Macroscopic Dna/Rna Epi-fluorescence (MaDRE) For Differentiated Detection of Bacterial, Viral and Fungal In *Small Fluid Volume Diagnostic (SFVD) Device:* InnovaBug™

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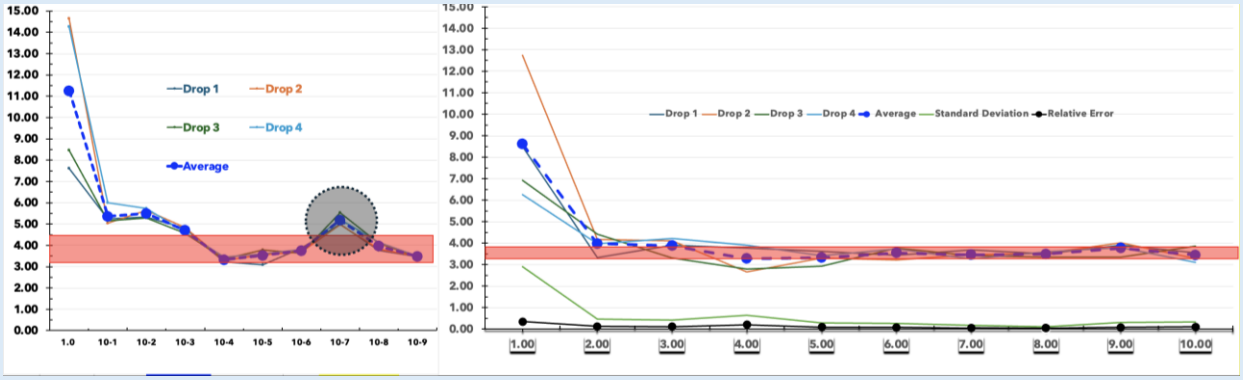
Dilution	Drop 1	Drop 2	Drop 3	Drop 4	Average	Standard Deviation	Relative Error
1.0	7.61	14.64	8.46	14.26	11.24	3.72	33.12%
10-1	5.25	5.03	5.15	6.00	5.36	0.44	8.17%
10-2	5.32	5.64	5.29	5.73	5.50	0.22	4.05%
10-3	4.80	4.83	4.56	4.62	4.70	0.13	2.82%
10-4	3.22	3.28	3.35	3.44	3.32	0.09	2.85%
10-5	3.10	3.57	3.79	3.61	3.52	0.29	8.37%
10-6	3.88	3.70	3.62	3.80	3.75	0.11	3.03%
10-7	5.00	4.97	5.54	5.22	5.18	0.26	5.08%
10-8	3.76	3.84	4.10	4.14	3.96	0.19	4.76%
10-9	3.48	3.49	3.45	3.50	3.48	0.02	0.62%
					5.00	0.55	7.29%
	BACKGROUND RATIO				3.9		
					0.7		

Table 1 Data table for bacterial fluorescence Experiment 1 with 300 Million CFUs/mL as initial 1.0 bacterial load. Average $R_{G/B}$ ratios for 4 drops over 10 serial logarithmic dilutions. Following statistical analysis, the relative error was ±7.3% which is meets the medical gold standard of maximal relative error of ±10%. In addition, using the dilutions of 10-4 to 10-9, the background ratio was calculated to be 3.9 ±0.7 with a relative error of 7.3%, which accounts for the correction of background fluorescence to calculate net fluorescence detection and lack of bacterial detection below a load of 50,000 CFU/mL.

Dilution		Inital number of E.Coli CFU/mL = 500 millions CFUs	Exponent of Dilution in Log Scale	Drop 1	Drop 2	Drop 3	Drop 4	Average	Standard Deviation	Relative Error
1.00	1.0000000000	5.00E+08	0.00	8.51	####	6.92	6.26	8.61	2.91	33.84%
10^-1	0.1000000000	5.00E+07	1.00	3.32	4.18	4.42	3.98	3.98	0.47	11.88%
10^-2	0.0100000000	5.00E+06	2.00	3.90	4.10	3.30	4.22	3.88	0.41	10.53%
10^-3	0.0010000000	5.00E+05	3.00	3.76	2.66	2.80	3.90	3.28	0.64	19.52%
10^-4	0.0001000000	5.00E+04	4.00	3.62	3.30	2.92	3.42	3.32	0.29	8.89%
10^-5	0.0000100000	5.00E+03	5.00	3.44	3.22	3.78	3.72	3.54	0.26	7.34%
10^-6	0.0000010000	5.00E+02	6.00	3.68	3.46	3.42	3.26	3.46	0.17	5.01%
10^-7	0.0000001000	5.00E+01	7.00	3.50	3.52	3.34	3.60	3.49	0.11	3.12%
10^-8	0.0000000100	5.00E+00	8.00	3.90	4.02	3.34	3.80	3.77	0.30	7.90%
10^-9	0.0000000010	5.00E-01	9.00	3.54	3.28	3.86	3.10	3.45	0.33	9.59%
								4.08		8.77%
				BACKGROUND RATIO				3.50		
								0.15		

Table 2 Data table for bacterial fluorescence Experiment 2 in a second, independent laboratory, corroborating the data from Experiment 1 in the first independent laboratory. The relative error is ±8.8% which meets the medical gold standard of a maximum relative error of ±10% and shows that ViroBug™ can detect bacterial infections and measure bacterial load to a load of 50k CFUs/mL. The background ratio for Experiment 2 matches Experiment 1 and is calculated to be 3.5 ±0.15, which overlaps the value for background fluorescence in Experiment 1 and thus validates the reproducibility of ViroBug™ for viral detection.



(a) (b)

Fig. 1 $R_{G/B}$ as a function of decreasing pathogen load in logarithmic scale matching the value of the dilution form Experiment 2 for bacterial detection and load in Experiments 1 from the data in Table 1 (a). The second graph in (b) shows $R_{G/B}$ as a function of decreasing bacterial load. Reproducibly of bacterial detection and load within ±10% corroborates the viability of MaDRE for diagnosing bacterial infections and load. The present study is to be repeated with more sensitive green fluorescent DNA dye to detect very low bacterial load with 100x more sensitive dye to 500 CFUs/mL below infection level.