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A Pilot Study in NYS to Perform Newborn Screening for Congenital Cytomegalovirus

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Outline

- Introduction to cytomegalovirus
- Newborn screening for congenital cytomegalovirus
- Pilot study in NYS
- Methodology used
- Validation data
- Workflow

Cytomegalovirus

- Cytomegalovirus (CMV) is a member of the Herpesviridae family
- CMV has a linear, double-stranded DNA genome
- The coding sequence of CMV is >230 kb
- The virus has an icosahedral capsid, a tegument layer and an outer, lipid bilayer envelope

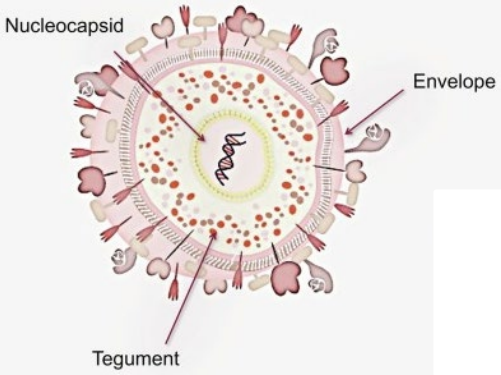

Figure from: Cytomegalovirus Mark R. Schleiss, in Maternal Immunization, 2020

CMV Infection

- Humans and other primates are natural hosts
- After infection, CMV remains with the host life-long in a chronic and then latent state
- In healthy people, CMV rarely causes disease
- In immunocompromised individuals or babies infected in utero it can cause serious disease
- CMV spreads through body fluids, such as blood, saliva, urine, semen and breast milk

Symptoms of CMV in Newborns

- Hearing Loss
- Visual impairment
- Premature birth
- Low birth weight
- Jaundice
- Petechiae – rashes or purple skin
- Microcephaly
- Enlarged spleen
- Pneumonia
- Seizures

Newborn Screening for Congenital CMV

- Since the 1990s there have been publications on detecting CMV in DBS from newborns
- Ontario NBS Program started screening in 2019
- Minnesota NBS Program started screening in 2023
- NYS NBS Program obtained funding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) to perform screening of all newborns born in NYS for cCMV for 1 year

Screening Tests

- Extraction of nucleic acid from dried blood spots, followed by qPCR

  Convenient but sensitivity is an issue. Most recent study indicates sensitivity of approx. 75% in DBS (Dollard et al., 2021)

- Diagnostic Test (Gold standard):

  Detection of CMV in urine by PCR

qPCR = quantitative polymerase chain reaction; DBS = dried blood spot

Pilot Study in NYS

- IRB approval – waiver of consent
- Investigate and validate an assay
- Obtain regulatory approval for performing the assay
- Collaborate with:
    - Specialty Care Centers in NYS
    - Early Hearing Detection and Intervention
- Educate providers:
    - Create educational material with translations
    - Webinar for hospitals

Actions for NBS Program

- Hired 2 laboratory and 1 Follow-up staff members
- Performed literature searches
- Selected and compared several nucleic acid extraction methods and qPCR methods
- Obtained and validated instrumentation
- Validated a screening method
- Developed SOPs and worksheets
- Set up testing in NBS LIMS (Neometrics)
- Provided opt-out options
- Submitted validation package to the NYS Clinical Laboratory Evaluation Program (CLEP)

LIMS = laboratory information management system

Factors Considered when Developing the CMV Molecular Assay

- Nucleic acid extraction method
- Number of dried blood spots
- qPCR method
- Automation
- Sensitivity/specificity/cost/throughput/practicality

Assay Controls

- CMV Controls:
  - Extracted CMV DNA (from cell culture)
  - Blood from adult CMV infected patients
  - DBS from babies who were suspected of having cCMV
  - Kit controls

- Internal Controls:
  - RNase P (commercially available)
    - Used in SCID/SMA assay
  - NeoMDx cCMV Real-time PCR Assay (Perkin Elmer/Revvity) internal control
    - RPP30 (Ribonuclease P/MRP Subunit P30, RNase P)

SCID/SMA = severe combined immunodeficiency/spinal muscular atrophy

Nucleic Acid Extraction Methods

- Home-brew: CASM method
  - Cheap
  - Easy
  - Already being used in the NBS Program for SCID/SMA
- Quanta Extracta DBS Buffer (QuantaBio)
- QIAamp 96 DNA Blood Kit (Qiagen #51161 or 51162) / QIAamp DNA Blood Mini Kit (Qiagen #51104 or 51106) / QIAGEN Buffer ATL (Qiagen #939011 or #19076) / QIAGEN Proteinase K (Qiagen #19131 or #19133)
- GenElute-E Single Spin Blood DNA 96 Kit (Millipore Sigma or Krackeler - #EC196)
- Perkin Elmer/Revvity Elution Solution

CASM = Home brew extraction method
SCID/SMA = severe combined immunodeficiency/spinal muscular atrophy

qPCR Methods

- Boppana et al., 2005 (targets CMV glycoprotein B [gB] gene)
- Boppana et al., 2010 (immediate early 2 [IE2] gene)
- Soetens et al., 2008 (UL83 region)
- Yun et al., 2003, modified per Dupuis et al., 2011 (polymerase POL gene)
- Thermo Fisher human CMV TaqMan assay Vi06439643_s1
- 2 Perkin Elmer/Revvity assays:
  - NeoMDx cCMV Real-time PCR Assay (wet chemistry)[UL122 gene region (regulatory protein IE2)]
  - Dry chemistry

