DNA Yield From Tissue Samples in Surgical Pathology and Minimum Tissue Requirements for Molecular Testing

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- **Context.**—Complex molecular assays are increasingly used to direct therapy and provide diagnostic and prognostic information but can require relatively large amounts of DNA.

- **Objectives.**—To provide data to pathologists to help them assess tissue adequacy and provide prospective guidance on the amount of tissue that should be procured.

- **Design.**—We used slide-based measurements to establish a relationship between processed tissue volume and DNA yield by A260 from 366 formalin-fixed, paraffin-embedded tissue samples submitted for the 3 most common molecular assays performed in our laboratory (EGFR, KRAS, and BRAF). We determined the average DNA yield per unit of tissue volume, and we used the distribution of DNA yields to calculate the minimum volume of tissue that should yield sufficient DNA 99% of the time.

- **Results.**—All samples with a volume greater than 8 mm³ yielded at least 1 μg of DNA, and more than 80% of samples producing less than 1 μg were extracted from less than 4 mm³ of tissue. Nine square millimeters of tissue should produce more than 1 μg of DNA 99% of the time.

- **Conclusions.**—We conclude that 2 tissue cores, each 1 cm long and obtained with an 18-gauge needle, will almost always provide enough DNA for complex multigene assays, and our methodology may be readily extrapolated to individual institutional practice.

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Molecular oncology assays are used increasingly to direct therapy for primary and metastatic tumors, and they can also provide important diagnostic and prognostic information. Assays performed on solid tumors can require substantial amounts of DNA, sometimes as much as 1 μg for comprehensive multigene assays based on next-generation sequencing. It is also becoming increasingly common for physicians to request directed biopsies for the sole purpose of molecular testing. If the DNA yield from a tissue sample is inadequate for testing, significant treatment delays can result, and patients may be subjected to the risk associated with a repeat biopsy. Studies published to date have provided limited information on DNA yield from formalin-fixed, paraffin-embedded tissue samples. Thus, there is a need for data to help pathologists make timely and reliable decisions about the adequacy of tissue samples for molecular oncology assays, and to enable them to provide prospective guidance to clinicians regarding the amount of tissue that should be procured.

In this study, we used simple measurements of tissue area and volume to assess whether samples will produce enough DNA to perform even the most complex multigene assays. In addition, we wanted to develop a method to provide a standard recommendation for the minimum tissue volume that should be obtained during directed tissue procurement for molecular testing.

**MATERIALS AND METHODS**

To help ensure that findings would be broadly applicable, we chose formalin-fixed, paraffin-embedded tissue samples processed for the 3 most common molecular oncology assays performed in our laboratory: EGFR mutational analysis, typically performed on small needle core biopsies of lung tissue; KRAS mutational analysis, typically performed on endoscopic mucosal biopsies or resections of colonic tissue; and BRAF mutational analysis, typically performed on larger skin or lymph node excisional biopsies. The study included a total of 366 samples received during a 3-year period (174 samples for EGFR, 115 samples for KRAS, and 77 samples for BRAF). In the study set of 366 samples, a total of 3 samples yielded insufficient DNA to provide a reportable result for the ordered test. These 3 samples were included in the study data set.

Samples were provided to the molecular laboratory as either (1) unstained tissue as curls (typically 10 curls, each 0.01 mm thick) or (2) a variable number of unstained tissue slices on slides (typically 0.01 mm thick). For both sample types, a hematoxylin-eosin–stained slide was used to estimate the area of submitted tissue and the percentage of tumor cells in the harvested tissue. All measurements were rounded to the nearest millimeter, and irregular shapes were estimated as a collection of regular shapes (eg, rectangles and triangles). The total estimated tissue volume (in mm³) used for DNA preparation was calculated as area of harvested tissue in mm² × number of slides or curls processed × section thickness in mm. If only a portion of the tissue on the slide was harvested in order to enrich for viable tumor cells, the calculation was based on the harvested area, rather than the total tissue area on the slide. The
precision of this method of estimation is limited by natural variation in tissue area that occurs with progressive leveling through the paraffin block, but empirical observation of tissue on the unstained slides suggests that this effect is limited.

