Technology Transition Workshop| *Robert O'Brien*

Validation of the MinElute® Post-PCR Cleanup System

Validation of the MinElute® Post PCR Cleanup

- This presentation covers the following topics:
  - Type of required validation
  - Types of required testing for validation
  - Number of samples needed for a successful validation
  - Factors affecting the validation
  - Satisfying audit requirements for the validation

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Type of Validation

- The two types of validation defined in the quality assurance standards (QAS) for DNA laboratories documents are:
  - **Developmental validation** – the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic and/or casework reference samples
  - **Internal validation** – the accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory

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Type of Validation

- Methodology is used to describe the analytical processes and procedures used to support a DNA typing technology
- For example:
  - Extraction methods
  - Quantification methods
  - Typing test kit
  - Platform

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Type of Validation

- Based on these definitions, the MinElute® PCR Purification Kit for post-PCR cleanup is not considered a new method
  - It is a concentration and cleanup process
  - No different than Microcon® and Centricon®
- For MinElute® an internal validation will be required

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Before Validation Begins

- Depending on the requirements of the laboratory and before beginning the validation, some preliminary testing must be performed
- If amplified product is retained for future testing then either the volume of amplified product cleaned up or the volume placed on the genetic analyzer must be determined
  - The fold increase expected from the process depends on which is chosen and the volume used

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Before Validation Begins

- If implementing the manual MinElute® procedure the number of washes to be used must be determined by the laboratory
  - The number of washes also affects the fold increase
- Determination of volumes and number of washes to be used in the manual protocol will correlate to an expected fold increase
- This fold increase will vary somewhat based on pipetting differences between analysts

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Before Validation Begins

- If implementing the QIAcube® robot procedure the number of washes will be two (unless a change is made by the manufacturer)
- Changing volumes will still affect the fold increase
  - However due to more precise and consistent pipetting by the robot there should not be much variation once an expected fold increase is determined
- Determination of the expected fold increase during validation will help the laboratory define the limits of use of the MinElute® system and when it should and should not be used

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Required Testing for an Internal Validation

- According to the new QAS DNA documents effective July 1st, 2009 the following types of testing must be conducted as part of an internal validation:
  - Known and non-probative evidence samples or mock evidence samples
  - Reproducibility and precision
  - Sensitivity and stochastic studies
  - Mixture studies
  - Contamination assessment

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Required Testing for an Internal Validation

- Sensitivity and stochastic studies:
  - The first study that should be performed is the sensitivity and stochastic study
  - This study is important to set the quantitation threshold at which the laboratory will use MinElute®
  - The threshold will vary from one laboratory to the next depending on their comfort level and how low they want to go to interpret data

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Required Testing for an Internal Validation

- Sensitivity and stochastic studies:
  - The results previously presented showed an instance where there was no data before cleanup but after cleanup there were peaks brought up to calling levels (75 RFU)
  - Some laboratories may choose to only use this cleanup system on low level peaks that are already visible but below threshold
  - The only question with setting a limit of quantitation is whether your quantitation system is accurate

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Quantitation Accuracy

- At the NFSTC what was thought to be a problem with the sensitivity of the 3130*xl* was found to be a problem with the quantitation standards
- When analyzing casework there is usually a large peak height range that is accepted even though every amplification usually targets the same concentration (e.g. 1 ng/µl)

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Quantitation Accuracy

- When conducting a kit study at NFSTC in which the sensitivity of the amplification kits were being compared to each other, a lot of fluctuation was seen in peak heights from one week to the next within the same kit
- This made the study difficult and lead the staff to believe the 3130*xl* was having a sensitivity problem

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Quantitation Accuracy

- The 3130*xl* was not losing sensitivity, the peak heights were simply varying for all samples that quanted at 1 ng/µl
- After the instrument was checked and reported as being in working order, other processes were looked at
- Apart from pipetting variations the only other factor could have been the accuracy of the quantitation results

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Quantitation Accuracy

- The data showed that the standards supplied with the quantitation kit were giving a very different result when compared to standards from the National Institute of Standards and Technology (NIST)
- The next two slides show differences in quant values based on which of these two standards was used to construct the quantitation standard curve:
  - Standard A from the Applied Biosystems Quantifiler® kit
  - NIST Human DNA Quantitation Standard (SRM 2372)