Final CMV Screening Assay

- Number of DBS: 2
- Extraction method: Quanta Extracta Buffer
- qPCR method: NeoMDx cCMV Real-time PCR Assay (Revvity/Perkin Elmer)
- qPCR instrument: EonisQ
- Automation: Janus Liquid Handler Workstation for some steps

DBS = dried blood spot

Validation Results - LOD

- Limit of Detection (LOD) of qPCR assay: 10 gene copies

CMV (gene copies)	RNase P 1	CMV Ct 1	RNase P 2	CMV Ct 2	RNase P 3	CMV Ct 3	RNase P 4	CMV Ct 4
1000	UND	27.52	UND	27.58	UND	27.64	UND	27.47
100	UND	31.57	UND	31.38	UND	31.49	UND	31.34
50	UND	32.55	UND	32.57	UND	32.33	UND	32.39
10	UND	34.23	UND	35.39	UND	33.4	UND	35.67
5	UND	35.05	UND	UND	UND	UND	UND	UND
1	UND	UND	UND	UND	UND	UND	UND	UND
0.1	UND	UND	UND	UND	UND	UND	UND	UND

UND = undetected

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Validation Results - Specificity

Agents	NeoMDx cCMV Real-time PCR Assay Results
Herpes simplex virus 1	UND
Herpes simplex virus 2	UND
Varicella zoster virus	UND
Epstein Barr virus	UND
Human herpes virus 6	UND
Streptococcus pneumoniae_1	UND
Legionella pneumophila	UND
Streptococcus pyogenes	UND
Adenovirus type 3	UND
Adenovirus type 4	UND
Adenovirus type 5	UND
Adenovirus type 8	UND
Adenovirus type 31	UND
Parvovirus B19	UND
Treponema pallidum	UND
Listeria monocytogenes	UND
HIV (positive newborn DBS)	UND
HIV (positive newborn DBS)	UND
POS (1000 gene copies)	28.8
POS (100 gene copies)	32.9
NTC	UND

UND = undetected; POS = positive control; NTC = no template control

- Primers and probes for the qPCR assay did not cross-react with other organisms in the specificity panel

Validation Results – No. of DBS

- Extracta DBS extraction consistently gave better results with 2 DBS compared to 1 DBS

Viral Load IU/mL	Extracta Extraction 1 DBS (%)	Extracta Extraction 2 DBS (%)
441	0/12 (0%)	1/16 (6.3%)
612	1/6 (16.7%)	2/10 (20%)
1700	3/13 (23.1%)	5/17 (29.4%)
1770	5/11 (45.4%)	7/15 (46.7%)
~2000	6/15 (40%)	10/19 (52.6%)
2250	6/15 (40%)	11/19 (57.9%)
7060	11/15 (73.3%)	17/19 (89.5%)

DBS = dried blood spot

Validation Results – Reproducibility

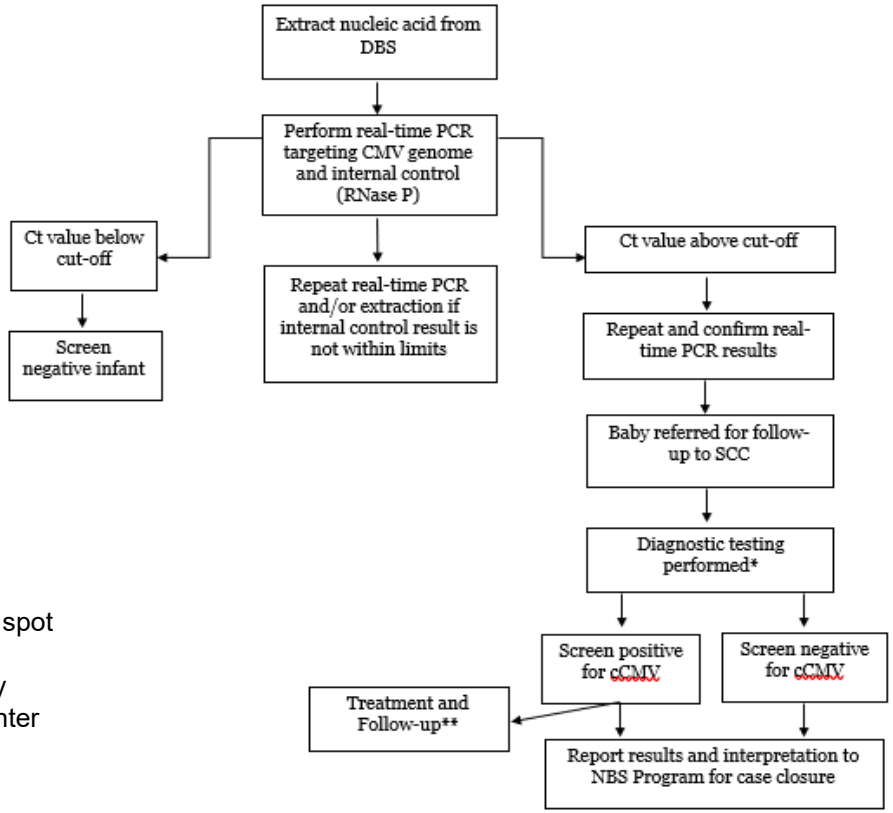
Reproducibility	%CV
Intra-assay	≤ 3.6
Inter-assay	≤ 4.0

CV = coefficient of variation

Validation Results – Retrospective Screening

	Number
Specimens screened	29,279
CMV Positive results	65
Incidence	1 in 450

Workflow Diagram



DBS = dried blood spot
*PCR of urine
**As determined by Specialty Care Center (SCC)

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Prospective Screening

- To start in October 2023 for a period of 1 year
- Approximately 210,000 babies will be screened and CMV positive babies will be referred to specialty care centers in NYS for follow-up

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