Alinity m HSV 1 & 2 / VZV – a new qualitative multi-plex PCR test

PP-156

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BACKGROUND

HSV-1, HSV-2 and VZV cause self-resolving skin or mucosal diseases and concurrently establish persistent latent infections in sensory ganglia neurons. HSV-1 primarily affects the oral area and is mainly transmitted through oral-to-oral contact; however, the virus can also cause genital herpes. HSV-1, HSV-2 and VZV often cause phenotypically indistinguishable skin blisters or lesions. VZV lesions typically localized in specific anatomical areas (dermatomes) can also spread to other areas, including the genital region.

We evaluated the performance of Alinity m HSV 1&2 / VZV, a new fully automated qualitive multiplex real-time PCR developed for simultaneous detection and differentiation of the three pathogens in clinician-collected swab specimens from cutaneous and mucocutaneous lesions.

METHODS

A HSV-1&2/VZV/Syphilis Positive Swab EQA sample (Microbix Biosystems Inc.) resuspended in 3 ml 0.9% NaCl was tested in triplicate with Alinity m HSV 1&2/VZV and Allplex Genital ulcer assays to compare their response to the three pathogens targeted by the Alinity assay. Assay linearity and precision were assessed by testing 10 replicates of a dilutional series prepared from HSV-1/2 and VZV EQA samples (2.000.000 cp/mL of inactivated virus each; Exact Diagnostics Verification Panel, BioRad) with concentrations ranging from 5000 to 20 cp/mL for each virus and mixture of the 3 viruses.

A total of 360 residual archived (-20°C) clinical samples (lesion swabs [N=206], urine [N=21] and pooled urine/swab samples [N=81] collected in Aptima Specimen Collection Kits (Hologic), pre-selected based on previous results established during laboratory routine testing with the Allplex Genital ulcer Assay (Seegene), detecting HSV-1/2, VZV, *T. pallidum*, *H. ducreyi*, LGV and CMV and the Allplex STI Essential Assay (Seegene), detecting *C. trachomatis, N. gonorrhoeae, M. genitalium, M. hominis, T. vaginalis*, *U. urealyticum* and *U. parvum*. All specimens were parallel-tested with Alinity m and Allplex Genital ulcer assays; 42 samples with insufficient volume were diluted with Aptima Collection fluid (maximum factor 1:2) prior to testing.

RESULTS

Both assays detected the Herpes viruses in the EQA positive swabs at high concentrations with the lower Ct-values for Alinity (3-5 cycles, see Tab. 1). Both assays demonstrated high dilutional sensitivity and linearity, with slightly lower sensitivity for VZV. No compensatory effects were seen in triple-positive samples compared to single-positive samples. The mean Ct-values of the ten measurements of the 5000 cp/mL dilution were identical in the single virus measurement and the mixture of all three viruses. Overall, precision was higher with the Alinity assay (see Tab. 2).

All samples pretest-negative for HSV-1/2 and VZV [N=100] tested negative with the Alinity assay. No cross-reactivity for non-targeted sexually-transmitted pathogens present in 144 clinical samples (most often *U. urealyticum* and *C. trachomatis* and up to four different additional microorganisms) in the absence or presence of HSV-1/2 or VZV was observed with the Alinity test. For HSV-1, 101 of 109 pretest-positive samples were confirmed by at least one subsequent test. For HSV-2 127 of 128, and all VZV were confirmed. Overall-agreement between Alinity and Allplex for qualitative detection of HSV-1, HSV-2 and VZV in clinical samples was 95.4% (104/109), 98.4% (126/128), and 100% (30/30), respectively (see Figures 1-3). Alinity identified 4 infections missed by Allplex, while 3 infections were only detected by Allplex.

CONCLUSIONS

The Alinity m HSV 1 & 2 / VZV assay demonstrated excellent efficacy in detecting the Herpes viruses, exhibiting no discernible competitive effects in samples with co-infections. The test showed good linearity and higher sensitivity for VZV and HSV-2 compared to the Allplex Genital Ulcer Assay. The random-access capability of the Alinity platform is a valuable tool in clinical routine.

ACKNOWLEDGEMENTS: The study was funded by Abbott GmbH, Wiesbaden, Germany.

Tab. 1: EQA swab tested in triplicates with Allplex and Alinity

EQA sample	Allplex HSV-1	Allplex HSV-2	Allplex VZV	Alinity HSV-1	Alinity HSV-2	Alinity VZV
1	23,98	20,78	23,94	18,58	16,13	20,28
2	24,08	20,91	23,75	18,83	16,28	20,30
3	24,00	20,72	23,69	18,69	16,17	20,15
Allplex TP	28,00	28,10	28,20	-	-	-

