APPLYING A MINIMUM AMPLIFIABLE DNA THRESHOLD TO SEXUAL ASSAULT SAMPLES

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Significant backlogs of untested sexual assault kits remain in many crime laboratories. Also contributing to the U.S. backlog are thousands of sexual assault kits collected but never submitted to a lab by the law enforcement agency. Each of these untested kits represents an opportunity to identify a perpetrator and to assist in other investigations. Streamlining sexual assault kit processing can make it easier to eliminate backlogs. Male / human quantification can be used to replace conventional serology for sexual assault kits by allowing samples with insufficient male DNA for STR typing to be terminated after quantification.

Previous work from our laboratory showed that detecting male DNA at the quantification stage using Quantifiler Duo, and subsequent microscopic ID of sperm during extraction was significantly more accurate in identifying male DNA than using conventional serological screening on sexual assault samples (Welch 2010). In addition to increased sensitivity in semen detection, this approach also allows for the detection of male DNA from non-semen sources such as saliva or touch DNA. Because a determination of semen is still important, PSA and spermatozoa detection are now performed as part of our differential extraction process. To streamline the processing of increased numbers of differential samples using this approach, we developed a procedure to automate the initial decisions for downstream processing of differential samples after quantification.

Samples from sexual assault kits are cut for extraction with no initial screening for Alkaline Phosphatase (AP), Prostate Specific Antigen (PSA), or spermatozoa. Portions of each swab are combined in one tube for extraction. The differential extraction procedure includes an initial incubation of the sample in PSA buffer prior to extraction for PSA testing if no sperm are observed microscopically during differential extraction. This has the added benefit of increasing the total yield of male DNA obtained from the samples. After extraction, all samples are quantified using Quantifiler Duo.

As a general rule, samples containing less than 0.06 ng of amplifiable human DNA are not processed past quantification. For differential samples, if at least 0.06 ng total human DNA is present in a sample, the termination decision is then based on the amount of male DNA in the sample. If either the non-sperm (epithelial cell) fraction or the sperm fraction contains at least 0.03 ng male DNA, then both fractions are amplified. If both fractions contain less than 0.03 ng male DNA, then both are terminated after the quantification stage regardless of the amount of human DNA present in the samples. The samples that are terminated are automatically placed on a list so that a determination can be made as to whether it is useful to process these samples for STR profiling even without male DNA present (for instance, if the perpetrator were female or if female DNA on suspect items is of interest). We employ a series of linked spreadsheets that incorporates decision making for differential samples after the quantification stage using data imported directly into the spreadsheet from the ABI 7500.

By performing the steps outlined above for the testing of sexual assault kit swabs and stains from clothing or bedding, we have increased our ability to detect male DNA in sexual assault cases while eliminating the need to amplify and interpret samples containing only female DNA. By terminating samples that contain no male DNA after the quantification stage, we save equipment time and reagent costs.

After substantial validation, all automatic Excel links, formulas, calculations, and macros within the spreadsheet template have been verified. The template has been implemented at HCIFS and used in routine casework since June of 2010.

Reference:

Welch, K., Best, T., Timanova, A., Gefrides, L., and Kahn, R. An Efficient, Systematic Approach to Serology Screening of Sexual Assault Evidence. Proceedings of the AAFS (2010) 16:38