

APPENDIX 11.

Preparation method for the *C. trachomatis* DNA dilutions used for EQA

SH is our code name for a preparation of nucleic acid from a high titre preparation of tissue culture grown L2 strain *C. trachomatis*. The number of plasmid copies in the preparation has been measured by comparison with a known amount of cloned chlamydial plasmid * using quantitative PCR in the Roche LightCycler™. Stock freeze dried vials of SH contain 1.2×10^6 copies of plasmid DNA

* Kindly supplied by Professor Ian Clarke of Southampton University.

Preparation of DNA dilutions

A panel of 10 coded dilutions is prepared for use in each test as follows:

- i. Raise a vial of SH in 240 μ l of sterile dH₂O to give 5000 molecules plasmid/ μ l.
- ii. Dilute x 25 (x 5 then x 5) to give 200 molecules/ μ l. (This is the lowest dilution = 500 molecule input- 2 dilutions in panel)
- iii. Dilute this x 10 to give 50 molecule input- (2 dilutions in panel)
- iv. Dilute again x 2.5 to give 20 molecule input- (4 dilutions in panel)
- v. Dilute finally x 2 to give 10 molecule input.- (2 dilutions in panel)

Dispense as 75 μ l aliquots for testing Cobas and 150 μ l aliquots for the BD Probetec. (These volumes allow testing to be repeated in the case of a failed run.)

Code the 10 aliquots in the panel A-J and record code.

Method for testing DNA panel in the Roche Cobas Amplicor PCR.

In the Cobas assay, urine pellets are raised in 250 μ l of lysis buffer plus 250 μ l of specimen diluent and 50 μ l of this is used as input to the assay (1/10th of initial volume).

Thus for each dilution of DNA, add 25 μ l to the tube followed by 238 μ l of lysis buffer and, after 15 minutes incubation, 238 μ l of specimen diluent. 50 μ l of this solution is used for the assay as normal.

Record the results of the panel on the EQA result sheet. Return to GUIRL Bristol for decoding.

Method for testing DNA panel in BD Probetec ET SDA Assay.

In the BD assay, urine pellets are raised in 2mls of ET specimen diluent, lysed by heating for 30, then 150 μ l of this is used in the priming stage and then finally 100 μ l is used as input into the assay. (1/20th of initial volume)

Therefore take 50µl of each DNA dilution into an ET sample tube and add 1950µl of ET specimen diluent, proceed to lysis and then input into priming and the assay as normal.

Record the results of the panel on the EQA result sheet. Return to GUIRL Bristol for decoding.

Reports containing the decoded results for both assays will be returned to the two labs and to the Project Co-ordinator. (It is important that they are kept confidential until a decision has been made on publication policy as such information can be a sensitive issue for the assay manufacturers).