Tissue from scraped slides or curls was pooled in a single tube, and paraffin was removed by extraction with CitriSolv (VWR, Brisbane, California); the tissue was then dried with 100% ethanol. The DNA from the isolated tissue was extracted using a Gentra Puregene Tissue kit (part 158667, Qiagen, Valencia, California) via the following protocol: cell lysis in the presence of DNA stabilizers was followed by RNAse treatment and salting out of proteins. The DNA was precipitated with ethanol, pelleted, and eluted into 10 mM Tris-HCl and 1.0 mM EDTA (pH 7.5). Eluted DNA was quantitated by absorbance at 260 nm on a NanoDrop 1000 instrument (Thermo Scientific, Wilmington, Delaware), which has a coefficient of variation of 3.3% at a DNA concentration of 110 μg/mL. It is important to note that absorbance measurements typically overestimate the amount of DNA by a factor of 2 relative to fluorescence assays, so the 1-μg figure we selected is the equivalent of 0.5 μg in a laboratory using fluorescence assay to quantify the amount of extracted DNA. This was confirmed by quantifying DNA in a subset of samples using the Qubit fluorescence assay (Invitrogen, Grand Island, New York).

To determine the average DNA yield per unit of tissue volume, we performed linear regression analysis on DNA yield versus tissue volume for each of the 3 sample types included in the study. The average DNA yield per mm³ of tissue is equal to the slope of the regression line (y = mx). Average DNA yields and linear regression values did not change significantly on log-log plots or when the trend line was adjusted to pass through zero.

RESULTS

Average DNA Yield as a Function of Estimated Tissue Volume

The average DNA yield as a function of estimated tissue volume for all 366 samples is summarized in the Table.

<table>
<thead>
<tr>
<th>Average DNA yield, μg/mm³</th>
<th>EGFR</th>
<th>KRAS</th>
<th>BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples</td>
<td>174</td>
<td>115</td>
<td>77</td>
</tr>
<tr>
<td>Average DNA yield</td>
<td>1.2</td>
<td>3.1</td>
<td>3.9</td>
</tr>
<tr>
<td>No. (% of samples &lt; 1 μg)</td>
<td>41 (23)</td>
<td>20 (17)</td>
<td>10 (13)</td>
</tr>
<tr>
<td>Minimum volume to obtain &gt; 1 μg DNA, mm³</td>
<td>8</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

Samples for EGFR testing (typically from lung) yielded an average of 1.2 μg DNA/mm³ of tissue (y = 1.2x, R² = 0.25); in this group, 23% of the samples (41 of 174) yielded less than 1 μg of DNA. Samples for KRAS testing (typically from colon) yielded an average of 3.1 μg/mm³ of tissue (y = 3.1x, R² = 0.48), and 17% of these samples (20 of 115) yielded less than 1 μg of DNA. Samples for BRAF testing (typically from skin or lymph node) yielded an average of 3.9 μg of DNA per mm³ of tissue (y = 3.9x, R² = 0.19), and 13% of these samples (10 of 77) yielded less than 1 μg of DNA. All tissues for EGFR and KRAS with volume greater than 8 mm³ and all samples for BRAF with volume greater than 6 mm³ yielded more than 1 μg of DNA. The vast majority (80%) of samples that produced less than 1 μg of DNA came from samples with less than 4 mm³ tissue.

Variability of DNA Yield for a Given Estimated Tissue Volume

The Figure, A through C, shows the variability of DNA yield as a function of estimated tissue volume. The horizontal lines delineate the 10th and 50th percentiles in each panel. A DNA yield of 1.0 μg or greater was obtained from 90% of the tissue samples with volume 4 mm³ or greater and 100% of tissue samples with volume 8 mm³ or greater.

Calculations of Minimum Tissue Requirements for Directed Biopsies

Based on the results summarized in the Figure, A through C, we calculated the minimum amount of tissue required to give at least 1 μg of DNA either 90% or 99% of the time for several commonly tested tissue types. If the desired failure rate is 10% or less, this corresponds to a tissue volume of 4 mm³; for a failure rate of 1% or less, the corresponding minimum volume is 8 mm³.
volume is 8 mm³. Assuming a loss of 1 mm³ of tissue during histologic preparation of slides (an estimate developed in consultation with experienced histologic technicians at our institution), one would need to obtain a minimum volume of either 5 or 9 mm³ to achieve these goals.

