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

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

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[redacted]s relating to OQI's 19477, 19768 and 19349 (Table 1) indicate potential instances of well-to-well cross contamination during automated DNA IQ™ extraction on the MultiPROBE® II PLUS HT EX platforms. Investigations performed so far, including repeating samples through extraction, quantitation, amplification and capillary electrophoresis protocols, have not been able to identify the exact cause of the contamination. A memo was released on 15 July 2008 to communicate these findings to DNA Analysis staff members.

Table 1. List of OQI's that document possible recent instances of well-to-well cross contamination.

OQI	Description	Investigation Summary
19477	A negative extraction control (ID 346795477) generated a DNA profile that was confirmed after reprocessing.	A DNA concentration value of 0.008ng/µL was detected during quantitation. A peak at Amelogenin was discovered. After performing a Microcon concentration, amplification and analysis at 30RFU, a full 9-loci profile was observed. The profile matched to 8 other samples (all from the same case) on the extraction batch. Three of these samples were found to be possible sources of the contamination.
19768	A negative extraction control (ID 346796064) generated a DNA profile that was confirmed after reprocessing.	A DNA concentration value of 0.005ng/µL was detected during quantitation. A peak at Amelogenin was discovered. After Microcon concentration, amplification and analysis at 30RFU, 15 discernable alleles were elucidated and matched to 2 samples from the same off-deck lysis and extraction batches.
19349	A negative extraction control (ID 346790262) generated a DNA profile that was confirmed after reprocessing.	The negative control did not yield a DNA concentration value. The control generated peaks below the 75RFU threshold. After re-preparation and reamplification followed by analysis at 30RFU, the profile detected was found to match the DNA profile of the positive control. The exact point where the contamination occurred could not be elucidated.

An audit team consisting of the authors was formed in order to perform a process audit of the automated DNA IQ™ protocol that is performed on two MultiPROBE® II PLUS HT EX platforms. The aim of the audit team was to identify any steps in the protocol where a potential for quality breakdown was present, and also to identify areas of improvement that may benefit the protocol. This report documents the findings and recommendations of the audit team.

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 b[redacted]

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

Batch ID	Protocol type	MP II Platform	Auditor[s]	Date
[redacted]	Off-deck (retained supernatant)		IAM, AC	15 July 2008
	STORstar lysate		IAM	15 July 2008
	Off-deck (no retained supernatant)		IAM, AC	15 July 2008
	Automated DNA IQ (Reference)	MP II B	IAM	16 July 2008
	Automated DNA IQ (Reference)	MP II A	AC	16 July 2008
	Off-deck (no retained supernatant)		PAC	16 July 2008
	Off-deck (no retained supernatant)		PAC	16 July 2008
	Automated DNA IQ (Casework), elution	MP II B	IAM	17 July 2008
	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*.

2.1.2. When adding Sarcosyl, the reagent should be decanted from the stock bottle into a smaller “working” container (e.g. Falcon tube or 10mL tube) to avoid potential contamination of the stock solution. This should be specified in the SOP. The Proteinase K is appropriately aliquoted into individual tubes.

[redacted]3. The operators were concerned regarding inconsistencies in label types: sometimes they would be of the 3-part type, and other times they would be of the Nunc type. When requiring extra labels, the operator is often required to print a 3-part label, which they considered to be wasteful. The labelling on some tubes appeared to come off at [redacted]edges, but this was most likely due to inadequate pressure being [redacted]lied during label application.

[redacted] top of tubes containing the retained supernatant is labelled with [redacted]text “sup” as appropriate to identify them as retained supernatant, [redacted]ber the SOP.

2.1.5. Some details that should be added to the SOP include:
- Step 11, a pipette mix should be performed.
- Step 18, store lysates in a 4ºC fridge immediately after performing the incubation (and not stored on the bench).

Although some suggestions are made to improve the process, we did not observe any steps where a potential quality breakdown could occur and cause sample-to-sample cross contamination when the protocol is performed following the SOP.

2.2. *Off-deck lysis (without retained supernatant)*

IAM and AC both reviewed off-deck lysis batch CWIQLYS20080714_02. The protocol was performed by the same trainee and trainer as per RSPNT20080714_02. This off-deck lysis batch had a new configuration type as detailed in the memo of 15 July 2008. Briefly, the lysis batch contained 1 positive and 1 negative control, with 47 samples and 47 blanks (containing nanopure water) arranged in checkerboard format. This new batch format was chosen in order to better identify any occurrences of well-to-well cross contamination. As per 2.1., the DNA IQ™ training module does not contain KPC’s related to this component of the process.

