newborn screening programs start screening for congenital CMV, we will continue to learn the best way in which to screen for cCMV

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Thank you!

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