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- very thorough
- only minor recs to improve

Audit 8227. Process Audit of the Automated DNA IQ™ System (including Off-Deck Lysis)

2008

exact date? → 08/08/2008

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Forensic And Scientific Services

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2. Findings and Observations

A series of process audits on the complete DNA IQ™ protocol (including off-deck lysis and use of the STORstar instrument) were performed by the authors between 15-28 July 2008. The batches that were audited by the authors are listed in Table 2 below.

Table 2. List of batches and protocols that were reviewed in this audit.

Batch ID	Protocol type	MP II Platform	Auditor[s]	Date
RSPNT20080714_02	Off-deck (retained supernatant)		IAM, AC	15 July 2008
CWIQ20080711_01	STORstar lysate		IAM	15 July 2008
CWIQLYS20080714_02	Off-deck (no retained supernatant)		IAM, AC	15 July 2008
RFIQEXT20080711_01	Automated DNA IQ (Reference)	MP II B	IAM	16 July 2008
LNIQEXT20080715_01	Automated DNA IQ (Reference)	MP II A	AC	16 July 2008
CWODL20080715_01	Off-deck (no retained supernatant)		PAC	16 July 2008
CWODL20080715_02	Off-deck (no retained supernatant)		PAC	16 July 2008
CWIQEXT20080710_01	Automated DNA IQ (Casework), elution	MP II B	IAM	17 July 2008
RFIQEXT20080724_02	Automated DNA IQ (Reference)	MP II B	PAC	28 July 2008

The training records of 16 staff members from the Analytical and Automation Project teams were also reviewed. This included three staff members who have left DNA Analysis.

2.1. *Off-deck lysis (with retained supernatant)*

IAM and AC both reviewed off-deck lysis batch RSPNT20080714_02. The protocol was performed by a trainee under supervision by a trainer (the trainer's *Competent to Train* statement for the DNA IQ™ protocol was submitted to SSDU on 27 June 2008). While performing the protocol, the trainee had access to a controlled copy of the most recent version of the SOP (QIS 24897 R3). When questioned about the SOP, the trainee said that the SOP was easy to follow, but suggested separating the off-deck lysis protocol from the remainder of the SOP, which was relatively large at 33 pages. It must be noted that the DNA IQ™ training module (QIS 24896) does not contain any KPC's relating to the off-deck lysis component. The protocol was performed in a sterilised fume hood with the appropriate PPE.

Observations that were made are listed below.

2.1.1. The worksheet used for the protocol is inappropriate because a Chelex® extraction worksheet is used, as configured in AUSLAB for the RSPNT batch type, which may lead to quality issues (e.g. forgetting to record the TNE incubation step). However, it is understood that configuration of the appropriate worksheets is in progress by LISS/PJAS. As a temporary measure, reagent lot numbers and operator details are recorded in a worksheet that forms part of the DNA IQ™ SOP: *Appendix 18.2. Reagent & Batch details recording tables.*

2.2.2. Some swab samples retained excessive lengths of swab shaft. Out of 47 samples on CWIQLYS20080714_02, 6 swabs required re-sampling, and one swab head was accidentally flicked during reprocessing and landed on the floor of Room 6120. The appropriate corrective actions (e.g. adding Specimen Notes) were performed by the operator.

2.2.3. The procedure for preparing water blanks was reviewed and the following recommendations have been made:

2.2.3.1. The water should be decanted from the stock bottle into a Falcon tube to avoid contaminating the stock reagent. ✓

2.2.3.2. If water is dispensed into individual tubes (labelled with the appropriate barcodes), different tubes (e.g. tubes with white caps) should be used in order to differentiate these from sample tubes and therefore removing the possibility of accidentally adding water to the samples instead of extraction buffer. The accidental addition of water to a sample may cause inefficient extraction of the affected sample.

2.2.3.3. As the water blanks do not actually undergo the off-deck lysis protocol, nanopure water could be added directly to the ABgene plate during STORstar of the lysates, with the appropriate barcodes being scanned from a roll/list. This removes any potential detection of contamination originating from the tubes, and enables the use of the water blanks to specifically diagnose and identify any well-to-well cross contamination events that may occur during automated DNA IQ™ processing. In this instance, only the positive/negative controls and 47 samples require sequence checking by another operator.