PAC reviewed off-deck lysis batches CWODL20080715_02 (performed by a trainer with trainee observing) and CWODL20080715_01 (steps 7 to 12).

Observations that were made are listed below.

2.2.1. Three steps where a possibility of either contamination or cross contamination can occur were identified:
- 2.2.1.1. Subsampling/re-processing of sample.
- 2.2.1.2. Incorrect use of a multistep pipettor.
- 2.2.1.3. Transferring substrate from lysis tube to spin basket.

These are described in more detail below.

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.

[redacted]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.

[redacted] 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

Bunsen burner. Using forceps may cause cross-contamination in downstream samples if forceps are not sterilised correctly.

2.2.6.2. Using autoclaved twirling sticks to either:
- Assist in tipping the substrate into the spin basket.
- Pickup the substrate and transfer to spin basket using a "chopstick" method.

[redacted]2.2.6.3. Some standardisation of the method for transferring substrates to spin baskets should be considered. The different methods should be assessed to determine which one is the best method with the least potential for contamination.

2.2.6.4. Cross contamination from sample-to-sample via droplets is unlikely because only one sample tube is open at any one time and each sample is processed individually.

[redacted]as noted that 2mL 4N6 tubes are designed with a wide bottom, therefore 500µL of Extraction Buffer may not always immerse the mple substrate (e.g. a swab head). This may potentially cause fficient extraction of DNA from the sample.

2.2.8. Feedback from questioned staff indicates that a high level of workload exists in the Analytical Section, coupled with frequent protocol changes, resulting in overworked and tired staff members that may result in a quality breakdown.

2.2.9. It was observed that newly trained staff members have access to the bench copy of SOP (QIS 24897 R3) when processing ODL batches but are not regularly referring to the SOP for guidance. A tick box checklist system is recommended for re-assurance that the process is followed methodically.

Although there are several steps where opportunities for contamination or mislabelling exist, staff consider that there are appropriate and sufficient quality control measures in place that minimise these risks. We recommend that the issue of overworked staff be investigated further.

2.3. *STORstar of lysate*

The *automate.it* STORstar system is used as an automated sequence checking system to ensure that:
- A sample lysate is transferred from a sample tube to the correct well in a 96-deep well plate;
- The correct Nunc™ Bank-It DNA extract storage tube is positioned in the correct grid coordinates for any sample ID.

DNA Analysis FSS has purchased two STORstar units: one is located in the Workflow Area for sequence checking DNA extract tubes, while the other is located in the DNA Suite for both sequence checking of extract tubes and also transfer of sample lysate. The STORstar system utilises an electronic platemap generated by AUSLAB that contains a list of unique sample identifiers linked to a specific grid coordinate. The electronic file is imported into AUSLAB as a log file.

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.

[redacted]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 [redacted]sterilised between samples using the same reagents.

[redacted]r scanning the barcode on each sample tube, the operator [redacted]firms that the sample ID displayed in the bottom right hand corner [redacted]he OVERLORD software displays the sample ID that is on the [redacted]e. This is a commendable QC measure to ensure that the correct [redacted]ple 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.

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

acceptable because this reagent is not required until towards the end of the protocol.

2.4.2. As mentioned in point 2.1.1, the DNA IQ™ worksheet has not been configured in AUSLAB and therefore the operator was required to use the worksheet in Appendix 18.2 of QIS 24897 R3.

[redacted]3. There is no controlled copy of the DNA IQ™ SOP (QIS 24897) in Room 6122. The operator normally calculates the required volumes in Room 6125 and brings their worksheet into Room 6122 prior to reagent preparation.

[redacted] uncontrolled copies of Appendix 18.1 from QIS 24897 R3 were nd on a shelf in Room 6122. One uncontrolled copy of page 5 from 24897 R2 (archived document) containing handwritten changes no initials were also found in Room 6122. IAM removed these.

hould be noted that prior to commencing the procedure, an operator mentioned that the last time they were rostered on to the Extraction MP II platform was around 3-4 months ago, and they had not been retrained since changes have been made to the DNA IQ™ protocol. The operator had never performed an automated REF FTA protocol before.

2.4.6. Appendix 18.1 of QIS 24897 R3 had a note underneath each table, instructing operators to calculate *n* to the nearest multiple of 8. An operator did not note this because the note was not clearly visible, and had to recalculate all reagent volumes before commencing to prepare reagents.

