Background

Following a request from the Director, Mr Greg Shaw, a review of procedures was conducted by Drs Sloots and Whiley (the reviewers) at the Forensic and Scientific Services laboratory, Clinical and State-wide Services, Coopers Plains, pertaining to the extraction of nucleic acids from samples submitted for analysis.

The reason for this review related to a previous episode in the laboratory which resulted in anomalous results and which appeared to be linked to the operation of robotic instrumentation utilised in the nucleic acid extraction process.

During their visit, the reviewers were made aware of the operations applied in the general laboratory from receipt of specimens to issuing of results, and then examined in detail the bench process relating to the pre-digestion of specimens and the extraction of nucleic acids using the Perkin Elmer MultiPROBE II PLUS HT EX with Gripper Integration Platform.

All aspects of these operations were scrutinised including staff input and instrument operation.

Findings

It was obvious to the reviewers that extensive measures were applied by all staff to prevent the misidentification or cross contamination of samples. There was appropriate use of personal protection equipment and other protective measures to prevent contamination of the work environment with extraneous nucleic acid.

The procedures currently in place for the Off-Deck Lysis and MPII extraction appeared to be adequate and specifically designed to prevent cross contamination of test samples.

We agree with the Forensic Services Management team that the previous issue of possible cross-contamination of samples most likely related to the use of adhesive film in sealing the deep-well plates used in the Off-Deck lysis procedure. The type of plate used, and the period of storage at reduced temperatures have in our experience caused similar problems in molecular diagnostics. The subsequent decision to change this procedure to the use of capped tubes has clearly solved this problem.

The use of robotic equipment for the extraction of nucleic acids has some considerable benefits for a busy laboratory, and prevents human error introduced as a result of repetitive actions. However, the efficient use of such instruments requires the proper maintenance and calibration be performed at the requisite time intervals. These appeared to be adequately performed at the time of review.

It may appear that the original issue concerning the cross-contamination of samples in the deep-well plates could have been prevented if this change in procedure had been fully validated against existing protocol when the new method was introduced. Although most

laboratories would have considered this change to be minor and therefore accepted without validation, it clearly demonstrates that all changes in procedure, no matter how minor, need to be validated according to a standardised protocol before their introduction as standard operating procedure.

Items for Further Consideration

During the review process some items were identified which may require further consideration by the management staff of the Forensic and Scientific Services laboratory.

These are:

1. Develop a standard validation protocol for each procedure based on the guidelines described by J Butler (www.promega.com; September 2006). Incorporate these into the Standard Operating Procedures for the laboratory.
2. We advise that the number of negative controls included in each batch of extractions be increased to comprise at least 10% of the total number of specimens tested. These controls should ideally be distributed randomly over the plate. Currently one negative control is included with 47 samples.
3. Quality assessment might be increased by testing a control plate once every 3-4 weeks on each of the MultiPROBE II PLUS platforms. We would suggest alternating between the soccer ball, zebra and checkerboard formats.
4. It was noted that the magnetic particles used for the nucleic acid extraction had a tendency to settle quickly, thereby blocking the filter tip and potentially producing a false-negative result. At the time of review this was not a problem as the attending operator was diligent in observing all stages of this process. We would like to reiterate however, that constant observation by the operator of all processes leading up to and including the addition of magnetic particle is necessary to ensure that failure of the robotic system does not occur.
5. Finally, it was noted that the laboratory design allowed traffic from the amplification/post-amplification area into the lysis/extraction areas. Presently this carries moderate contamination risk, as the amplification protocol is limited to 28-32 cycles. However, if this protocol is changed in the future to detect low copy nucleic acid (greater than 32 cycles) the risk of carrying post-PCR product into the extraction area would be high, and work-flow dynamics must then be carefully examined to minimise that risk. Likewise, sample cross-contamination during specimen handling and extraction processes will assume greater relevance when contemplating detection of low copy nucleic acid, and would necessitate stringent validation of all steps.

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