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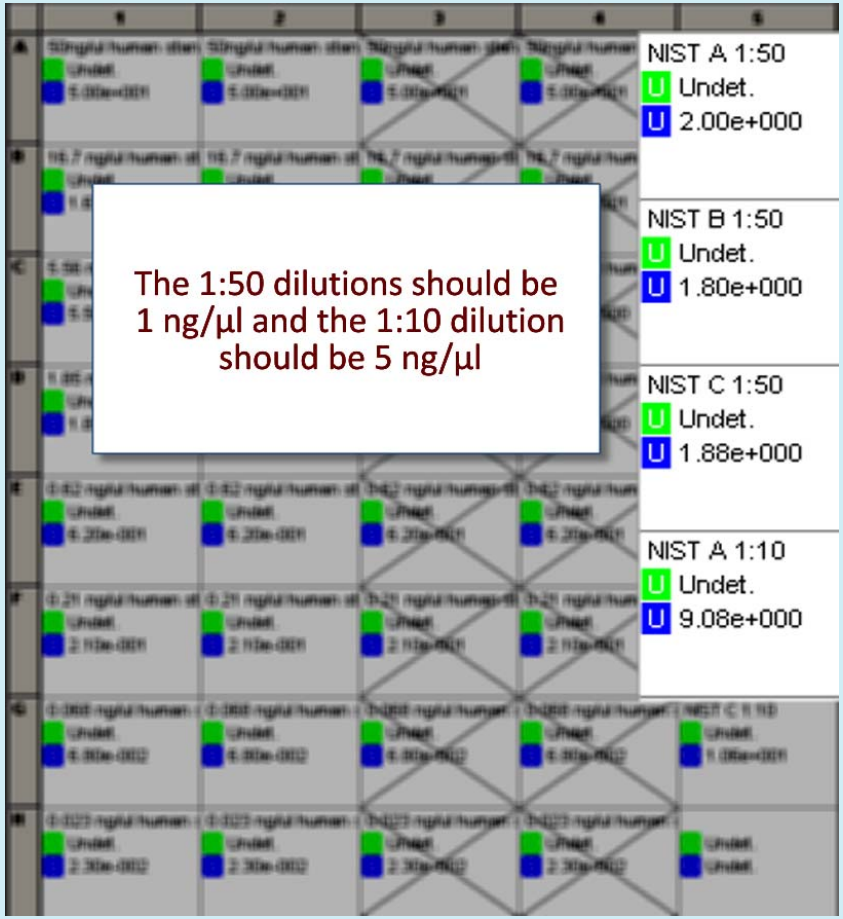
Quantitation Accuracy

Quantitation values using Standard A from the Applied Biosystems Quantifiler® Kit to construct the quantitation standard curve



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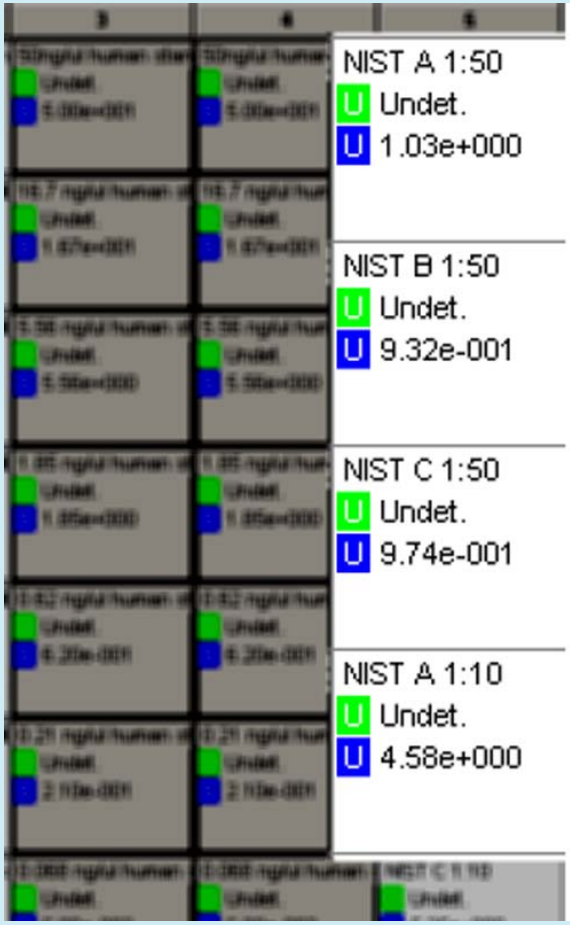
Quantitation Accuracy