2.4.7. In Table 7 of Appendix 18.1 of QIS 24897 R3, the formula to calculate the volume of Elution Buffer is incorrect. There should be a minimum volume of 3mL in each channel if the batch size is small due to the large dead volume required for this type of labware. Having volumes less than 3mL will cause inaccurate pipetting of volumes. Although this has been identified as a mistake within the SOP, comments had not been entered into QIS.

2.4.8. The results from calculating reagent volumes are not checked by a different operator in order to confirm that the calculations are correct.

2.4.9. Reagent volumes are measured using gradations on a Falcon tube, which are not calibrated. This is acceptable for bulk reagents (e.g. Lysis Buffer, Wash Buffer, Elution Buffer), but should not be used for reagents that need to contain a certain concentration of a reagent (e.g. TNE Buffer, which is then mixed with Proteinase K to a validated concentration).

2.4.10. After completing the reagent-making process, the operator carries all the reagents (in Falcon tubes or Schott bottles) from Room 6122 into

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.

[redacted]

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 [redacted] ment has been made in QIS. The operator was able to show all documentation and testing associated with the new version.

[redacted] servations associated specifically with the automated method are [redacted] ned below.

[redacted] 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

increase the probability of a splash back and therefore increase the risk of cross contamination, especially if the volume of lysate exceeded 500µL. This step is redundant and should be removed.

2.4.13.8. Operators report that the ABgene plate that is currently used is not 100% identical to the Slicprep™ device, as it requires clicking into the magnet. Resin loss, and therefore sample loss, occurs if the plate is not clicked in properly.

2.4.13.9. When transferring supernatant to the storage plate, the first transfer is always performed efficiently but the second transfer exhibits bubbles that are possibly caused by aspirating large air gaps, however this problem may only potentially contaminate the storage plate (and not the sample plate). However, if bubbles pop towards column 12 of the storage plate, potential cross contamination may occur in columns 1 or 2 of the Nunc storage tubes that sit adjacently (note that extraction controls are located in column 1). A second set of tips should be used for the second transfer to remove this problem.

2.4.13.10. On MP II Platform B, a blockage of tip 2 was identified due to the consistent appearance of frothing. The operator noted this but did not inform the Supervising Scientist. Although not perceived to be a source of cross contamination, maintenance on this tip should be performed.

2.4.13.11. The rack that is used to store lids from decapped Nunc tubes is cleaned daily using bleach. Racks should be cleaned daily in Decon solution prior to washing. New, cleaned racks should be used daily: the operator can collect cleaned racks from the Workflow Area at the beginning of a run, and return the used rack into a Decon bucket at the conclusion of a run.

2.4.13.12. For the RFIQ worksheet, there are no fields to record positive, negative and blank control barcodes, which are instead affixed onto the general surface of the worksheet which causes inconsistencies. There are no fields to record lot numbers for Slicprep™ devices, etc.

2.4.13.13. After incubation at 65°C, the deep-well plates warp due to heating. The plate needs to cool down before the operator can click it on to the magnet appropriately. The waiting period is generally 1-2 minutes and the operator generally enters the hood about 2-3 times to check if the plate can click onto the magnet. The plate should not be forced onto the magnet as splash back can potentially occur. This process is not described in the SOP.

2.4.13.14. During this time, the eluate appears to be evaporating as evidenced by condensation forming on the tops of the wells. The risk of possible contamination should be evaluated. To avoid evaporation, aluminium foil can be applied onto the plate. To avoid causing cross contamination when peeling the film, pierceable foil can be used. Additionally, a septa may be appropriate.

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.

[redacted]

[redacted]

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.

Video footage of the entire double elution processes was captured and included with this report as appendices:

- Video 1: the entire elution process (Platform B extraction.mpeg).
- Video 2: detail into the formation of 2 bubbles during the elution process (bubbles.mpeg).

[redacted] appropriate timestamps in parentheses for footage of each observation.

From Video 1, we observed:

Elution 1

[redacted] robot was unable to automatically click sample plate into the [redacted]gnet after 65°C incubation due to plate warping, therefore requiring [redacted]rator intervention (0:06:41, 0:07:04, 0:07:29 – 0:07:45).
[redacted]iration of first eluate using non-conductive disposable tips did not [redacted]ntify any problems, with uniform volumes of aspirate (and transport [redacted]gap) in each tip (e.g. 0:09:02).
[redacted]7 ejection problem was encountered (0:09:12), requiring operator intervention (0:09:24).

