Evaluation of a Multiplexed, Sample-to-Answer, Microarray System for the Detection of Encephalitic Viruses

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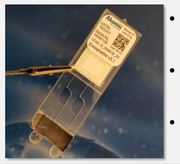
Monday - 15

Background

- Identification of the cause of encephalitis can prove challenging as cerebrospinal fluid (CSF) is an invasive specimen to collect, with small sample volumes and commonly low viral titers.
- Molecular assays, with high sensitivity and the potential for multiplexing are advantageous.
- We developed an encephalitis microarray platform in an all-plastic lateral flow cell, for the detection of HSV1, HSV2, VZV, CMV, HHV6, enterovirus, and West Nile virus (WNV).
- Lateral flow cell designed for simplified workflow, including simultaneous amplification and microarray detection within a single reaction chamber, and in a fully contained, closed amplicon system.
- The analytical and clinical sensitivity of this assay was evaluated from extraction, through amplification, hybridization, and imaging, to assess its performance relative to that of individual real-time assays for the same targets.

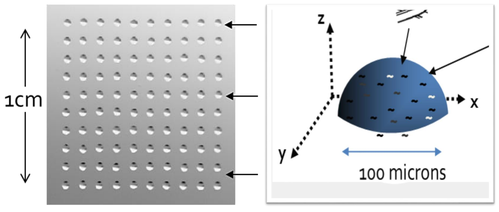
Akonni Biosystems Encephalitis TruArray Panel



- Lateral Flow Array (LFA) – Plastic slide containing two flow chambers houses low-density "gel-drop" microarrays
- Gel elements are copolymerized with specific target capture probe and have a 3D structure allowing increased hybridization efficiency
- Amplification, hybridization, and imaging all occur onboard the LFA within a closed system

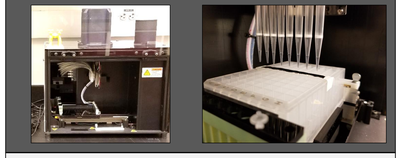
8-Plex Primer Pool

Target	Gene Target
HSV1	UL44 Envelope glycoprotein C
HSV2	UL3 Nucleophosphoprotein
VZV	ORF28: DNA Polymerase catalytic subunit
CMV	UL54 DNA Polymerase
HHV6	U38 DNA Polymerase
Enterovirus	5' UTR
WNV	Envelope
GFP	GFP Transcript



Methods

Extraction

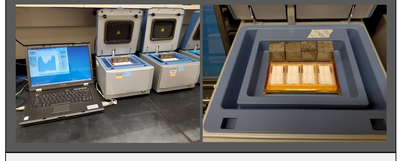


- Artificial CSF amended with quantified viral isolates
- Serially diluted, divided into multiple replicates
- Spiked with GFP RNA transcript as an extraction, amplification, and inhibition control.
- Extraction and onboard reverse transcription performed using Akonni TruTip Technology on Automated Work Station

Master mix

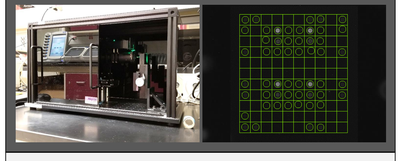
- Amplification and hybridization with Platinum Multiplex PCR Master mix (ThermoFisher).
- Master mix for encephalitis TruArray Panel utilizes an 8-plex primer pool containing self-avoiding molecular recognition system (SAMRS) primers for all targets except VZV

Amplification & Hybridization



- Quantified RT products amplified and hybridized simultaneously on plastic lateral flow cell microarrays
- Asymmetric PCR with 10:1 primer ratio. 10X primers are Cy-3 labeled for single-stranded amplicon binding and imaging
- Performed on heat blocks, modified with a copper strip to isolate heat transfer to LFA.

