

## MICROBIOLOGY

## A reliable and easy to transport quality control method for chlamydia and gonorrhoea molecular point of care testing

S. G. BADMAN<sup>1</sup>, L. M. CAUSER<sup>1</sup>, R. GUY<sup>1</sup>, H. WAND<sup>1</sup>, B. DONOVAN<sup>1,2</sup>,  
S. N. TABRIZI<sup>3,4,5</sup>, D. SPEERS<sup>6,7</sup>, M. D. SHEPHARD<sup>8</sup>, A. VALLELY<sup>1</sup> AND  
D. WHILEY<sup>9,10</sup>, ON BEHALF OF THE TTANGO INVESTIGATORS<sup>11</sup>

<sup>1</sup>Kirby Institute, UNSW Sydney, Sydney, NSW, <sup>2</sup>Sydney Sexual Health Centre, Sydney, NSW, <sup>3</sup>Department of Microbiology and Infectious Diseases, The Royal Women's Hospital, Melbourne, Vic, <sup>4</sup>Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Vic, <sup>5</sup>Murdoch Children's Research Institute, Parkville, Vic, <sup>6</sup>Pathwest Laboratory Medicine WA, Nedlands, WA, <sup>7</sup>School of Medicine and Pharmacology, University of Western Australia, Crawley, WA, <sup>8</sup>International Centre for Point-of-Care Testing, Flinders University, SA, <sup>9</sup>UQ Centre for Clinical Research, The University of Queensland, Brisbane, Qld, and <sup>10</sup>Pathology Queensland Central Laboratory, Brisbane, Qld, Australia; <sup>11</sup>members are listed in the Acknowledgements

### Summary

Quality control (QC) is an essential component of point-of-care testing programs. In the context of a randomised-controlled trial (TTANGO) using GeneXpert (Xpert) *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (CT/NG) point-of-care testing in remote areas of Australia, we aimed to develop and utilise a stable positive control material.

Bacterial cultures of CT and NG were resuspended together to provide cycle threshold ( $C_t$ ) values of approximately 25 cycles for both CT and NG when tested on the Xpert CT/NG assay. These positive control suspensions were dried in aliquots, heat inactivated, and then provided to 12 participating health services as research-only QC samples in kit form. At each service, a QC sample was resuspended and tested each month on the Xpert. QC results, including Xpert  $C_t$  values, were analysed from each site over 30 months and we calculated costs per QC sample.

Overall, at 12 health services there were 89 QC samples tested (average of 8 tests per site per year). Mean  $C_t$  values for the 89 controls samples were 25.25 cycles (SD = 1.15) for CT, 24.04 cycles (SD = 1.400) for one NG target and 23.35 cycles (SD = 1.55) for the other NG target. No significant differences in  $C_t$  value for CT or NG controls were observed over a trial period of 30 months.

Positive QC samples for research use in a trial of a molecular point-of-care assay were inexpensive to produce and stable when stored at 2–8°C. For routine use, additional requirements such as meeting National Association of Testing Authority (NATA) regulations and Therapeutic Goods Administration (TGA) approval will need to be achieved.

**Key words:** GeneXpert; chlamydia; gonorrhoea; DNA; quality; control; assurance; point-of-care.

Received 4 June, revised 27 September, accepted 27 September 2017  
Available online: xxx

### INTRODUCTION

Nucleic acid amplification testing (NAAT) is now a common laboratory method used to diagnose a range of bacterial and viral infections. For many pathogens NAATs have the advantage of shortening the window period to diagnosis, and for others they are the only way of determining if the infection is active versus a past exposure. However, use of NAAT-based point-of-care (POC) tests by primary care services has been mainly limited to tuberculosis (TB), with the scope beginning to expand to other infections.<sup>1–5</sup> Availability of molecular POC devices provides an opportunity for accurate test results to be generated by clinical staff, rather than laboratories, and more timely treatment<sup>6</sup> which has advantages for clinics that are significant distances from laboratories or where there are high rates of patients lost to follow up.<sup>7</sup>

With increasing use of POC assays has come the need to ensure that quality management frameworks, including quality control (QC) and external quality assurance (EQA) testing, are available. Quality management frameworks are important to monitor the reliability of results, alert operators if a change in test performance has occurred, and reduce the risk of misdiagnosis.<sup>6</sup> In Australia, the National Pathology Accreditation Advisory Council (NPAAC) guidelines recommend QC be run regularly to ensure that all testing is performed using instruments, reagents and consumables which are working correctly and according to specifications. They also recommend that POC testing devices should achieve an acceptable standard of performance in external proficiency testing programs whereby the provision external quality assurance (EQA) panels are provided for testing.<sup>8</sup> The EQA panels typically comprise a series of positive and negative samples in a panel for testing at the location of the device. Operators are usually blind to the results, and the results are returned to the provider for review and assessment of test accuracy when compared to other laboratories who participate in the same peer program.