2.5.5. No resin was transferred to the elution tubes (except towards 0:13:00).
2.5.6. Bubble formation was observed at the end of a disposable tip during transfer into elution tube (0:11:15).
2.5.7. Nunc tubes were picked up by the tips after dispensing eluate (as the 8-tip arm retracted), causing the tubes to raise in the rack, requiring operator intervention (0:13:03). If the operator did not intervene, there was a risk of the 8-tip arm crashing into the Nunc rack.
2.5.8. Bubble formation was observed at the end of a disposable tip after dispensing eluate into elution tube (0:13:30).
2.5.9. Resin was transferred to elution tube (0:14:17).
2.5.10. Droplet formation was observed at the end of a disposable tip after dispensing eluate into elution tube (0:14:24). The droplet subsequently came off at the tip chute (0:14:38).

Elution 2

2.5.11. Shaking on DPC shaker appears to shake resin sufficiently in wells (0:15:31).
2.5.12. Condensation formed on the sides of each well as the plate was cooling on the magnet (0:18:14).
2.5.13. Operator intervention was required to click in the warm plate onto the magnet (0:18:34 – 0:19:09).
2.5.14. Aspiration of second eluate using non-conductive disposable tips did not identify any problems, with uniform volumes of aspirate (and transport air gap) in each tip (e.g. 0:20:44).
2.5.15. Droplet formation was observed at the end of a disposable tip after dispensing eluate into elution tube (0:23:48).
2.5.16. Resin was transferred into elution tube (0:24:07).
2.5.17. A Nunc tube was lifted off the rack after dispensing eluate into the elution tube, requiring operator intervention (0:24:38).

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).

[redacted] 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. [redacted]al of condensation may cause cross contamination when the [redacted]moved.

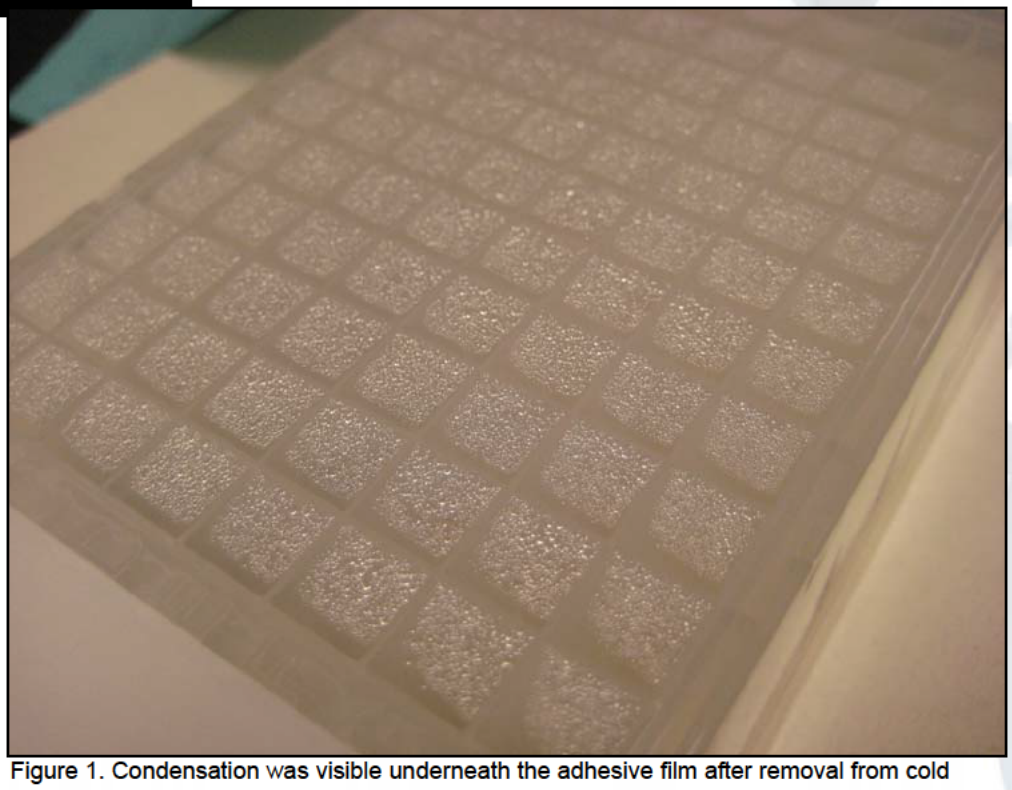

Figure 1. Condensation was visible underneath the adhesive film after removal from cold storage, before centrifugation.

