

## Controls for DNA Analysis

### Casework

Four controls are processed with each case:

A. Reagent Blank (RB). Per QAS: A reagent blank is an analytical control sample that contains no template DNA and is used to monitor contamination from extraction to final fragment or sequence analysis. This control is treated the same as, and parallel to, the forensic and/or casework reference samples being analyzed.

A reagent blank (RB) is processed with each set of extractions. The volume of the RB used for the amplification step must be the same as (or greater than) the maximum volume used to amplify the evidentiary samples. Re-amplification of the RB may be omitted when re-amplifying the same volume (or less) of the evidentiary sample(s). When re-amplifying a greater volume of the evidentiary sample(s), the RB must be re-amplified with that volume. With differential extractions, the RB is subjected to the same processing steps as the evidence. When processing non-differential samples and differential samples in the same set, the RB-A will also serve as the reagent blank for non-differential samples as well as for the epithelial-rich samples. RB-B will be the reagent blank for the sperm-rich fraction of the differential samples. There may be multiple RBs in an extraction set. The RB may be used as the amplification negative, if necessary, with TL approval.

B. Laboratory Positive Control. A laboratory extraction positive control (EP1 or EP2) is processed for each set of extractions. The EP1 extraction control is carried through all STR analysis steps. The EP2 is stopped at quantitation. The EP1 extraction positive control may be omitted for re-amplifications if expected results were previously generated for the extraction set. Previously used laboratory extraction positive controls are RKO, TMP, and KJL. If all evidence samples are halted in an extraction set at quantification, the EP1 (associated with those samples) does not have to be carried through to CE if the reagent blank is quantified. If the RB is not quantified, then the EP1 will be carried through with the RB. The EP1 may be used as an amplification positive control if necessary, with TL approval.

C. Negative Control. Per QAS: An amp negative control is used to detect DNA contamination of the amplification reagents. This control consists of only amplification reagents without the addition of template DNA. An amplification blank (NEG) is processed with each set of amplifications. The maximum volume (dH<sub>2</sub>O) possible for each kit is used for the amplification step.

D. Kit Positive Control (POS). A kit amplification positive control is processed with each set of amplifications.

E. Each RB and extraction control extraction tube will be labeled with a unique identifier. An

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example could be RB-date initials. The unique identifier information for the control can be found on any of the DNA extraction worksheets.

Under the Sample # field in line with the appropriate Control. The unique identifier will be used for any extract tubes that will be stored in long-term freezer boxes. This also is typically the batch number.

**Known Processing**

Based on internal validation the following QA parameters are set:

**For database known samples:**

1 RB, 1 NEG, and 1 POS run on a plate. 3 EP1s on a full plate and at least 1 EP1 on a partial plate.

If called alleles arise in the RB or NEG, interpret with caution and see CODIS administrator. If necessary, re-amplify or re-extract affected samples.

Barcodes and QC samples will continue to be used to prevent sample switches and assure proper plate orientation.

**Casework known samples:**

1 RB, 1 NEG, 1 POS, and at least 1 EP1 per plate.

If called alleles are in the RB or NEG, re-amplify or re-extract samples as necessary.

For both types of known processing, an RB and NEG must be amplified and injected to reflect the largest amplification volume and longest injection times for the samples.