

#### Results of LOD validation of qPCRs used in table 4.

Samples were spiked with viruses (grown in the laboratory or NIBSC international virus standards) at different loads per volume extracted, and expressed as TCID50 or IU when international standard were available (B19, HBV, HCV), see Material and Methods). The limit of detection (LOD) is the lowest virus load that can be detected within a stated confidence limit (< 5%).

#### HBV

Spiked HBV LOD determination			
Number of International Units (UI) spiked in the volume extracted	Experiment	Number of detected replicates	Frequency
$10^4$	1	3/3	100%
	2	3/3	
	3	3/3	
$10^3$	1	2/3	77.7%
	2	3/3	
	3	2/3	
$10^2$	1	1/3	11%
	2	0/3	
	3	0/3	
$10^1$	1	0/3	0%
$10^0$	1	0/3	0%

In this validation, the LOD was defined as the lowest amount of target sequence that can be detected in at least 2 out of 3 replicates. Based on these results, the LOD for spiked HBV was defined at 1000 UI per extraction (280µl) or 3571 UI/ml

## HCV

Spiked HCV LOD determination			
Number of International Units (UI) spiked in the volume extracted	Experiment	Number of detected replicates	Frequency
$10^{3.47}$	3	2/2	100%
$10^3$	1	2/2	100%
	2	3/3	
	3	3/3	
	4	2/2	
	5	2/2	
$10^2$	1	2/2	90%
	2	3/3	
	3	2/3	

The LOD for spiked HCV UI was defined as 300 UI per extraction (280 $\mu$ l) or 1071 IU/ml, as the  $10^3$  UI and  $10^2$  UI were detected respectively with a frequency of 100% and 90%.

## H3N8

Spiked H3N8 LOD determination			
Number of genome copies (gc) spiked in the volume extracted	Experiment	Number of detected replicates	Frequency
$10^4$	1	3/3	100%
	2	3/3	
	3	3/3	
$10^3$	1	3/3	100%
	2	3/3	
	3	3/3	
$10^2$	1	3/3	100%
	2	3/3	
	3	3/3	
$10^1$	1	1/3	11.1%
	2	0/3	
	3	0/3	

The LOD for spiked H3N8 virus was defined as  $10^2$  gc per extraction (280  $\mu$ l) or 357 cp/ml.

### FLUBV-B/LEE/40 LOD

Spiked FLUBV-B/LEE/40 LOD determination			
Number of copies (cp) spiked in the volume extracted	Experiment	Number of detected replicates	Frequency
10 <sup>4</sup>	1	3/3	100%
	2	3/3	
	3	3/3	
10 <sup>3</sup>	1	3/3	100%
	2	3/3	
	3	3/3	
10 <sup>2</sup>	1	3/3	88.8%
	2	2/3	
	3	3/3	
10 <sup>1</sup>	1	0/3	33.3%
	2	3/3	
	3	0/3	

In this validation, the LOD was defined as the lowest amount of target sequence that can be detected in at least 2 out of 3 replicates. Based on these results, the LOD for spiked FLUBV-B/LEE/40 viruses was defined as 10<sup>2</sup> copies per extraction (280 µl) or 357 copies/ml.

## RhCMV

Spiked RhCMV LOD determination			
Number of TCID <sub>50</sub> spiked in the volume extracted	Experiment	Number of detected replicates	Frequency
3.8	1	2/2	100%
0.38	1	2/2	100%
	2	2/2	
	3	2/2	
0.038	1	2/2	50%
	2	0/2	
	3	1/2	

The LOD for spiked RhCMV viruses was defined as 0.38 TCID<sub>50</sub> per extraction (140 µl) or 3 TCID<sub>50</sub>/ml.

## BVDV-1

Spiked BVDV LOD determination				
Number of TCID <sub>50</sub> spiked in the volume extracted	Experiment	Number of detected replicates	Ct values obtained	Frequency
200	1	2/2	29.18	100%
			29.42	
	2	2/2	29.04	
			29.18	
	3	2/2	30.94	
			30.95	
	4	2/2	31.22	
			31.27	
2000	1	2/2	22.41	100%
			22.19	
	2	2/2	23.24	
			22.83	

	BVDV Ct standards values								Eff.	R <sup>2</sup>	Validity
	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10	1			
<b>1</b>	18.84	22.11	26.02	29.46	32.67	35.58	38.89	n.d.	97.4%	0.999	<b>Yes</b>
	19.00	22.36	26.01	29.39	33.41	36.10	39.58	n.d.			
						35.58	39.32	n.d.			
<b>2</b>	19.09	22.21	25.12	29.00	32.21	36.50	39.99	n.d.	91.9%	0.996	<b>Yes</b>
	19.03	21.97	25.51	29.02	32.75	38.23	n.d.	n.d.			
	19.06	22.06	25.38	29.01	32.65	35.39	n.d.	n.d.			

n.d. : not detected

Cp values obtained for standards defined as genome copies number per qPCR reaction.

The extraction of 200 PFU led to 29.00 -31.27 Ct , corresponding to approximately 10<sup>4</sup> genome copies in the dose-effect curve (Ct values ranged between 29.00 and 29.46), corresponding to 100-fold the LOD (close to 100 genome copies per qPCR reaction).

We have thus established the LOD of spiked BVDV viruses at 200UFP/100 = 2 UFP per extraction (280 µl) or 7.14 PFU/ml, rounded to 10 TCID<sub>50</sub>/ml.

### Had-5

	HAde-5 Cp standards values								Eff.	Error	Validity
	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10	1			
<b>Experiment 1</b>	18.68	22.36	26.29	29.93	33.77	36.10	>40	n.d.	86.7%	0.0178	<b>Yes</b>
	18.57	22.37	26.51	29.83	33.30	37.22	n.d.	n.d.			
	18.65	22.46	26.85	29.96	33.24	37.21	n.d.	n.d.			
<b>Experiment 2</b>	18.09	22.75	25.90	29.59	33.75	38.88	n.d.	n.d.	81.9%	0.0242	<b>Yes</b>
	18.16	22.79	25.92	29.74	34.11	37.14	>40	n.d.			
	17.91	22.63	26.06	29.88	33.78	37.25	n.d.	n.d.			

Cp values obtained for standards defined as genome copies number per qPCR reaction.

n.d. : not detected

The qPCR LOD was defined at 10<sup>2</sup> copies per qPCR reaction with Cp included between 36 and 39.

HAde-5 Cp standards values				
Number of TCID <sub>50</sub> units spiked in the volume extracted	Experiments	Number of detected replicates	Cp values obtained	Frequency of detection
<b>10<sup>2</sup></b>	1	3/3	>40.00	100%
			39.64	
			>40.00	
	2	3/3	38.66	
			38.29	
			>40.00	
<b>10<sup>3</sup></b>	1	3/3	37.36	100%
			36.14	
			36.06	
	2	3/3	35.74	
			35.21	
			35.50	
<b>10<sup>4</sup></b>	1	3/3	32.77	100%
			32.30	
			32.58	
	2	3/3	31.77	
			32.22	
			32.14	

The LOD was defined at 10<sup>2</sup> TCID<sub>50</sub> per extraction (280 µl) or 357 TCID<sub>50</sub>/ml. Although a smaller quantity of TCID<sub>50</sub> (<10<sup>2</sup> TCID<sub>50</sub>/extraction) has not been tested, the Cp values obtained were high enough (Cp >38) to be sure that the non tested dilution would have been negative.