Detection of Congenital Cytomegalovirus Infection on High-Risk Newborn Population

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BACKGROUND

- Up to 0.5% of babies in the U.S are born with congenital CMV infection
- 10% of these babies can have clinical symptoms such as hearing loss
- Leading environmental cause of hearing loss
- More common than most diseases in the current RUSP
- Antiviral medicines are available, can reduce the risk of developing hearing loss
- Targeted screening on high-risk newborn population is mandated in several states
- Universal screening on all newborns is under consideration

METHODS

- A high-throughput qPCR assay was developed for the detection of CMV infection using DBS
- The cCMV kit provides reagents for both DNA extraction and qPCR
- Two 3.2mm DBS punches were used for a simple buffer exchange DNA extraction
- The qPCR reaction was setup and performed on the Roche LightCycler 480 instrument.
- Primers and probes for a human housekeeping gene, the RPP30 gene, are included as a quality/quantity indicator of DNA isolated from DBS

LIMIT of DETECTION (LoD)

- CMV Analytical Q panel from Qnostics was used to determine the limit of detection of this assay.
- Viral load range from 0 to 50,000 IU/ml.
- Samples were tested for a total of 12 times.
- The lowest viral load (5,000 IU/ml) that was detected >95% of times was determined to be the LoD.

Sample ID	Viral Load (IU/ml)	Detected/Total Run # (% detection rate)
CMVAQP02-S01	50,000	12/12 (100%)
CMVAQP02-S02	15,000	12/12 (100%)
CMVAQP02-S03	5,000	12/12 (100%)
CMVAQP02-S04	1,500	4/12 (33%)
CMVAQP02-S05	500	1/12 (8%)
CMVAQP02-S06	150	0/12 (0%)
CMVAQP02-S07	50	0/12 (0%)
CMVAQP02-S08	15	0/12 (0%)
CMVAQP02-S09	0	0/12 (0%)

ANALYTICAL SENSITIVITY & SPECIFICITY

- 2019 QCMD proficiency samples and 15 CMV negative DBS samples were used.
- These samples were tested for a total of 12 times.
- Results of 2019 QCMD samples listed in table below
- All 15 CMV negative DBS samples were negative on all 12 repeat runs
- Analytical specificity was calculated to be 100%.
- Analytical Sensitivity was calculated to be 95.24%.

Sample ID	Expected Result	Detected/Total run #
CMVDBS20S-01	**Positive**	**12/12 (100%)**
CMVDBS20S-02	**Positive**	**12/12 (100%)**
CMVDBS20S-03	**Negative**	**0/12 (0%)**
CMVDBS20S-04	**Positive**	**10/12 (83%)***
CMVDBS20S-05	**Positive**	**10/12 (83%) ***
CMVDBS20S-06	**Positive**	**12/12 (100%)**
CMVDBS20S-07	**Positive**	**12/12 (100%)**
CMVDBS20S-08	**Positive**	**12/12 (100%)**

*False negative results were likely due to the viral load was at or near the LoD of the assay.

DETECTION OF cCMV ON HIGH-RISK NEWBORN POPULATION

- This assay was performed on DBS specimens from newborns who failed hearing screening (started on 09/21/2021).
- A total of 48 samples were run (by 02/172022).
- Two were detected positive.
- Possible reasons for those negative results include:
  - Hearing loss caused by genetic factors
  - Hearing loss caused by other environmental factors
  - Viral loads below the LoD of the assay at the time of sample collection
  - False positive hearing screening results.

CONCLUSION

- Next Generation Sequencing (NGS) based hearing loss panels are now commonly ordered but they do not detect the non-genetic cause of hearing loss such as CMV infection.
- This assay is now used for a high-risk population which includes newborns who fail the newborn hearing screen
- Once adopted on an automated liquid handlers system, this assay can be used for population based newborn screening for cCMV.

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