Image Analysis

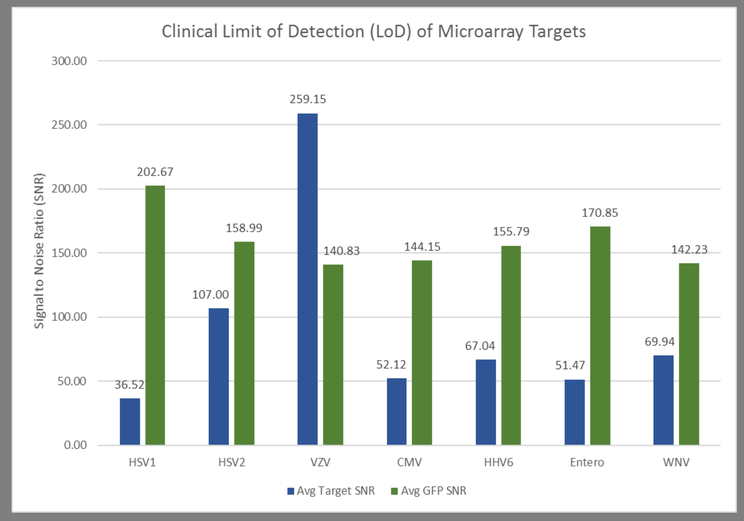


- LFA's washed and imaged directly on the slide with 1000ms exposure
- Amplicon signal is measured against background to generate a signal to noise ratio (SNR) for each gel spot

Encephalitis TruArray v.3.1 Chip Map

- (Left) Sample loading and LFA washing conducted through a single port onboard the array
- (Right) Example of HHV6 Positive microarray
  - Targets are measured in quadruplicate per microarray
  - Green Fluorescent Protein (GFP) used as internal positive control
  - Cy3 beacons on microarray used for positional reference

Clinical Sensitivity



Viral Target	HSV1	HSV2	VZV	CMV	HHV6	Entero	WNV
Avg Target SNR	36.52	107.00	259.15	52.12	67.04	51.47	69.94
Avg GFP SNR	202.67	158.99	140.83	144.15	155.79	170.85	142.23
Dilution gc/ml	1.03E+04	5.21E+04	1.62E+03	6.55E+03	1.28E+04	1.05E+04	1.07E+04
Average gc/ul	14	56	8	10	9	8	3
Average gc/rxn	58	242	36	43	40	36	13
Average CT	35.18	33.42	37.11	33.15	31.43	33.35	33.45
# Positive	39/40	40/40	40/40	40/40	40/40	40/40	55/56
% Positive	97.50%	100%	100%	100%	100%	100%	98.21%

- Limits of detection (LoD) – determined as lowest viral DNA concentration in which at least 95% of extracted replicates were detected on the array (starting from extraction)
- Quantitation of virus determined by real-time PCR standard curves using in-house developed standards

Analytical Sensitivity

Viral Target	HSV1	HSV2	VZV	CMV	HHV6	Entero	WNV
Avg SNR	45	43	300	178	25	65	63
GC/rxn	90.00	195	< 25	43	22	4	5
SNR range	42-48	3-107	262-327	114-252	3-47	44-86	31-95
# Positive	2/2	8/8	4/4	4/4	4/4	2/2	2/2

- Analytical sensitivity – determined as lowest viral DNA concentration in which all replicates tested positive
- Determined using nucleic acid from high titer in-house controls

Analytical & Clinical LoD Comparison

Viral Target	Analytical Sensitivity (gc/rxn)	Clinical Sensitivity (gc/rxn)
HSV1	90	58
HSV2	195	242
VZV	< 25	36
CMV	43	43
HHV6	22	40
Entero	4	36
WNV	5	16

[a] Detection of reverse transcribed product from viral nucleic acid
[b] Detection of reverse transcribed product after automated TruTip extraction and RT of virus-amended samples

Conclusions/Discussion

- A multiplexed amplification microarray system has been developed for the detection of viral encephalitis targets in clinical CSF samples, with sensitivities below 50 gene copies/reaction for VZV, CMV, HHV6, enterovirus, and WNV in both analytical and clinical sensitivity experiments.
- Slightly higher detection limits of 60 and 200 gene copies/reaction were observed for HSV1 and HSV2 respectively.
- Additional optimization experiments are ongoing to improve the HSV1 and HSV2 sensitivity.
- The evaluation demonstrated the effective performance of all subparts of the fully integrated and automated sample-to-answer system, with high sensitivity and clinical utility in the multiplexed format.
- Further studies are planned to evaluate assay specificity, the effect of interfering substances, and array performance with clinical CSF samples.

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