

Table 4. Combination of fluctuation tests and growth kinetics assays for *V. cholerae* O1 CVEC in environmental water samples, compared with a laboratory-grown *V. cholerae* O1 strain of environmental origin

Date of collection and sample no.	Description of sample*		<i>V. cholerae</i> O1 counts (cfu/ml) [†] on TTGA plates containing streptomycin, after enrichment for 1–6 h in BP medium					
			1 h	2 h	3 h	4 h	5 h	6 h
Aug. 2, 2005 Sample 1664	Undiluted sample (5 ml)		0	0	4.5×10^1	8.2×10^2	6.3×10^3	2.8×10^4
	Aliquots (500 μ l) of 10-fold diluted sample	Fraction 1	0	0	0	0	5.1×10^1	2.7×10^3
		Fraction 2	0	0	0	0	0	0
		Fraction 3	0	0	0	0	0	0
		Fraction 4	0	0	0	0	0	0
		Fraction 5	0	0	0	0	0	0
		Fraction 6	0	0	0	0	0	0
		Fraction 7	0	0	0	0	4.2×10^2	2.7×10^4
		Fraction 8	0	0	0	0	0	0
		Fraction 9	0	0	0	0	0	0
Fraction 10	0	0	0	3.2×10^1	9.2×10^2	1.6×10^4		
Aug. 9, 2005 Sample 1674	Undiluted sample 5 ml		0	0	0	1.8×10^2	5.6×10^3	3.3×10^4
	Aliquots (500 μ l) of 10-fold diluted sample	Fraction 1	0	0	0	0	0	0
		Fraction 2	0	0	0	0	0	0
		Fraction 3	0	0	0	2.1×10^2	6.9×10^3	2.8×10^4
		Fraction 4	0	0	0	0	0	0
		Fraction 5	0	0	0	2.4×10^1	1.2×10^3	5.6×10^3
		Fraction 6	0	0	0	0	0	0
		Fraction 7	0	0	0	9.0×10^1	5.2×10^2	2.6×10^3
		Fraction 8	0	0	0	0	0	0
		Fraction 9	0	0	0	5.0×10^1	6.9×10^2	3.7×10^3
Fraction 10	0	0	0	0	0	0		
Aug. 14, 2005 Sample 1684	Undiluted sample (5 ml)		0	0	5.0×10^1	8.2×10^2	1.2×10^4	4.8×10^4
	Aliquots (500 μ l) of 10-fold diluted sample	Fraction 1	0	0	0	0	0	0
		Fraction 2	0	0	0	0	0	0

Date of collection and sample no.	Description of sample*		<i>V. cholerae</i> O1 counts (cfu/ml) [†] on TTGA plates containing streptomycin, after enrichment for 1–6 h in BP medium					
			1 h	2 h	3 h	4 h	5 h	6 h
		Fraction 3	0	0	0	0	0	0
		Fraction 4	0	0	2.5×10^1	3.9×10^2	6.5×10^3	2.7×10^4
		Fraction 5	0	0	0	0	0	0
		Fraction 6	0	0	0	0	0	0
		Fraction 7	0	0	0	0	0	0
		Fraction 8	0	0	0	8.2×10^1	7.8×10^2	7.8×10^3
		Fraction 9	0	0	0	0	0	0
		Fraction 10	0	0	0	0	0	0
Oct. 16, 2005 Sample 1774	Undiluted sample (5 ml)		0	0	0	4.6×10^2	9.2×10^3	3.5×10^4
	Aliquots (500 µl) of 10-fold diluted sample	Fraction 1	0	0	0	0	0	0
		Fraction 2	0	0	0	0	0	0
		Fraction 3	0	0	0	3.2×10^1	2.9×10^3	1.5×10^4
		Fraction 4	0	0	0	2.3×10^1	1.5×10^3	7.2×10^3
		Fraction 5	0	0	0	0	0	0
		Fraction 6	0	0	0	0	0	0
		Fraction 7	0	0	0	0	0	0
		Fraction 8	0	0	0	0	0	0
		Fraction 9	0	0	0	0	0	0
Fraction 10		0	0	0	0	0	0	
Nov. 28, 2005 Sample 1825	Undiluted sample (5 ml)		0	0	7.0×10^1	9.1×10^2	2.3×10^4	9.0×10^4
	Aliquots (500 µl) of 10-fold diluted sample	Fraction 1	0	0	0	0	0	0
		Fraction 2	0	0	0	0	0	0
		Fraction 3	0	0	0	0	0	0
		Fraction 4	0	0	0	0	0	0
		Fraction 5	0	0	0	0	0	0
		Fraction 6	0	0	0	0	0	0

