Slide Ordering

For a standard molecular order of 10 unstained 5 micron thick slides, please order the following task protocol in Care Connect:

1) Unstained 5u and Send to Molecular x10

If additional unstained slides are needed, please add on the following task protocol in Care Connect.

1) Recut Unstained 5u and Send to Molecular (enter number of additional unstained slides)

Please see figures 1 and 2 for examples of orders for a total of 10 unstained slides and for a total of 20 unstained slides, respectively. **This ordering scheme is also designed to accommodate the availability of pre-cut unstained molecular slides (e.g. for lung biopsies).**

Figure 1. Example of order for total of 10 unstained slides.

Figure 2. Example of order for total of 20 unstained slides.
Slide Assessment

Macrodisssection

1) If macrodissection is not requested, all of the material on the unstained slides will be scraped off and submitted for molecular testing.

2) If macrodissection is requested, an attempt will be made to scrape off only material corresponding to circled areas identified on the submitted H&E (see Figure 3 for macrodissection examples).

3) Avoid circling small areas.
   a. Macrodisssection is a manual process where areas are grossly identified and scraped off by hand using a razor blade.
   b. Due to difficulty identifying and scraping small circled areas on unstained slides:
      i. “Wanted” material inside but near the borders of circled areas may be missed.
      ii. “Unwanted” material outside but near the borders of circled areas may be scraped.

4) Avoid macrodissection for biopsies.
   a. Tissue landmarks present on the H&E slide may be difficult to identify or may not be present on deeper unstained slides for orientation purposes.
   b. Tumor foci may change size, disappear, or appear in different locations on deeper unstained slides.
   c. If necessary, macrodissection can be considered for biopsies showing readily identifiable foci of tumor that are likely to be present in the deeper unstained slides (see Figure 3c for a difficult case).

5) DNA integrity may be reduced in samples with necrosis.
   a. Macrodisssection should be considered for large specimens with necrosis if focal areas of viable tissue are present.

Estimated Tumor Percentage

1) If macrodissection is not requested, provide an overall tumor percentage for the whole slide.

2) If macrodissection is requested, provide an overall tumor percentage only for the material in the circled area(s).

3) Tumor percentage = # tumor cells / (# normal cells + # tumor cells).
   a. The counts are based on the numbers of cells.
   b. Ignore cell size.
      i. Small cells (e.g. lymphocytes) are assumed to contain the same amount of DNA as larger cells (see Figure 4).
   c. All background cells including inflammation should be included when counting the number of normal cells.
   d. Do not include necrotic cells in the count.

4) To calculate the overall tumor percentage:
   a. Examine multiple fields and provide a single overall estimate based on an average of the tumor percentages seen in these fields (see Figure 5).
   b. As a single overall value (e.g. 15%) may be difficult to estimate, the low and high ends of an estimated range (e.g. 10-20%) can be reported.

5) While the limit of detection for next-generation sequencing is approximately 10% tumor (corresponding to approximately 5% of an alternate allele in a background of reference alleles), tumor percentages down to a few percent can be reliably detected.

Adequacy of Material

1) The total material scraped from the unstained slides (or from areas corresponding to circled areas) needs to contain at least 20,000 viable cells (including both normal and tumor cells) in order to provide the typical amount of DNA needed for testing.
a. If there are 2,000 or more cells on the H&E (if no macrodissection is requested) or in the circled areas (if macrodissection is requested), place a standard molecular order of 10 unstained 5 micron slides.
b. If there are fewer than 2,000 cells on the H&E (if no macrodissection is requested) or in the circled areas (if macrodissection is requested), order the standard 10 unstained 5 micron thick slides plus additional unstained slides to reach a total of 20,000 viable cells (including both normal and tumor cells) on the total number of unstained slides ordered.
   i. Typically, a standard 10 additional slides are ordered if needed to provide extra material.
   ii. Fifteen (15) additional slides are occasionally ordered.
   iii. Please consult with MDL if more than 15 additional slides (i.e. 25 total slides) are being ordered.

