

FRA 2 assay

Dye	Standard curves										Single-plex																			
	Multiplex					ABY					VIC					FAM					ABY					VIC				
	<i>B. bronchiseptica</i>					<i>C. felis</i>					<i>M. felis</i>					<i>B. bronchiseptica</i>					<i>C. felis</i>					<i>M. felis</i>				
Target (copies/ μ l)	33.5577	34.0506	33.7113			33.7928	33.6334	33.6483			33.0819	32.6959	32.2194			33.7561	33.8686	34.3714			32.3522	32.4897	33.1156			32.85	32.99	33.43		
1.00E+02	30.7328	30.6835	30.5749			29.9029	30.4174	30.1991			29.771	29.7001	29.9736			31.0498	31.0324	31.1507			30.087	30.059	30.047			30.36	30.35	30.46		
1.00E+04	27.6941	27.689	27.5289			26.92	27.0355	27.0385			26.8033	26.7661	26.932			27.8491	27.7302	27.7464			26.672	26.4225	26.7141			27.14	26.94	27.1		
1.00E+05	24.4306	24.3833	24.327			24.2148	23.981	23.883			23.4505	23.5128	23.4545			24.762	24.6219	24.6045			23.5755	23.075	23.3601			24.03	23.78	23.9		
1.00E+06	21.1536	21.1684	21.1341			21.1821	20.6355	20.7838			20.2486	20.3229	20.1449			21.5439	21.4355	21.3727			20.3064	20.297	20.1868			20.82	20.65	20.59		
1.00E+07	17.515	18.0547	18.0329			17.9898	18.0549	17.7396			17.1012	17.1033	17.059			18.2983	18.2826	18.2682			17.1757	17.0932	17.1736			17.51	17.46	17.49		