2.2.4. The operators for each of the batches reported difficulties using the 3-part labels that may lead to confusion during labelling and possible mislabelling events, as the barcodes contained extra information that crowds the label. However, sequence checking of samples ensures detection of any mislabelling events.

2.2.5. The use of a multistep pipettor to dispense aliquots of reagent may be a potential source of cross contamination from splashing or aerosolisation if the multistepper is not used correctly. As only one sample tube is opened and processed at any one time, the potential contamination can only occur if incorrect use of the multistepper has caused extract to splash on to the multistepper syringe, and subsequently carried-over to the next sample. This hypothetical event would only potentially contaminate downstream samples. Appropriate and adequate training in the use of a multistep pipettor is deemed sufficient to prevent this event from occurring.

2.2.6. Different methods for transferring substrate matrices to spin baskets exist in the laboratory:

2.2.6.1. Using stainless steel forceps that are sterilised in between samples using 10% bleach, 70% ethanol and flaming with a

The DNA IQ™ training module does not contain any KPC's related to the STORstar process. The STORstar protocol for the DNA IQ™ process is described in QIS 24256 R1, separate to the DNA IQ™ SOP (QIS 24897 R3).
Observations that were made are listed below.

2.3.1. Lysate tubes are vortexed and centrifuged briefly for 30 sec – 1 min prior to transfer.

2.3.2. The instrument is sterilised appropriately using 10% bleach and 70% ethanol between uses. The sample aperture on top of the unit can also be sterilised between samples using the same reagents.

2.3.3. After scanning the barcode on each sample tube, the operator confirms that the sample ID displayed in the bottom right hand corner of the OVERLORD software displays the sample ID that is on the tube. This is a commendable QC measure to ensure that the correct sample lysate is transferred to the correct well.

2.3.4. Operator feedback indicates that they are satisfied with the performance and ease of use of the instrument. Operators also feel that the environment around the instrument is sufficient to maintain integrity of the process.

2.3.5. The operator suggested to decrease the diameter of the aperture to allow only one well to be exposed at any one time, but did not feel that the current design (coupled with the protocol) compromises sample integrity.

2.3.6. When transfer is complete, the deep well plate is sealed using adhesive film. It was noted in downstream processes that condensation can collect and stick on the adhesive film. — need to spin before removing film

2.4. ***Automated DNA IQ™ Protocol (reference)***

From various reference batch types performed, the authors observed the following:

2.4.1. When preparing reagents for automated DNA IQ™ processing, the operator is required to constantly move from one room to another in order to access reagents. Reagent preparation occurs in the fume hood within Room 6122 of the DNA Suite, which is commonly used for FTA® washing preparation. Normally, the operator prepares reagents in the shared fumehood workspace, however TNE buffer, sarcosyl and reagents that require fridge/freezer storage are located in Room 6120, and therefore the operator is required to travel between the two rooms to fetch the required solutions. The reagent-making process cannot be combined in Room 6120 because it requires the use of a fumehood. When IAM was observing, the operator travelled back and forth for a total of 3 times. The Elution Buffer is stored in Room 6127, which is

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Room 6125. When entering Room 6125, the operator does not have dedicated space to place the reagents while the operator prepares their PPE.

2.4.11. The operator adds reagents to specific troughs. A different operator does not check to ensure that the reagents have been poured into the correct troughs.

2.4.12. When IAM was observing, a new version of the MP II program was being used in order to enable shaking of plates without the use of a Wallac Isoplate support. This version is not in the current SOP but a comment has been made in QIS. The operator was able to show all the documentation and testing associated with the new version.

2.4.13. Observations associated specifically with the automated method are outlined below.

2.4.13.1. Automated scanning of barcodes on 96-well plates does not work 100% of the time on both MP II platforms, requiring operator intervention.

2.4.13.2. On MP II Platform B, heating tile #1 (45W) is broken on the right-hand side, and the operator is required to click the plate into place prior to commencing the incubation. This information is not present in the SOP.

