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Know Error DNA Specimen Provenance Assignment (DSPA) Process

4 Stages

Stage 1: DNA Extraction

DNA is extracted from the tissue/buccal swab by lysing the cells. The amount of DNA that is present is typically quantified; depending on the amount of DNA available, the sample may be concentrated or diluted to optimize the amplification reaction. Formalin fixed, paraffin embedded tissues are not an optimal source for DNA, as the fixation process is known to both degrade DNA and to cause chemical crosslinking that can impede downstream tests. We use specialized reagents and techniques specifically designed to work with this difficult sample type.

Stage 2: Amplification

Very specific locations (loci) in the DNA are copied (amplified) millions of times; each copy has a fluorescent tag attached. This amplification process is very sensitive. If non-patient DNA is present, for example, from extraneous tissue in the water bath or from a cutting station that is not cleaned between samples, the non-patient DNA will be amplified along with the patient DNA, and readily discernable in our test result—even if the foreign cells are not distinguishable under microspic examination.

Stage 3: Separation & Analysis

Capillary electrophoresis separates the DNA based on fragment size and charge. The fluorescent tag on each fragment is excited by a laser and the emission is captured by an optical detection system. The combination of fragment size and fluorescent tag allows the assignment of DNA fragments to specific alleles for the 16 individual loci that were tested. Even if useful data is only obtained on a faction of the loci examined, assignment of provenance is generally possible.

Stage 4: Interpretation & Reporting

The DNA profile from each tissue sample is compared to the DNA profile from the corresponding reference sample of known provenance. The comparison includes identifying the loci having data that are acceptable for comparison, identifying the alleles at each locus and calculating the random match probability of the profile. The random match probability is a statistical calculation of the likelihood that the same profile will be observed in another individual chosen from the population at random (i.e. how specific is the identification). The results of each comparison can be categorized most simply as Match, Non-match or Inconclusive (QNS).

- **MATCH –** There are at least 5 loci that are identical between the reference and the tissue, no conflicting loci observed and a random match probability indicating that the profile statistically can be expected only once in a population of at least one hundred thousand people.
- **NON-MATCH –** the reference and the tissue do not match one another. This can be due to a transposition (the reference and the tissue come from different people), contamination (DNA from two or more people in a single sample) or severe genetic anomalies resulting from the cancerous nature of the tissue.
- **INCONCLUSIVE –** the data obtained from the tissue, the reference or both is of insufficient quantity or quality such that a meaningful comparison cannot be made.

In the case of non-match or inconclusive results, the treating physician and/or pathologist must consider whether assignment of provenance for that specimen is necessary to render a complete and accurate diagnosis or treatment plan. If so, we recommend that the pathology lab re-submit a new confirmatory tissue scroll from the same paraffin block for which provenance is unverified for repeat DSPA testing, and re-read a newly prepared microscopy slide from the same block in order to confirm the original pathology interpretation. Conversely, if the provenance of the particular tissue can be disregarded without impacting diagnosis or treatment, then repeat testing may not be necessary (for example, if the provenance of five high grade cancer specimens has been confirmed via DSPA, but a 6th specimen yields an inconclusive DSPA result).