Tab. 2: Dilution series with Exact Diagnostics Verification Panel

HSV-1 (cp/ml)	Allplex Det Rate	Allplex Mean	Allplex SD	Allplex %CV	Alinity Det Rate	Alinity Mean	Alinity SD	Alinity %CV
5000	100%	27,48	0,72	2,62%	100%	25,04	0,65	2,60%
500	100%	31,22	1,79	5,73%	100%	28,14	0,41	1,46%
100	50%	33,04	1,66	5,32%	50%	30,52	0,93	3,05%
50	20%	32,86	0,49	1,48%	20%	30,73	0,31	1,01%
20	20%	33,49	0,53	1,61%	0%			
HSV-2 (cp/ml)	**Allplex Det Rate**	**Allplex Mean**	**Allplex SD**	**Allplex %CV**	**Alinity Det Rate**	**Alinity Mean**	**Alinity SD**	**Alinity %CV**
5000	100%	32,92	0,65	1,97%	100%	26,43	0,24	0,91%
500	60%	36,7	0,84	2,29%	95%	30,09	0,45	1,50%
100	25%	37,7	0,6	1,63%	50%	32,18	0,47	1,46%
50	5%	37,6			35%	32,3	0,26	0,80%
20	5%	37,4			10%	32,94	0,01	0,03%
VZV (cp/ml)	**Allplex Det Rate**	**Allplex Mean**	**Allplex SD**	**Allplex %CV**	**Alinity Det Rate5**	**Alinity Mean2**	**Alinity SD3**	**Alinity %CV4**
5000	95%	32,73	0,99	3,02%	100%	30,2	0,38	1,26%
2500	40%	33,39	0,26	0,78%	100%	31,53	0,3	0,95%
2000	80%	34,32	0,46	1,38%	40%	32,59	0,02	0,06%
1500	0%				30%	32,62	0,31	0,95%
1000	0%				40%	32,4	0,02	0,06%
500	0%				5%	32,75		
100	0%				0%			

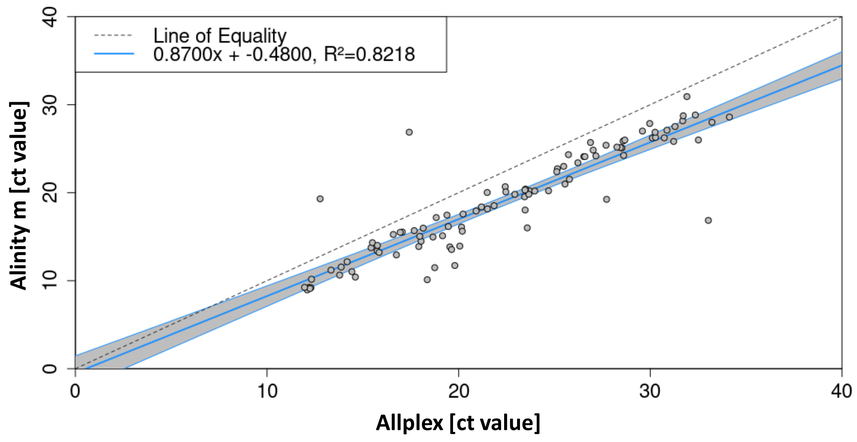

Fig. 1: Linear regression analysis for HSV-1

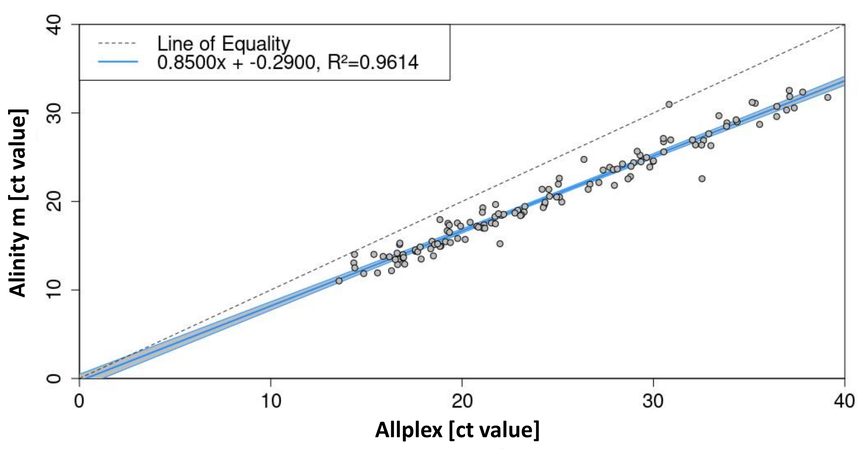

Fig. 2: Linear regression analysis for HSV-2

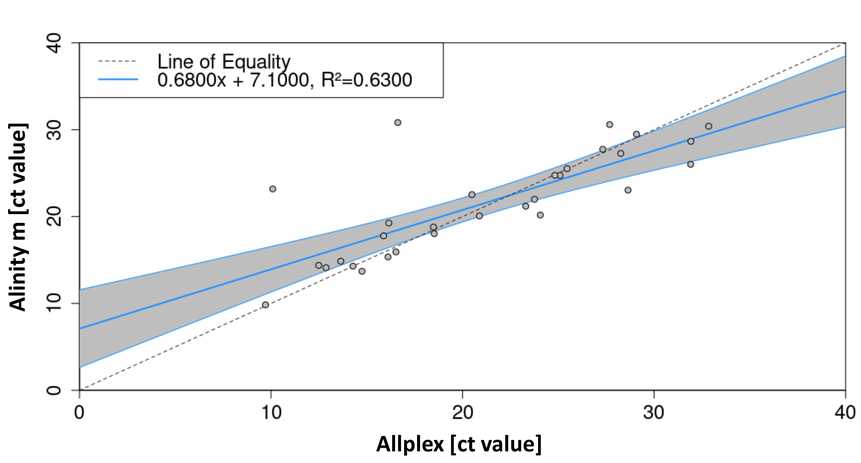

Fig. 3: Linear regression analysis for VZV

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ESCV 2024