2.4.13.3. The aluminium foil that is used needs to be properly sealed on to the plate to minimise the risk of cross contamination due to evaporation or condensation. Sealing should be performed using the supplied brown plastic tool and pressing gently to ensure a perfect seal.

2.4.13.4. There is not much room for the operator to move within the MP II hood when adding the DNA IQ™ resin manually which may become an OH&S hazard. When adding the resin to the deep-well plate, the resin should be dispensed onto the side of the plate without touching or mixing with the lysate in order to remove the potential for contamination.

2.4.13.5. When adding Wash Buffer to its specific trough, the operator is required to reach over the plate containing samples.

2.4.13.6. Operators report difficulties with the tip chute receptacle (the tip catcher). Because of rusting, tips can become stuck in the catcher and cause subsequent tips to flick out during ejection, and possibly cause contamination of plates that are in close vicinity to the tip chute. Tip loss may also cause contamination of the workspace.

2.4.13.7. Addition of Lysis Buffer to the sample lysate, followed by pipette-mixing using disposable tips, is a crucial step and takes approximately 1hr to perform for a full plate. The subsequent transfer and shaking on the DPC Shaker platform does not create a vortex of suitable intensity to mix the resin. Furthermore, because the volume within each well is considerably full at this stage, the shaking process may

2.4.13.15. During transfer of eluate from the plate to individual tubes, one bubble and one drop was observed, with both popping at the tip chute. The formation of bubbles may be attributed to either:
- Warm liquid is being transferred, causing the polypropylene tip to expand during movement and causing the movement of air into the spaces, which then form bubbles as the eluate is being dispensed.
- Inefficient programming of the pipetting step. In this case, the performance file for the tip should be examined, and perhaps reducing the volume of system air gap may remove the formation of bubbles.
- The use of non-conductive versus conductive tips.
- The use of tips with smaller aperture may decrease bubble formation and should be investigated.

2.4.13.16. When the procedure is finished, the operator applies aluminium foil to seal the Slicprep™ sample plate and the storage plate. Each plate is then placed into different Ziploc bags, and Nunc tubes are recapped manually. Plates are then transferred to the Workflow Area for storage.

2.4.13.17. There is insufficient storage space in the freezers.

2.4.13.18. Washing and decontamination of the labware is inconsistent and should be standardised in order to minimise the risk of contamination from the tip chute. The tip chute and tip catcher are washed and dried in a rack adjacent to reagent troughs and reagent bottles, or dried on positions in a rack that is not officially reserved for reagent troughs.

2.4.14. The MP II maintenance log for each MP II platform is well maintained and is used effectively to document maintenance schedules that are performed, including replacement of any components.

2.4.15. The syringe plungers on the MP II platforms appeared dirty, which may increase the likelihood of bubble formation that affects pipetting accuracy. Syringes are not normally replaced until they break down.

2.4.16. The PC hard drives for both instruments contain archived performance files and electronic plate maps that should be archived to disc on a monthly basis.

2.4.17. Although environmental cleaning is regularly performed monthly, the top of the MP II hood appeared to be quite dusty. An appropriate cleaning method for hard to reach areas should be investigated.

2.5. *Automated DNA IQ™ Protocol (casework)*

Most of the observations for the reference protocol also apply to the automated DNA IQ™ casework protocol.

From Video 2, we observed some details from Video 1:

2.5.18. Bubble formation from 2.5.6 (0:00:06).
2.5.19. Droplet formation from 2.5.10 (0:00:22).
2.5.20. Also from 2.5.10, the droplet was caught onto the outer surface of the tip chute (0:00:35).

In addition, operators have identified that sample plates often display condensation at the top of the wells and underneath the adhesive film after prolonged storage in the fridge (Figure 1). The condensation was not removed after one cycle of centrifugation (Figure 2), and therefore may require further centrifugations. Incomplete removal of condensation may cause cross contamination when the adhesive film is removed.