Date of collection and sample no.	Description of sample*		<i>V. cholerae</i> O1 counts (cfu/ml) [†] on TTGA plates containing streptomycin, after enrichment for 1–6 h in BP medium					
			1 h	2 h	3 h	4 h	5 h	6 h
	Fraction 7		0	0	7.2×10^1	9.8×10^2	1.2×10^4	5.0×10^4
	Fraction 8		0	0	0	0	0	0
	Fraction 9		0	0	0	0	0	0
	Fraction 10		0	0	0	0	0	0
Dec. 6, 2005 Sample 1844	Undiluted sample (5 ml)		0	0	5.5×10^1	5.6×10^2	2.9×10^4	1.5×10^5
	Aliquots (500 µl) of 10-fold diluted sample	Fraction 1	0	0	0	0	0	0
		Fraction 2	0	0	0	7.2×10^1	2.5×10^3	1.9×10^4
		Fraction 3	0	0	0	0	0	0
		Fraction 4	0	0	0	5.0×10^2	9.5×10^4	3.2×10^5
		Fraction 5	0	0	0	0	0	0
		Fraction 6	0	0	0	0	0	0
		Fraction 7	0	0	2×10^1	3.2×10^2	7.5×10^3	2.3×10^4
		Fraction 8	0	0	0	0	0	0
		Fraction 9	0	0	0	0	0	0
Fraction 10		0	0	0	0	0	0	
Dec. 13, 2005 Sample 1847	Undiluted sample (5 ml)		0	0	5.2×10^1	7.3×10^2	2.1×10^4	8.2×10^4
	Aliquots (500 µl) of 10-fold diluted sample	Fraction 1	0	0	0	0	0	0
		Fraction 2	0	0	0	0	0	0
		Fraction 3	0	0	0	0	0	0
		Fraction 4	0	0	0	0	0	0
		Fraction 5	0	0	0	0	0	0
		Fraction 6	0	0	6.0×10^1	7.5×10^2	7.5×10^3	2.7×10^4
		Fraction 7	0	0	0	0	0	0
		Fraction 8	0	0	0	0	0	0
		Fraction 9	0	0	2.0×10^1	8.4×10^2	1.6×10^4	7.8×10^4
Fraction 10		0	0	0	0	0	0	
Laboratory-grown strain 1734 diluted 10^6 -fold in PBS and 500-µl aliquots used for each assay	Fraction 1	2.9×10^2	5.3×10^2	2.3×10^3	8.9×10^3	3.2×10^4	9.8×10^4	

Date of collection and sample no.	Description of sample*	<i>V. cholerae</i> O1 counts (cfu/ml) [†] on TTGA plates containing streptomycin, after enrichment for 1–6 h in BP medium					
		1 h	2 h	3 h	4 h	5 h	6 h
	Fraction 2	2.5×10^2	4.2×10^2	1.3×10^3	6.1×10^3	2.1×10^4	1.1×10^5
	Fraction 3	1.7×10^2	3.2×10^2	1.1×10^3	5.0×10^3	1.9×10^4	7.7×10^4
	Fraction 4	2.1×10^2	2.9×10^2	1.4×10^3	5.2×10^3	2.0×10^4	7.5×10^4
	Fraction 5	2.3×10^2	4.8×10^2	1.7×10^3	5.3×10^3	2.2×10^4	9.1×10^4
	Fraction 6	1.9×10^2	3.7×10^2	1.4×10^3	4.7×10^3	2.1×10^4	1.2×10^5
	Fraction 7	2.1×10^2	3.8×10^2	1.8×10^3	5.0×10^3	1.7×10^4	7.2×10^4
	Fraction 8	1.8×10^2	4.1×10^2	1.2×10^3	3.7×10^3	1.5×10^4	6.3×10^4
	Fraction 9	2.0×10^2	3.2×10^2	9.5×10^2	4.2×10^3	1.0×10^4	5.1×10^4
	Fraction 10	2.4×10^2	3.6×10^2	1.2×10^3	5.0×10^3	1.8×10^4	8.2×10^4

TTGA, taurocholate–tellurite–gelatin agar.

*For the fluctuation test, the water sample was diluted 10 times in BP medium, and 500 μ l of the diluted sample was used in each fraction (effective volume of water used was equivalent to 50 μ l of undiluted sample).

[†]A colony count of zero means that no colonies were detected on plating of 100- μ l aliquots of enriched culture. In theory, the concentration should be <10 colonies per ml.