

## affigene® HSV 1/2 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 Herpes Simplex Virus (HSV) was distributed in February 2010 to 216 participants from 32 different countries. 227 qualitative datasets were reported to QCMD of which 79 were analysed with commercial real-time PCR assays. In total, three affigene HSV1/2 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 HSV panel 2010 (HSVDNA10) consisted of 10 samples in total. Four positive samples were included for each HSV-1 and HSV-2, with expected viral loads ranging from 115 to 9016 copies/ml and 69 to 4710 c/ml for HSV-1 and HSV-2 respectively. The two remaining samples were negative for HSV-1 and HSV-2, however one was positive for Varicella-Zoster Virus to check for cross reactivity 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 as duplicates by real-time PCR using affigene HSV 1/2 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. For each sample, incorrect results give between 1 and 3 points depending on the sample concentration.

#### Qualitative results

The affigene HSV 1/2 tracer dataset achieved the best possible overall scoring of 0 points, correctly reporting all samples as positive or negative respectively. Four of the low titer samples were detected despite being under the established Limit of Detection of the assay. The affigene performance in relation to the best attainable score and the average reported score is shown in Table 1.

Average score commercial real-time PCR (n=79)	In-house score affigene® HSV 1/2 tracer (n=1)	Best score attainable
2.8	0	0

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

A correct result gives 0 points for the panel. 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 panel score for the 79 qualitative commercial real-time PCR datasets, and the score for the Cepheid affigene® HSV 1/2 tracer dataset.

The lowest sample, HSV-2 69 c/ml was only detected in 23% of the commercial real-time PCR datasets. Of all qualitative datasets, only 19% reported all samples correctly. The results of the qualitative panel for individual samples are reported in Table 2 below.

**Performance on core proficiency samples**

QCMD has designated six panel members as ‘core proficiency samples’ to serve as a measure of performance. Correct analysis of these key samples are expected in order to achieve ‘required level of proficiency’ for this panel. affigene HSV 1/2 tracer reported all core proficiency samples correctly. The proficiency samples are indicated below in Table 2.

QCMD EQA Panel HSV10DNA Core Proficiency Samples (✓)				affigene HSV 1/2 tracer (n=1)			Commercial real time PCR (n=79)
Sample	Content	HSV c/ml	Expected	HSV-1 Repl.1/Repl.2	HSV-2 Repl.1/Repl.2	Reported	Correctly reported
1 ✓	HSV-2	4 710	HSV-2 +	-/-	+/+	HSV-2 +	96.2%
2 ✓	HSV-1	968	HSV-1 +	+/+	-/-	HSV-1 +	94.9%
3	HSV-2	69*	HSV-2 +	-/-	+/+	HSV-2 +	22.8%
4 ✓	VZV	0	neg	-/-	-/-	neg	100%
5 ✓	HSV-2	1294	HSV-2 +	-/-	+/+	HSV-2 +	94.9%
6	HSV-1	129*	HSV-1 +	+/+	-/-	HSV-1 +	77.2%
7 ✓	HSV-1	9 016	HSV-1 +	+/+	-/-	HSV-1 +	97.5%
8 ✓	-	0	neg	-/-	-/-	neg	97.5%
9	HSV-2	178*	HSV-2 +	-/-	+/+	HSV-2 +	69.6%
10	HSV-1	116*	HSV-1 +	+/+	-/-	HSV-1 +	78.5%

\* Sample concentration below LOD as determined for affigene® HSV 1/2 tracer

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

The table lists the details of the HSV10DNA panel including content, 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 results of the affigene HSV 1/2 tracer dataset samples are listed. Four of the samples had concentrations below the LOD for the assay, however these samples were all detected. The percentage of correctly reported datasets from commercial real-time PCR assays are listed for each sample.

**Conclusion**

- The affigene HSV 1/2 tracer assay shows excellent performance in the QCMD HSV proficiency panel 2010.
- All samples were correctly reported as positive or negative respectively for HSV-1 and HSV-2.
- The sample positive for the closely related Varicella-Zoster virus was correctly reported as negative for both HSV-1 and HSV-2. This sample was included to test the assay specificity.
- affigene HSV 1/2 tracer shows no cross-reactivity between HSV-1 and HSV-2 samples.
- affigene HSV 1/2 tracer reported 100% correct results on the six core proficiency samples.