Quantitation values using the NIST Human DNA Quantitation Standard (SRM 2372) to construct the quantitation standard curve



The correct values were obtained for the 1:50 and 1:10 dilutions (1 ng/µl and 5 ng/µl, respectively)

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Quantitation Accuracy

- On the last two slides, the quanted samples were prepared from the NIST Human DNA Quantitation Standard
- The NIST SRM 2372 components (A, B and C) are prepared to have a DNA concentration of 50 ng/µl
  - A 1:50 dilution should give a quantitation value of approximately 1 ng/µl
  - A 1:10 dilution should give a quantitation value of approximately 5 ng/µl

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Quantitation Accuracy

- By using the Quantifiler® Standard A to generate the quantitation standard curve, the quant results obtained for these samples were almost double what they actually are
- Based on this, when you think you are amplifying a target of 1 ng/µl you may actually be amplifying 0.5 ng/µl
- This overestimation by double is not consistent

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Quantitation Accuracy

- When the Quantifiler® Standard A is used to construct the standard curve, its quantitation accuracy varies from lot number to lot number
  - Sometimes the result was double, sometimes it was less
- This inconsistency will make it difficult for laboratories to set a limit of quantitation

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Quantitation Accuracy

- Using the NIST Human DNA Quantitation Standard to make the standard curve is not cost effective, because of the price of the standard
  - When the Quantifiler® Standard A is used to generate the quantitation standard curve, dilutions of a NIST SRM component(s) can be run on each plate to help normalize the quantitation values for samples
  - Alternatively, the NIST SRM can be used to obtain an accurate value for each lot number of Quantifiler® Standard A, so designated quant values for each standard curve dilution point will be accurate

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Required Testing for an Internal Validation

- Sensitivity and stochastic studies:
  - Based on the MinElute® validation study results the limit of detection of the laboratory's quantitation sytem will also have to reevaluated
    - Alleles will now be detected and can be easily distinguished from the baseline at much lower amplification concentrations when samples are subjected to MinElute®
    - MinElute® will increase the limit of detection
    - Alleles previously not distinguishable from baseline will now be easily recognizable

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Required Testing for an Internal Validation

- Sensitivity and stochastic studies:
  - Stochastic threshold will also change with implementation of MinElute®
  - The stochastic effects caused by low level amplification will become more visible with MinElute® as the low level peaks are brought to callable peak heights
  - Based on their validation results, it will be up to each laboratory to decide what data they are willing to interpret and report out and what they will consider inconclusive

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Required Testing for an Internal Validation

- Sensitivity and stochastic studies:
  - Whether a laboratory wants to interpret data that exhibits stochastic effects is up to the laboratory and their comfort level
  - With new rulings on what is considered a low copy number sample, it will be important for a laboratory's interpretation protocol to take into account the increase in stochastic effects that will now be visible when using MinElute®
  - This protocol should be backed up by validation studies

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Required Testing for an Internal Validation

- Remainder of validation studies:
  - After the sensitivity and stochastic studies have been completed the other required validation studies can be performed
  - The interpretation guidelines that were set based on the sensitivity and stochastic studies will be followed for the remainder of the validation

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Required Testing for an Internal Validation

- Reproducibility and precision studies:
  - By analyzing samples in triplicate, reproducibility and precision can be checked

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Required Testing for an Internal Validation

- Mixture study:
  - The Mixture study will show the limitations of the MinElute® cleanup process
  - MinElute® will not work well for samples that have a high major contributor and a low minor contributor
  - MinElute® PCR Purification kits for DNA cleanup will still be useful on mixtures when the entire mixture is at a low level

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Required Testing for an Internal Validation

- Mixture study:
  - When the laboratory chooses to use MinElute® on mixtures with major and minor contributors:
    - Testing must be conducted during validation to determine the maximum RFU level the major can be at prior to cleanup
    - By determining the maximum RFU level for the major contributor prior to cleanup, the laboratory will ensure the fold increase in the resulting MinElute® data does not cause an increase in artifacts that interfere with interpretation of the minor contributor