2) For small biopsies with significant necrosis with concerns about viability, order 10 additional slides even if 2,000 or more cells are present (see Figure 6).

3) For small biopsies with scanty appearing tissue which may disappear on deeper recuts, order 10 additional slides (see Figure 7) even if 2000 or more cells are present.
a. The circled area containing tumor is adequate for macrodissection and should be straightforward to identify and scrape from the unstained slides.

b. The two circled areas containing tumor are adequate for macrodissection, but more challenging to identify and scrape due to the irregular and focally narrow shapes.

c. The two circled areas containing the only tumor present in the specimen are suboptimal for macrodissection. The smaller bottom right fragment with a floater-like appearance will potentially disappear with deeper recuts.

Recommendations:
1) See if another specimen is available to test.
2) When counting cells for adequacy, give less weight to the small fragment which may disappear.
3) Order at least 10 additional slides.
4) Consider submitting the whole specimen in case other foci appear in deeper sections.

Figure 3. Macrodissection Examples
Figure 4. The left field shows high % tumor. The right field shows a smaller but somewhat similar number of tumor cells, but significantly lower % tumor due to the presence of many lymphocytes. Although small, each lymphocyte is assumed to contribute the same amount of DNA as a larger cell.

Figure 5. Averaging over multiple representative fields resulted in an overall estimated % tumor of 5-10%. This lung biopsy specimen was submitted without macrodissection.
Figure 6. DNA integrity may be reduced with necrosis. In cases with necrosis, consider ordering more slides for small biopsies and requesting macrodissection for large specimens.

Figure 7. For biopsies showing scanty tissue, order additional slides even if 2000 or more cells are present on the H&E, as the tissue may disappear on deeper slides.
<table>
<thead>
<tr>
<th>Please fill in all fields</th>
<th>Notes</th>
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<tbody>
<tr>
<td>Macrodissection (Answer Yes/No)</td>
<td>If answer is “No,” the entire surfaces of the unstained slides will be scraped off and submitted for processing. Do not send H&amp;E slide to MDL when the answer is “No”. If answer is “Yes,” only areas on the unstained slides corresponding to circled areas on the H&amp;E slide will be scraped off and submitted for processing. Send the H&amp;E slide with circled areas to MDL when the answer is “Yes”.</td>
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<tr>
<td>Estimated % Tumor (Low End)</td>
<td>Low end of tumor percentage estimate (e.g. for an approximate tumor percentage of 10-20%, this would be 10%). If no macrodissection is requested, tumor percentage = # tumor cells / (# normal cells + # tumor cells) across entire H&amp;E slide. If macrodissection is requested, tumor percentage = # tumor cells / (# normal cells + # tumor cells) in circled areas of H&amp;E slide. Exclude necrotic cells when calculating estimate.</td>
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<tr>
<td>Estimated % Tumor (High End)</td>
<td>High end of tumor percentage estimate (e.g. for an approximate tumor percentage of 10-20%, this would be 20%).</td>
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<tr>
<td>2000 or More Cells Per Slide, or in Circled Areas if Macrodissection (Answer Yes/No)</td>
<td>If no macrodissection is requested, are there 2000 or more cells (including both normal and tumor cells) present on the H&amp;E slide? If macrodissection is requested, are there 2000 or more cells (including both normal and tumor cells) present in the circled areas of the H&amp;E slide? Exclude necrotic cells from count.</td>
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| Total Unstained Slides Ordered | Enter total number of unstained slides that have been ordered and are being sent to MDL for processing. If answer is “Yes” to 2000 or More Cells question, order standard 10 unstained 5 micron thick slides for molecular. If answer is “No” to 2000 or More Cells question, order standard 10 unstained 5 micron thick slides plus additional unstained slides to reach a total of 20,000 viable cells (including both normal and tumor cells) on the unstained slides ordered.  
  • Provide the total number of unstained 5 micron thick slides (standard 10 + number of extra slides you are ordering). |
| Reviewing Pathologist | Enter surgical pathologist involved with assessment. |

Please consult MDL if any questions or concerns about specimen adequacy for testing.