

Supplementary Material

Reduced sensitivity from pooled urine, pharyngeal and rectal specimens when using a molecular assay for the detection of chlamydia and gonorrhoea near the point of care

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Table S1. Cycle threshold (C_t) values from the GeneXpert for specimens positive for Chlamydia by individual sample testing but negative by the pooling method

Participant	Site of infection	Individual cycle threshold		Pooled cycle threshold	
		CT1		CT1	
1 P3	Anorectal	37.5		0.0	
2 P3	Anorectal	36.7		0.0	
3 P3	Anorectal	37.8		0.0	
4 P3	Anorectal	44.1		0.0	
5 P3	Anorectal	32.1		0.0	
Median		37.5		n/a	

n/a = not applicable

Table S2. Cycle threshold (C_t) values from the GeneXpert for specimens providing positive results for Gonorrhoea by individual sample testing but negative by the pooling method. Note that the GeneXpert has two gonorrhoea targets, NG2 and NG4, both of which must be positive for the GeneXpert to call a sample positive for Gonorrhoea

Participant	Site of infection	Individual cycle threshold		Pooled cycle threshold	
		NG2	NG4	NG2	NG4
5 P3	Pharyngeal	33.1	35.4	0.0	0.0
6 P3	Pharyngeal	37.2	38.9	0.0	0.0
7 P3	Pharyngeal	31.4	32.0	0.0	35.1
8 P3	Pharyngeal	32.6	32.7	37.5	0.0
Median		32.9	34.1	n/a	n/a

n/a = not applicable

Table S3. Individual and pooled chlamydia and gonorrhoea cycle threshold (C_t) values by anatomical site for all samples providing positive results by the pooling method (i.e. excluding the samples in Table S1 and S2)

Target	Anatomical site	Count	Median C _t	Minimum C _t	Maximum C _t
CT1	Urine	9	28.2	22.4	35.3
CT1	Rectum	40	26.7	18.3	44.1
CT1	Pharynx	8	32.1	24.4	37.2
CT1	Pooled	45	29.0	19.9	37.9
NG2	Urine	6	20.2	18.4	21.4
NG2	Rectum	18	20.7	16.3	37.4
NG2	Pharynx	30	24.3	18.1	37.2
NG2	Pooled	35	23.9	16.5	35.6

CT1 = GeneXpert *Chlamydia trachomatis* target; NG2 = GeneXpert *Neisseria gonorrhoeae* target; C_t = cycle threshold.

Table S4. Chlamydia (CT) experimental dilution series

Experiment	Experiment sample number															Total detected
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
A	38.6	38.4	0.0	38.7	38.5	38.6	37.5	0.0	0.0	0.0	37.7	38.5	0.0	38.3	0.0	9
B	0.0	0.0	38.8	0.0	37.1	36.7	38.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	38.6	0.0	1

A = 2mL base dilution: a known CT-positive clinical sample that was diluted in Cepheid transport medium to provide a C_t value of approximately 37.5 cycles.

B = 2 mL base dilution + 1mL neat urine experiment.

C = 2 mL base dilution + 7mL neat urine experiment.

Values reported are GeneXpert CT detection cycle threshold (C_t).