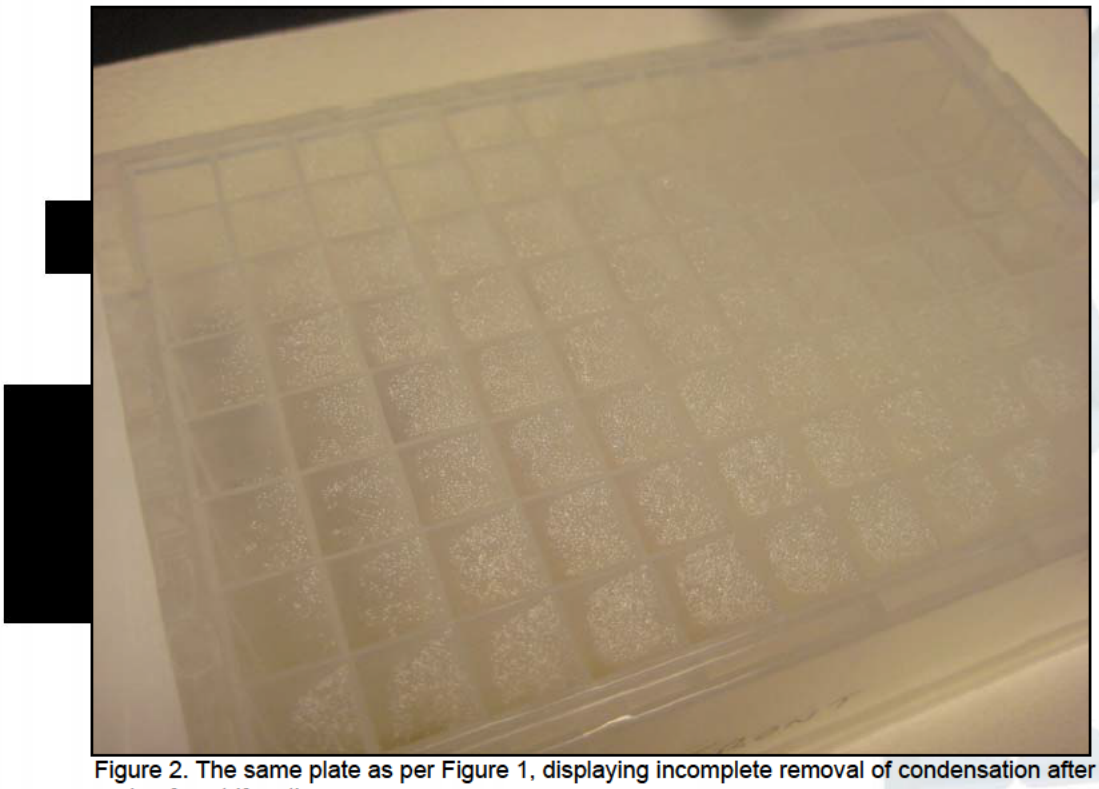

Figure 2. The same plate as per Figure 1, displaying incomplete removal of condensation after one cycle of centrifugation.

3. Trends

We identified various trends throughout the audit process.

3.1. KPC's for the off-deck lysis and STORstar components are not included in the DNA IQ™ training module (QIS 24896 R0), but are integral to the DNA IQ™ protocol.

3.2. The majority, but not all, training records (e.g. QIS 24450 Operation and Maintenance of the MultiPROBE® II PLUS HT EX Robotic Platform TM and QIS 24896 Automated DNA Extraction with the DNA IQ Kit TM) for staff members were either available in the QIS Professional Development module or ready for upload to QIS.

3.3. Staff in the Automation Project team, involved in the development of the SOP (QIS 24897) and Training module (QIS 24896), have either completed the training modules or possessed "Statements of Competence" records.

3.4. "Statement of Competence to Train" records were available for some but not all trainers. All trainers have previously attended Train the Trainer.

3.5. Staff members generally do not have a checklist system to ensure that they have performed a specific step within any particular protocol.

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 [redacted]NA 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.

[redacted]cedures within the automated DNA IQ™ protocol, e.g. (1) transfer [redacted]atant to the storage plate and (2) the double elution steps, require [redacted]and optimisation due to apparent inefficient pipetting parameters.