replicat #	LOD95% Detection rate limit & Ct cut-off																				Single-plex																																							
	Multiplex										ABY										VIC										FAM										ABY										VIC									
	<i>B. bronchiseptica</i>										<i>C. felis</i>										<i>M. felis</i>										<i>B. bronchiseptica</i>										<i>C. felis</i>										<i>M. felis</i>									
1	24.8895	27.9261	31.5557	37.6133	Undeterm	Undeterm	24.291	27.2508	31.2009	35.5471	38.8676	Undeterm	23.9994	26.949	30.808	35.0648	Undeterm	Undeterm	28.5081	31.8663	37.8994	39.299	Undeterm	28.6059	32.3042	37.405	Undeterm	Undeterm	28.7611	32.1436	34.9064	38.5339	Undeterm	Undeterm																										
2	24.8841	27.501	31.217	35.105	Undeterm	Undeterm	24.2426	26.6939	30.6532	33.5705	37.26	Undeterm	24.0545	26.4283	30.2957	33.5539	38.1363	Undeterm	Undeterm	28.372	31.6505	35.9713	Undeterm	Undeterm	28.7554	33.7011	35.3529	Undeterm	Undeterm	28.8949	33.4344	37.2247	Undeterm	Undeterm																										
3	24.4968	27.9611	31.8141	34.1051	Undeterm	Undeterm	23.8372	27.3168	30.9315	33.535	Undeterm	Undeterm	23.68	26.9395	30.6998	33.159	Undeterm	Undeterm	28.3144	33.0836	37.4347	Undeterm	Undeterm	28.6825	31.1675	34.9914	Undeterm	Undeterm	29.0663	32.9811	36.076	Undeterm	Undeterm																											
4	24.5403	27.6041	31.5793	36.8406	Undeterm	Undeterm	24.0518	26.8156	30.8582	35.2326	Undeterm	Undeterm	23.7403	26.6559	30.7633	35.4194	Undeterm	Undeterm	28.1414	32.7673	35.494	Undeterm	Undeterm	28.1071	32.0886	33.2204	Undeterm	Undeterm	29.1744	32.9879	34.0923	Undeterm	Undeterm																											
5	24.6828	27.8625	31.8229	34.7107	Undeterm	Undeterm	24.2183	26.9405	31.2975	33.8984	Undeterm	Undeterm	24.0285	26.7912	30.9799	33.6374	Undeterm	Undeterm	28.1012	32.6678	35.0193	Undeterm	Undeterm	28.3587	32.278	35.4141	Undeterm	Undeterm	29.1048	33.3894	34.9079	Undeterm	Undeterm																											
6	24.7471	27.2995	31.7807	33.6709	Undeterm	Undeterm	24.1134	26.4686	31.1803	32.9735	33.2296	Undeterm	23.9947	26.2799	30.8545	32.7608	33.0268	Undeterm	Undeterm	28.3638	32.4033	36.9997	Undeterm	Undeterm	28.4061	32.2939	36.1778	36.7042	Undeterm	29.0392	32.0658	36.0197	Undeterm	Undeterm																										
7	24.6415	27.6527	31.4427	34.1107	Undeterm	Undeterm	24.0012	26.8972	30.5161	33.3171	Undeterm	Undeterm	23.9029	26.6674	30.4539	33.2386	39.2714	Undeterm	Undeterm	28.2964	32.5496	34.8302	Undeterm	Undeterm	27.8638	32.0047	36.7217	Undeterm	Undeterm	29.3501	32.3181	35.2355	Undeterm	Undeterm																										
8	24.5124	27.3591	31.3091	34.3876	Undeterm	Undeterm	23.9238	26.4014	30.8524	33.6176	39.3626	Undeterm	23.7667	26.2828	30.6257	33.3731	Undeterm	Undeterm	28.1589	31.8871	35.5259	Undeterm	Undeterm	28.2699	32.7606	34.481	Undeterm	Undeterm	28.9977	31.9468	36.5522	Undeterm	Undeterm																											
9	24.649	27.6502	31.6078	34.61	Undeterm	Undeterm	23.7981	27.0518	31.0148	33.6954	37.3851	Undeterm	23.7646	26.6911	30.8002	33.3325	36.7002	Undeterm	Undeterm	28.5147	32.32	35.5	36.3093	Undeterm	28.4584	31.0269	36.4804	Undeterm	Undeterm	28.9075	33.0798	38.5525	Undeterm	Undeterm																										
10	24.7058	27.8764	31.3665	34.246	37.8834	Undeterm	24.0396	27.0564	30.6219	31.1914	35.7798	Undeterm	23.9151	26.7888	30.4373	33.1872	35.4628	Undeterm	Undeterm	28.238	31.3455	36.4778	39.7575	Undeterm	28.4213	32.2679	34.7771	Undeterm	Undeterm	28.8619	32.4355	35.8298	Undeterm	Undeterm																										
11	24.645	27.9205	31.0367	33.7931	35.9166	Undeterm	24.2002	27.1118	30.624	33.1333	35.6211	Undeterm	23.9258	26.8307	30.271	32.8248	34.6987	Undeterm	Undeterm	28.3163	32.5647	36.3225	Undeterm	Undeterm	28.6505	32.483	37.4123	Undeterm	Undeterm	29.2009	32.8711	37.43	Undeterm	Undeterm																										
12	24.7762	28.0511	31.378	33.9043	Undeterm	Undeterm	24.0413	27.5353	30.6364	36.573	Undeterm	Undeterm	23.9323	27.1376	30.3251	36.4637	Undeterm	Undeterm	28.4724	32.4218	36.705	37.2097	Undeterm	28.0598	32.3634	33.8054	Undeterm	Undeterm	29.0849	32.0289	34.026	Undeterm	Undeterm																											
Average	24.66	27.72	31.49	34.76	36.90	#DIV/0!	24.06	26.96	30.87	34.02	36.79	#DIV/0!	23.89	26.70	30.61	33.83	36.22	#DIV/0!	28.32	32.29	36.18	38.14	#DIV/0!	28.39	32.23	35.52	36.70	#DIV/0!	29.04	32.64	35.90	38.53	#DIV/0!																											
STDEV	0.11	0.25	0.25	1.23	1.39	#DIV/0!	0.16	0.33	0.26	1.13	2.10	#DIV/0!	0.12	0.26	0.24	1.16	2.29	#DIV/0!	0.14	0.51	0.96	1.65	#DIV/0!	0.27	0.69	1.35	#DIV/0!	0.16	0.54	1.38	#DIV/0!	#DIV/0!																												
Ct cut-off	25	28	32	38	41	#DIV/0!	25	28	32	37	43	#DIV/0!	24	27	31	37	43	#DIV/0!	29	34	39	43	#DIV/0!	29	34	40	#DIV/0!	#DIV/0!	30	34	40	#DIV/0!	#DIV/0!																											
LOD95%	11						7						9						9					15																																				

Intra-run variability	
a	-3.159
b	37.27
1	3.98
2	3.92
3	4.04
4	4.03
5	3.98
6	3.96
7	4.00
8	4.04
9	4.00
10	3.98
11	4.00
12	3.95
13	3.99
AVERAGE	3.99
STDEV	0.03
%CV	0.85

Inter-run variability	
replicat	1.00E+05
1	24.76
2	24.62
3	24.6
1	3.96
2	4.00
3	4.01
AVERAGE	3.98
STDEV	0.02344377
%CV	0.59