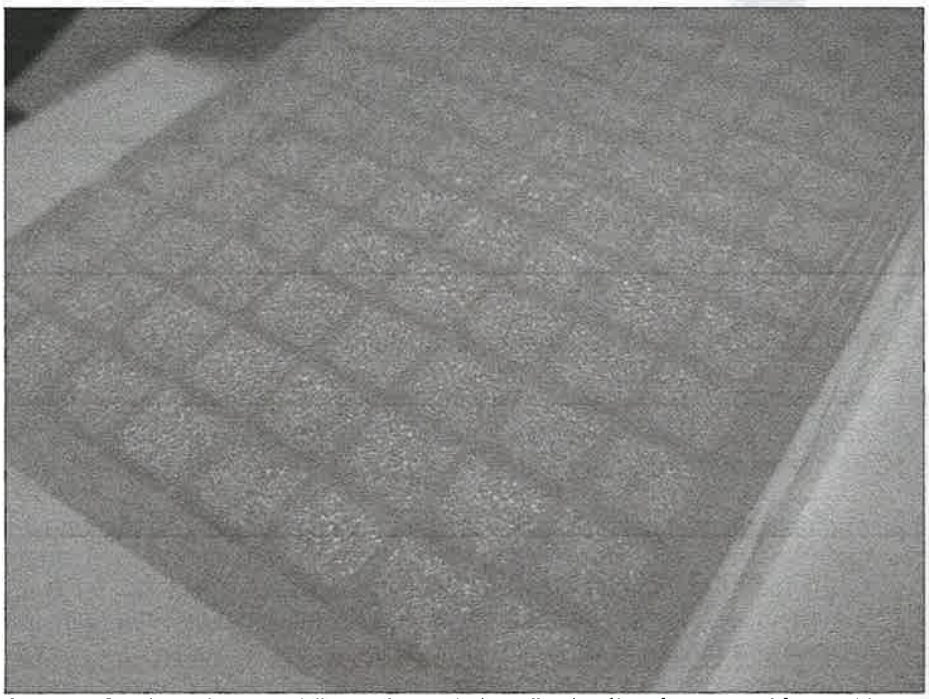

Figure 1. Condensation was visible underneath the adhesive film after removal from cold storage, before centrifugation.

3.6. Staff members use different methods to transfer substrate matrices into spin baskets.

3.7. Volume calculations for DNA IQ™ reagents are not checked by a different operator to confirm calculation results. The worksheet to record calculations (Appendix 18.1 of QIS 24897 R3) is often not used or not included with the DNA IQ™ worksheet (Appendix 18.2 of QIS 24897 R3).

3.8. The volume of critical reagents (e.g. TNE buffer) is not measured using calibrated volumetric devices.

3.9. Some procedures within the automated DNA IQ™ protocol, e.g. (1) transfer of supernatant to the storage plate and (2) the double elution steps, require a review and optimisation due to apparent inefficient pipetting parameters.

3.10. Operators are consistently required to manually secure the 96-well plate on to the magnet when performing the automated DNA IQ™ protocol.

3.11. The MP II maintenance log for each MP II platform is used effectively to document maintenance schedules that are performed, including any work performed by the PerkinElmer engineer. Day-to-day work and observations is recorded appropriately in specific logs for each platform.

3.12. Some staff members that were questioned feel that they are frequently exposed to changes in protocols and methods, and are required to adapt quickly. Although some staff members were comfortable with this environment, others feel slightly overwhelmed.

4. Summary and Recommendations

This review was unable to determine the exact source of contamination as reported in OQI's 19477, 19768 and19349. Although some risks for mislabelling, contamination or cross contamination exist in the procedure, there are appropriate and sufficient quality control measures in place to minimise these risks. Although we observed bubbles and droplets forming at the end of disposable tips during the automated DNA IQ™ protocol, these were not observed to have dripped into any wells and were discarded in the tip chute. Bubble formation can be reduced and eliminated by further optimising the pipetting parameters within the protocol. A follow-up of samples processed in checkerboard format on batch CWIQLYS20080714_02 did not show any instances of well-to-well cross contamination, as evidenced by the absence of DNA profiles in all of the water blanks.