The Test-Treat And Go (TTANGO) cluster randomised trial was implemented from June 2013 to December 2015 in 12 remote Australian Aboriginal and Torres Strait Islander

primary health services to determine the acceptability and cost-effectiveness of POC testing for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) and the operational performance of the POC test in the real-world. The trial was implemented in remote Aboriginal communities where there is a high sexually transmitted infection (STI) prevalence, and coinfection rate in young people<sup>9,10</sup> and delays in treatment, with up to 25% of patients not receiving treatment and an average time to treatment of 3 weeks with laboratory-based testing.<sup>11</sup> Full details of the TTANGO trial protocol are provided elsewhere.<sup>12</sup> The POC device chosen for the TTANGO trial was the GeneXpert CT/NG (Xpert) (Cepheid, USA) due to ease of use, dual detection of CT and NG by NAAT and demonstrated accuracy.<sup>4,13,14</sup> Xpert CT/NG is also approved for testing female endocervical swabs, patient-collected vaginal swabs and for female and male urine specimens.<sup>15</sup>

A quality framework was established in the study, including QC and EQA. For EQA, we purchased CT/NG external EQA panels manufactured by the National Reference Laboratory (Melbourne, Australia) for the trial. In regards to QC, the Xpert has three in-built QC measures for each test conducted. These are used to assess the adequate performance of critical processes in each test reaction, and include: a sample adequacy control (SAC); testing for the presence of human DNA, a sample processing control (SPC); testing for polymerase chain reaction (PCR) inhibition or extraction failure by the use of internal control DNA and a Probe Check Control (PCC), which verifies reagent rehydration, the PCR tube filling in the cartridge, probe integrity, and dye stability. All must process correctly for the CT/NG test to be valid. The probes in the Xpert detect one sequence for CT (CT1) and two different sequences for NG (NG2 and NG4). Both NG targets must be detected for the Xpert to return a positive NG result.<sup>16</sup> Independent CT and NG controls, however, are not provided with the Xpert CT/NG kit to assess the performance of these test reactions.

In this study, we sought to identify an independent positive control for the Xpert CT/NG test that combined both CT and NG DNA (for simultaneous confirmation of both CT and NG test performance) within the one reaction for the purpose of QC testing and clinical staff training. The control sample also needed to be inexpensive and ideally be produced in a dried tube format (DTF) such that it could be mailed to health services in the conventional postal system at an affordable cost and be easily adapted for staff training purposes. To our knowledge, the only commercially available QC option available at the start of the trial was the ZeptoMetrix NATrol (USA) CT/NG material which required CT and NG positive controls to be purchased separately at a combined cost of AU\$126 and in a liquid format. Based on the total number of samples needed for the trial, including those required to support staff training, the cost for this commercial option was considered to be outside of our available budget and in a format not suitable for our needs. Therefore, we developed in-house CT and NG positive DTF control samples and determined the cost of production and stability over the course of the trial (Table 1).

## METHODS

### Ethics

Ethical approval for this trial was provided by UNSW Sydney, and the Children's Health Services, Queensland Human Research Ethics Committee.

The trial is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613000808741). Written consent to publish de-identified QC test data was obtained from participating health services prior to the commencement of this trial.

### Development of the CT/NG in-house quality control samples

The positive control samples were prepared using bacterial cultures of CT and NG and comprised a local wild-type clinical CT strain grown in HEp-2 cells. As the Xpert SAC requires the presence of human DNA to provide a valid result,<sup>17</sup> it was advantageous that the CT was cultured from a human-derived cell line. The NG culture was a local wild-type clinical strain and grown on selective agar. CT and NG cultures were initially tested individually with the Xpert to provide cycle threshold ( $C_t$ ) value estimates for each. Based on these  $C_t$  values the CT and NG cultures were then combined and diluted such that a 10  $\mu$ L aliquot (when made up to 1 mL with sterile water as outlined below) would provide  $C_t$  values of approximately 25.0 cycles for both CT and NG when tested using the Xpert assay. In doing so it should be noted that the controls were provided as research-use-only for the TTANGO Trial.

### Pretesting of in-house quality control samples

A 10  $\mu$ L aliquot of the dilution was added to 10 separate 2 mL sample tubes. All tubes (with caps removed) were placed in a heater block at 95°C for 10 min to dry and render the material non-infectious.<sup>18</sup> After cooling, the tubes were capped. To prepare the controls for testing, each 2 mL dry tube sample was reconstituted using 1 mL of sterile water, agitated briefly by hand, allowed to settle and then transferred into the Xpert CT/NG cartridge. The Xpert CT/NG test was then performed on each of the 10 samples as per manufacturer's instructions and results kept for stability record purposes.

### QC and training sample kits

A total of 144 QC samples were provided to health services in a kit form, which comprised a re-sealable plastic bag containing 12 in-house CT/NG QC samples and 12 tubes containing 3 mL of sterile water for reconstitution. Using the same CT/NG dilution, each site also received a separate training kit with five positive CT/NG DTF samples designated for training and a matching number of tubes of sterile water for reconstitution, plus five positive air dried CT/NG swabs for use with Cepheid swab collection tubes. On delivery, it was recommended that both the controls and training samples be refrigerated immediately at 2–8°C until ready for use.

### Delivery to sites

QC and training kits were transported by air freight to health services along with Xpert cartridges in insulated foam containers. Given the significant distance to these remote service locations, up to 3740 kilometres, and the fact that ambient daytime temperatures at airline transit locations and the final service destination can exceed 40°C during the summer months, a temperature logger to monitor transport temperature history was included with each shipment.

### POC test operator training

Clinical staff underwent comprehensive Xpert competency based training as a part of this trial, including a specific component on the reconstitution and testing of QC samples. A specific standard operating procedure (SOP) for this process and a visual wall aid were developed; the latter included photographic thumbnails, which guided test operators through the process of preparing and testing the controls.

A hard-copy training manual, which covered the Xpert testing system, was available at each site and newly trained operators were encouraged to use the QC section as a primary reference source. Xpert test results, including those performed using the controls, were monitored using real-time, remote login software. A paper-based record of each monthly QC test event was also maintained by operators at each service.

This software allowed trial coordinators to remotely monitor testing and test performance directly from the Xpert laptop computer at each health service and to provide telephone support to services as needed. The monitoring system was also used to identify when device maintenance was required and to determine when calibration of the Xpert machine should take place.

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