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The power to reach higher in Real-time PCR

affigene® VZV tracer in QCMD proficiency program 2010

Background

Quality Control for Molecular Diagnostics (QCMD) specialises in the standardisation and quality control for molecular diagnostics and genomic technologies. A proficiency panel for Varicella Zoster Virus (VZV) was distributed in February 2010 to 177 participants from 27 different countries. 190 qualitative datasets were reported to QCMD of which 69 were analysed with commercial real-time PCR assays. In total, six affigene VZV tracer datasets were reported to QCMD. One of these datasets was submitted by Cepheid AB and is presented in this report.

Material and methods

The QCMD VZV panel 2010 (VZVDNA10) consisted of ten samples in total. Eight samples were positive for either VZV strain Ellen or 9/84, with expected viral loads ranging from 21 to 3639 copies/ml. The two remaining samples were negative for VZV, however one was positive for Herpes Simplex Virus 1 to check for cross reacticvity with a closely related virus. At the time of analysis, sample types and concentrations were unknown to the operator. The QCMD panel was prepared using affigene DNA extraction. Subsequently, the samples were analysed in duplicates by real-time PCR using affigene VZV tracer on the Mx3000P instrument (Agilent/Stratagene). The samples were analysed and reported to QCMD.

Results and discussion

QCMD uses a scoring system ranging from 0 points (‘highly satisfactory‘) to 3 (‘highly unsatisfactory‘) A correct result for the qualitative performance gives 0 points. Depending on the sample concentration, incorrect results give between 1 and 3 points per sample.

Qualitative results

All samples except for one were correclty determined as positive or negative respectively by affigene VZV tracer. Only the lowest sample was undetected, but with a concentration of 21 c/ml this is below the Limit if Detection (LOD) of the assay, i.e. the titer level where 95% of all replicates of a sample are determined as positive.

The remaining four low titer samples were detected, despite being under the established LOD. The affigene performance in relation to the best attainable and the average reported score is shown in Table 1.

Average score commercial real-time PCR (n=69)	In-house score affigene **VZV tracer (n=1)**	Best score attainable
2.2	1	0

Table 1 Qualitative QCMD scores for commercial real-time PCR assays.

A correct result gives 0 points. Depending on the sample concentration, incorrect results give 1-3 points. For the complete ten sample panel, the best attainable score is 0 and the worst 30. The table shows the average values for the 69 qualitative commercial real-time PCR datasets, and the value for the Cepheid affigene VZV tracer dataset.

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The results of the qualitative panel for individual samples are summarised in Table 2 below.

Performance on core proficiency samples

QCMD has designated six panel members as 'core proficiency samples' in order to establish a measure of performance. Correct analysis of these key samples are expected in order to achieve 'required level of proficiency' for the panel. affigene VZV tracer reported all core proficiency samples correctly. The proficiency samples are indicated below in Table 2.

QCMD EQA Panel VZV10DNA Core Proficiency Samples (✓)				affigene VZV tracer (n=1)		Commercial real time PCR (n=69)
Sample	Strain	VZV c/ml	Expected	Replicates	Reported	Correctly reported
1	9/84	165*	pos	+/+	pos	84.1%
2 ✓	-	0	neg	-/-	neg	98.6%
3	9/84	90*	pos	+/-	pos	71.0%
4	Ellen	63*	pos	+/+	pos	34.8%
5 ✓	HSV-1	0	neg	-/-	neg	97.1%
6 ✓	9/84	408	pos	+/+	pos	97.1%
7 ✓	Ellen	202*	pos	+/+	pos	95.7%
8 ✓	9/84	3639	pos	+/+	pos	98.6%
9	Ellen	21*	pos	-/-	neg	44.9%
10 ✓	Ellen	1941	pos	+/+	pos	97.1%

** Sample concentration below LOD as determined for affigene VZV tracer*

Table 2 Summary of qualitative results including perfromance on core proficiency samples

The table lists the details of the VZV10DNA panel including strain, sample concentration and expected qualitative result for each panel member. The six key samples designated as core proficiency samples are indicated in the table (✓). The qualitative result of the two affigene VZV tracer replicates are displayed as well as the reported result for this dataset. Five of the samples had concentrations below the LOD of the assay, however only the lowest of these samples was undetected. The percentage of correctly reported datasets from commercial real-time PCR assays are listed for each sample.

Conclusion

- The affigene VZV tracer assay performed very well in the QCMD VZV proficiency panel 2010, detecting samples from both included strains VZV 9/84 and VZV Ellen.
- The assay shows excellent performance in the qualitative analysis, correctly reporting all samples expect one sample below the LOD of the assay that was not detected.
- affigene VZV tracer reported 100% correct results on the six core proficiency samples.

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