Technical Brief

DNA Fingerprinting Analysis for Specimen Identification

Background
Specimen misidentification or cross contamination (“floaters”) are potential sources of error in the surgical pathology lab. Many instances of specimen misidentification and cross-contamination are easily resolved through clinical and laboratory correlation, but others are not. In particular, it may be quite difficult to determine whether or not small neoplastic fragments embedded in the tissue block truly belong within the block and ultimately to the patient. In one study, up to 3% of slides contained extraneous tissue, with up to 28% embedded in the tissue block, and up to 14% representing neoplastic tissue. DNA-based identity testing, or DNA fingerprinting, may be employed in these difficult-to-resolve instances.

Clinical Indications
Assessment of tissue identity by DNA fingerprinting is useful for:

1. Confirming possible tissue contaminants (“floaters”) identified on histologic slides.
2. Determining specimen identity in the case of suspected mislabeling.

Please Note: Cleveland Clinic does NOT perform paternity testing. See www.aabb.org for labs accredited by the American Association of Blood Banks for information regarding paternity testing.

Specimen Requirements
DNA fingerprinting can be performed on formalin-fixed, paraffin-embedded tissue, blood in EDTA, or fresh/frozen tissue. The submitting pathologist must designate the suspected contaminant/mix-up as well as the patient’s known sample with which to compare. It is optional to include the suspected source tissue.

Interpretation
Results are reported as the number of identical or non-identical alleles between the known and unknown samples. If the suspected source tissue is provided, an interpretation as to the identity of the unknown specimens also is given. If the suspected contaminant and patient’s known sample derived from unrelated individuals, there is a >99.9998% likelihood that one or more loci will be different.

Methodology
DNA fingerprinting analysis by PCR is a highly accurate technique for determining the patient identity of a tissue sample. This assay uses PCR amplification of short tandem repeats (STRs), which are short, repetitive DNA sequences. Each STR locus has two alleles, and each allele has a specific length that is stably inherited. Microdissection is performed as needed. PCR amplification of 15 highly polymorphic STR loci (D3S1358, TH01, D21S11, PentaE, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, vWA, D8S1179, TPOX, D18S51, FGA) and a sex chromosome specific locus (amelogenin) is performed. The fluorescently labeled PCR products are detected, analyzed, and quantified by capillary gel electrophoresis. Positive and negative external controls are included.

Limitations of the Assay
Fixatives that cause poor DNA quality such as mercury-based fixatives, picric acid-based fixatives and decalcifying agents will not yield interpretable results.

In the event that deeper levels have exhausted the tissue from the sample block and the only sample remaining for testing is tissue on the original stained slides, we will attempt to remove the coverslip and use the stained tissues for DNA testing. We will attempt analysis from any tissue fragment size, but success rates go down with progressively smaller samples.
The assay is composed of tetranucleotide and pentanucleotide repeats (microsatellites) that are susceptible to microsatellite instability. Microsatellite instability causes the generation of new alleles, which is also the basis of determination of tissue non-identity. If the tumor demonstrates high-microsatellite instability (a minority of colon, stomach, endometrium, and head and neck malignancies, among many others), interpretation of DNA identity testing may be less reliable.4,5

References


Test Overview

<table>
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<tr>
<th>Test Name</th>
<th>DNA Fingerprinting Analysis for Specimen Identification</th>
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<td>Methodology</td>
<td>Polymerase Chain Reaction</td>
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<td>Specimen Requirements</td>
<td>Tube/Container: See note</td>
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<td>Note: KNOWN SPECIMEN: 5-10 unstained 5 µm sections of a “known” tissue sample derived from the patient (formalin-fixed, paraffin-embedded tissue) on charged, unbaked slides OR the formalin-fixed paraffin block containing representative tissue. The corresponding H&amp;E marked to indicate the tissue of interest for testing is also required. A block that is separate from the block with the unknown tissue is requested, to reduce the risk of contamination and need for microdissection. ALTERNATIVELY, 4 mL of EDTA anticoagulated whole blood (not refrigerated, at ambient temperature only) may be used for analysis.</td>
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<td>UNKNOWN SPECIMEN: 5-10 unstained 5 µm sections containing the tissue of unknown identity on charged, unbaked slides OR the formalin-fixed paraffin block. The corresponding H&amp;E is also required, marked to indicate the tissue for identity testing.</td>
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<td>Special Information</td>
<td>Submit specimens with an Anatomic Pathology request form. Indicate DNA Fingerprinting Analysis on request form. In the event that deeper levels have exhausted the tissue from the sample block and the only sample remaining for testing is tissue on the original stained slides, we will attempt to remove the coverslip and use the stained tissues for DNA testing. We will attempt analysis from any fragment size, but success rates are not guaranteed for samples &lt;2 mm.</td>
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<td>CPT Codes</td>
<td>83891 (x2); 83901 (x2); 83894 (x2); 83912</td>
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