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Required Testing for an Internal Validation

- Contamination assessment study:
  - Because MinElute® does involve working with amplified product, the contamination assessment is very important, whether the lab elects to conduct the procedure manually or with the QIAcube®
    - Introduction of contamination can occur more readily when working with amplified product
  - Throughout the study blanks should be incorporated
    - These "MinElute®" blanks must be treated the same, and interpreted as stringently, as the analyzed samples

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Required Testing for an Internal Validation

- Contamination assessment study – QIAcube® protocol:
  - When using the QIAcube®, in at least one run the instrument should be set up with samples alternating with blanks to demonstrate that no contamination is occurring within each run
  - A cleanup run containing only blanks immediately following a QIAcube® run containing only samples should also be performed to demonstrate that no contamination is occurring from one run to the next

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Required Testing for an Internal Validation

- Contamination assessment study – manual protocol:
  - MinElute® manual procedure contamination can be assessed by running blanks after every sample to demonstrate that no contamination is occurring within each run

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Required Testing for an Internal Validation

- Known and non-probative evidence samples or mock evidence samples study:
  - After all other studies have been completed, the known and non-probative or mock samples can be run through the MinElute® cleanup process

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Samples Needed for a Successful Validation

- Most labs tend to over do their validation studies
- As long as the MinElute® validation covers all aspects of how the kit will be used by the laboratory, the validation should be sufficient
  - The validation should be adequately detailed to allow the lab to answer questions that arise in the future by referencing the validation
- No fixed number of samples are required, but for an internal validation as few as ten samples per study may suffice

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Factors Affecting Validation

- One of the major factors affecting validation of the MinElute® cleanup system is comfort level with respect to the lowest RFU value the laboratory is willing to use when interpreting data
  - By the end of the sensitivity study the laboratory will know what peak heights correspond to what quantity of DNA when using MinElute®
  - The lab will take this into consideration when writing their interpretation guidelines for samples processed with MinElute®
    - This data would previously have been below threshold and, therefore, not suitable for conclusions

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Factors Affecting Validation

- How to handle data that previously would not have been "looked at" will also affect the MinElute® validation
  - For example, what if a low level sample that previously would have been inconclusive but now because of the MinElute® cleanup, is pulled above the calling threshold shows that the suspect's profile is present?
    - Does the laboratory still report this out as inconclusive or do they report it as an inclusion?
    - The lab needs to ensure the MinElute® validation is sufficient to determine how they will interpret and report data defined by various scenarios

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Factors Affecting Validation

- The lab will need a sufficient amount of data to demonstrate that allelic drop in is not occurring as a result of / during the MinElute® process
- When defining the parameters of their MinElute® validation, each laboratory may also want to consider the impact of potentially lowering their calling threshold based on the results of the study
  - In some jurisdictions, this change may necessitate additional Frye or Daubert admissibility hearings
    - Admissibility hearing requirements may assist in determining the number of samples per study the laboratory wants to run

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Factors Affecting Validation

- The validation results must be sufficient to support a decision to adjust the lab's calling threshold for MinElute® processed samples
  - Following cleanup, peaks will be visible that were previously at 10 RFUs or lower due to the fold increase seen with MinElute®
  - While a traditional protocol RFU threshold of 100 (set based on LOD, LOQ, and stochastic effects) may be appropriate for that data, the lab may find that review of MinElute® validation data supports setting a lower calling threshold
    - Sufficient data must be generated during the MinElute® validation to support such a decision

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Audit Requirements for the Validation

- After the MinElute® validation is completed the laboratory must write up a summary
  - The technical leader must approve the validation summary
- Laboratory staff will then undergo training in the use of the adopted MinElute® protocol
- Before using MinElute® on casework, each analyst must successfully complete a competency test
  - With proper documentation and authorization, staff performing the validation may use their validation work in lieu of a competency test

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MinElute® Training

- MinElute® training and competency testing can be completed in a day or two regardless of whether the analysis is conducted manually or with the QIAcube® robot
- The ability to rapidly complete analyst training and competency testing is due to:
  - The simplicity of the MinElute® system
  - All techniques being consistent with current techniques used by analysts

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Questions?

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Note: *All images are courtesy of Rob O'Brien.*

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