To provide guidance for interventional radiology procedures, we estimated the corresponding length of tissue core required. For an 18-gauge needle commonly used in these procedures, the internal diameter is 0.838 mm, corresponding to an internal volume of 5.52 mm³ per mm of core length. Assuming that viable tissue of sufficient average tumor cellularity (>10%) is obtained and entirely submitted, a single core 9 mm long will produce 1 µg or more of DNA 90% of the time, and 2 or more cores with a combined length of 18 mm will produce 1 µg or more of DNA 99% of the time. For simplicity, one can therefore recommend obtaining two 1-cm cores to maximize the likelihood of obtaining enough DNA for even the most complex multigene molecular assays.

COMMENT

We retrospectively evaluated 366 formalin-fixed, paraffin-embedded tissue samples submitted for EGFR, KRAS, and BRAF testing to establish a relationship between processed tissue volume and DNA yield. All samples with a volume greater than 8 mm³ yielded at least 1 µg of DNA by A260 (equivalent to 0.5 µg by fluorescence assay), which is sufficient to perform even the most complex next-generation sequencing assays. Conversely, more than 80% of samples that produced 1 µg of DNA or less were extracted from less than 4 mm³ of tissue (similar across all 3 assays evaluated). From these data, we were able to calculate a recommended volume of tissue that should lead to a failure rate of 1% or less in directed tissue biopsies: a minimum of two 1-cm cores from an 18-gauge needle.

We note 2 potential limitations of this study. First, we did not formally analyze the average length and chemical integrity of the extracted DNA. However, the DNA extraction methods used routinely provide DNA of suitable quality for both amplification-based and capture-based next-generation sequencing assays; thus, the results should also be applicable to other laboratories performing complex multigene assays. Second, almost all the data were gathered from tissue samples processed at a single institution. However, our tissue fixation and DNA extraction procedures both follow widely accepted protocols and are therefore likely to be representative of methods used at the majority of institutions.

Despite these limitations, this study adds substantially to knowledge of DNA yield from surgical pathology samples. Fixative and processing effects on nucleic acids have been discussed in the literature, and there is a published study that prospectively evaluated the impact of needle gauge on breast biopsy interpretation, with smaller-gauge (ie, larger-bore) needles producing samples better suited to molecular studies.

Investigators have clearly established the utility of the directed needle core biopsy for histologic and molecular characterization of various tissues, but only one of these studies included data regarding the relationship between tissue volume and its DNA yield, and it was limited to analysis of a small set of lung biopsies. An additional publication describes DNA yields in fresh frozen tissue; again, the overall number of samples evaluated was small, and this study cannot be directly translated to formalin-fixed, paraffin-embedded samples, where there is further loss and degradation of nucleic acid during tissue fixation and processing.

Our study focused on simple measurements of tissue area that can be quickly and easily performed in any setting; however, the overall cellularity of the tissue (ie, nuclei per unit volume) will also affect DNA yield. Our data do show modest differences in average yield (twofold to threefold) among tissue types (see Results), but the average difference in yield among tissue types was overwhelmed by the variability of yield for individual samples. We therefore chose to use our pooled data for all sample types to estimate minimum tissue volumes required to obtain a given amount of DNA, because there is no practical method of measuring the density of nuclei per unit of tissue volume in the routine clinical setting.

Beyond DNA yield, there are other factors that influence whether a given specimen will be suitable for a given molecular assay. The percentage of neoplastic cells in the sample will influence the likelihood of missing a tumor-specific mutation, with different thresholds for different specific assay procedures; however, the pathologist can readily assess the tumor percentage and viability at the time the sample is also assessed for area and volume. It is more difficult to assess the quality of the DNA that will be obtained, that is, its average length or the chemical modification of purine or pyrimidine bases, which can affect the performance of downstream molecular assays. Here the best rule of thumb is to avoid all fixatives other than formalin for samples destined for molecular testing, and to be cautious in the use of severely necrotic tissue, in which the DNA is likely to be highly degraded.

CONCLUSIONS

We have provided guidelines to allow pathologists to make recommendations on how much tissue is needed for molecular assays and to rapidly assess the adequacy of samples received for molecular testing.

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REFERENCES