[redacted]s are consistently required to manually secure the 96-well plate on [redacted]gnet 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

describe any changes to protocols and methods. Changes to protocols should be disseminated generally to other staff either through meeting agendas (and is therefore recorded) and e-mail if appropriate, which is already the current procedure in place. Furthermore, the Minor Changes Register is used effectively to document specific changes within the laboratory, and can be accessed by all staff members. We noted that QIS also appears to be used appropriately for the purposes of r[redacted]uggestions and changes to protocols.

The authors have identified 28 points that may improve the automated DNA IQ™ extraction process, and have made the following recommendations:

[redacted]C's for off-deck lysis and STORstar components into the DNA IQ module (QIS 24896 R0).

of DNA IQ™ training delivery and the associated training module, ate more aspects into the background and theory of the system, g discussions on the composition and function of each buffer

4.3. A Training Delivery Plan needs to be developed for training in the automated DNA IQ™ extraction process. Note that a TDP already exists for training on the use of the MultiPROBE® II platforms (used in conjunction with QIS 24450).

4.4. "Statement of Competence to Train" records must be finalised for appropriate Automation Project team members.

4.5. Review the expected timeframes to complete training modules QIS 24450 (Operation and Maintenance of the MultiPROBE® II PLUS HT EX Robotic Platform) and QIS 24896 (Automated DNA Extraction with the DNA IQ Kit).

4.6. Trainers and supervisors need to progress the completion of training modules with staff. Consider adding progress reports as an agenda item in weekly team meetings or an appropriate alternative.

4.7. Apart from staff identified as trainers, it is recommended that all DNA Analysis staff attend *Trainer the Trainer*. This will assist with 1) trainer and trainee responsibilities, 2) adult learning styles, 3) introduces the FSS Learning and Development Manual (QIS 23651).

4.8. A re-evaluation of pipetting skills should be performed in order to benchmark and standardise techniques. The evaluation can incorporate demonstrations on differences in the pipetting behaviour of hot, warm and cool liquids; reagents containing a high proportion of solvents (e.g. ethanol), etc. A SOP and TM detailing and assessing pipetting techniques (e.g. forward versus reverse pipetting) should be created, if not yet available (e.g. see QIS 23899). The re-evaluation should also assess the use of multichannel and multistep pipettors in combination with various tip types.

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.

[redacted] 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).

[redacted] disation of the method for transferring substrates to spin baskets [redacted] e considered (see 2.2.6).

[redacted] ate the isolation of all DNA IQ™ reagents and off-deck lysis [redacted] s in one working area. The authors are aware, however, that the [redacted] 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.

Alternatively, the checklist can be configured in AUSLAB and printed out together with the batch worksheet.

4.20. Checking of calculation results for reagent volumes by a different operator should be introduced, as should the dispensing of reagents into the correct troughs.

[redacted]he use of “working” containers and aliquots should be enforced where appropriate so that the possible contamination of stock solutions is minimised.

[redacted]iate calibrated volumetric devices should be sourced to measure me of critical reagents such as TNE buffer.

[redacted]ss to change syringes more frequently at regular intervals should mented. Because of this, the process to calibrate or check new will be time consuming and therefore alternative calibration or verification systems should be sourced (e.g. Artel MVS).

4.24. The BSD Duet 600 instrument can be moved to a different location in order to decrease human traffic and increase the amount of working space available around the MP II extraction platforms. A portable biohazard hood can be introduced into Room 6125 to enable some sample processing outside of the MP II hoods (e.g. manual addition of DNA IQ™ resin).

4.25. Investigate the use of a tip catcher that is made of a material not prone to rusting (e.g. plastic).

4.26. The procedure for washing and drying the MP II tip chutes must be reviewed (see point 2.4.13.18). Designate a rack position or location for drying of the tip chute and tip catcher, separate from the rack used for reagent troughs. Furthermore, a spare tip chute can be made available for each MP II, therefore used tip chutes can be allowed to decontaminate in a Decon bucket to fully decontaminate the tip chute, without compromising throughput of the MP II.

4.27. The cleaning regime of the MP II, including surroundings and enclosure (e.g. top of MP II hood), must be reinforced.

4.28. As a continuous QA/QC measure, the supervisor should observe the DNA IQ™ protocols at regular intervals for critical assessment and possible re-evaluation of the impact and suitability of changes in the methods.

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
[redacted]	Automated DNA IQ™ process, including documentation	4.10 – 4.15, 4.19 – 4.22
	Enhancement of the MP II extraction platforms, including environment	4.16 – 4.18, 4.23 – 4.27
	Training and personnel related to the DNA IQ™ process	4.1 – 4.9, 4.28

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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.