We commend the department for actively engaging in a continual methods improvement process (either to improve QA/QC or increase ease-of-use and efficiency of a procedure), whereby staff input on method changes are investigated and eventually implemented if appropriate. To alleviate the feeling of being overwhelmed by frequent changes, staff members may benefit from a formal handover period as new task rotations occur at the beginning of a week, i.e. the previous operator rostered on a task will convene with the new rostered operator to

4.9. The issue of overworked staff in the Analytical Section needs to be investigated further.

4.10. In-tube sample submissions to the Analytical Section must contain the appropriate amount/length of sample in the first instance, in order to eliminate the need for reprocessing and reduce the risk of contamination.

4.11. If proceeding with a checkerboard format for DNA extractions, the method for preparing the water blanks must be reviewed and standardised (see point 2.2.3).

4.12. Standardisation of the method for transferring substrates to spin baskets should be considered (see 2.2.6).

4.13. Investigate the isolation of all DNA IQ™ reagents and off-deck lysis protocols in one working area. The authors are aware, however, that the current physical design of the DNA Suite may not allow this.

4.14. Investigate the advantages of separating the DNA IQ™ SOP (QIS 24897) into two separate documents, e.g. off-deck lysis (including STORstar) and automated DNA IQ™, and implement as appropriate. The SOP needs to be updated to reflect changes and correct minor errors (e.g. see points 2.4.7, 2.4.13.2, 2.4.13.13).

4.15. Finalise configuration of the appropriate AUSLAB worksheets for use throughout the DNA IQ™ method, so that operators are using the correct worksheets and are able to record all of the necessary batch details in designated fields.

4.16. The automated DNA IQ™ protocols must be reviewed and further optimised to increase liquid handling performance (e.g. incorporate the use of different syringe sizes and tip types) with the assistance of a qualified PerkinElmer specialist (e.g. see points 2.4.13.7, 2.4.13.9, and resin transfer in points 2.5.9, 2.5.16). The optimised protocol should be tested and verified prior to routine use, as per current practice.

4.17. Further to 4.16, the applicability of a different magnet in order to minimise the need to manually secure the plate to the magnet should be investigated. Alternatively, a 96-deep well plate that is not prone to heat warping should be sourced.

4.18. The option for using pierceable film or septa on plates during the automated DNA IQ™ protocol should be investigated (see point 2.4.13.14).

4.19. A procedural checklist should be considered for each protocol so that individual operators can keep track of each specific step as they are performed. This checklist can be added as an appendix to SOP's in QIS that can be printed out by operators prior to performing the procedure.

As an outcome of the recommendations, the authors have raised three OQI's that are listed in Table 3.

Table 3. List of OQI's generated from process audit 8227.

OQI	Description	Recommendations
20367	Automated DNA IQ™ process, including documentation	4.10 – 4.15, 4.19 – 4.22
20368	Enhancement of the MP II extraction platforms, including environment	4.16 – 4.18, 4.23 – 4.27
20369	Training and personnel related to the DNA IQ™ process	4.1 – 4.9, 4.28

5. Acknowledgements

The authors would like to acknowledge DNA Analysis (FSS) for the opportunity to conduct this audit. The authors would also like to thank all staff members in the Analytical Section for making themselves available throughout the duration of the audit process.

6. Documentation and Storage

A hard copy of this report, along with the footage on DVD, is stored with the Quality Management Team in DNA Analysis (FSS). An electronic copy of the report is available in PDF format from the authors and the Senior Scientist (Analytical Section). A summary of the audit findings is available in QIS for Audit 8227.

7. References

QIS 23651 R2 (2008). Forensic and Scientific Services Learning and Development Manual [Guideline]. Scientific Skills Development Unit, FSS: Coopers Plains, Brisbane, Australia.

QIS 23939 R2 (2008). Operation and Maintenance of the MultiPROBE® II Plus HT EX and MultiPROBE® II Plus HT EX with Gripper™ Integration platform [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 24256 R1 (2008). Procedure for the use of the STORstar unit for automated sequence checking [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 24450 R1 (2008). MultiPROBE® II PLUS HT EX Robotic Platform Training Module [Training Module]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 24896 R0 (2008). Automated DNA Extraction with the DNA IQ Kit Training Module [Training Module]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 24897 R3 (2008). Automated DNA IQ Method of Extracting DNA from Reference and Casework samples [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.