



NYC
**Office of Chief
Medical Examiner**

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From: Tiffany Vasquez, DNA Technical Leader, Nuclear DNA Operations

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Subject: Limit of Detection for Male Screening

This memo is regarding male screening results in use by the Department of Forensic Biology since 2017. The purpose of this screening was primarily to aid in the triage of the samples for downstream testing; in general, samples with higher amounts of male DNA would be slated for recut for an additional extraction or for STR typing. In December of 2023, it was brought to our attention that male screening results utilizing the phrase “male DNA detected,” may have been interpreted as always confirmatory for male DNA, even in the absence of STR typing results. For additional information, see associated memos from Timothy Kupferschmid dated December 18, 2023; and from Tiffany Vasquez dated January 4, 2024.

Reports from 2017 to 2023 relied on an estimated male DNA concentration threshold of 0.2pg/ μ L to report a sample as “male DNA detected.” To update our information regarding the 0.2pg/ μ L threshold, the sensitivity of the quantification for male DNA was re-evaluated using extraction methods currently in use at the OCME. The goal was to determine an estimated quantification threshold above which male DNA could be considered confirmed; confirmation was treated as the detection of STR alleles from a male after amplification. The false positive probabilities of the male DNA quantification at multiple thresholds were also determined. Similar studies and calculations were also performed for extractions that are not specifically used for male screening reporting. This additional data can inform the evaluation of male DNA in non-sexual assault cases, or if a sample from a sexual assault case bypasses the initial screening step, but the sample is insufficient for STR typing.

Consistent with previous validation studies, the estimates of total and male DNA concentration were observed to be more variable as the amount of DNA decreased. Given this inherent variability, all values listed below should be treated as estimated thresholds and probabilities.

To determine an estimated male concentration threshold above which male DNA can be confirmed with increased confidence, the estimated Limit of Detection (LoD) was calculated with a 95% upper bound using observed male concentrations, and in combination with the estimated Limit of Blank (LoB) using the male concentration values of presumed male negative samples (female oral swabs and negative controls).¹

¹ Method described in: Armbruster DA, Pry T. Limit of Blank, Limit of Detection and Limit of Quantitation. Clin Biochem Rev. 2008 Aug; 29(Suppl 1): S49-S52.



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| Extraction method | Male quantification threshold | Estimated probability of a false positive at or above this threshold |
|---------------------------------------|-------------------------------|--|
| Male screening extraction method | 0.70 pg/ μ L (LoD) | 0.0015% |
| | 0.20 pg/ μ L | 3.8% |
| Non-male screening extraction methods | 0.38 pg/ μ L (LoD) | 0.11% |
| | 0.20 pg/ μ L | 0.93% |

Since December 2023, all values above 0.0 pg/ μ L for male concentration are reported as presumptively positive for the presence of male DNA. If only the quantification data is being evaluated to opine as to whether male DNA is confirmed within a sample, prudence should be used when making conclusions regarding male concentration values below or approaching the relevant limit of detection. Above that threshold, there is increased confidence that such a value is representative of true male DNA. Confidence in this determination increases the higher the male concentration value.

Please review cases that report positive male screening results, particularly in the absence of DNA typing. If you feel this previous language may have impacted the adjudication of a case, please reach out.