

Molecular Pathology: Slide Ordering & Assessment

This tip sheet provides an overview of the workflows involved in solid tumor molecular testing, including the process by which surgical pathology places orders in Care Connect and how surgical pathology and the Molecular Diagnostics Laboratories (MDL) respond to orders initiated by in-house or Outreach clinicians.

This tip sheet also describes the process of morphologic assessment of solid tumor cases performed by surgical pathology which is required for every case. Of note, molecular testing on a case cannot be started until the assessment information is received by MDL.

On occasion when an assessment cannot be directly entered into Care Connect as described below, an assessment form is included at the end of this tip sheet which can be separately filled out and delivered to MDL.

Please additionally note that the workflows are designed such that the slides to be tested are ordered by surgical pathology or Outreach. These slides should be ordered according to the steps given below to avoid delays to testing.

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Pathologist Order: Ordered by surgical pathology during or after case signout

- The surgical pathologist/resident/fellow will place the solid tumor molecular order.
- The surgical pathologist/resident/fellow will assess an H&E slide and fill in the assessment fields on the solid tumor molecular order (see below). These are the same fields as are on the paper req.
- The surgical pathologist/resident/fellow will request the appropriate number of unstained molecular slides. The unstained slides will be sent directly to MDL.
- Items to be sent to MDL by surgical pathologist/resident/fellow:
- If macrodissection is not requested, nothing needs to be sent.
- If macrodissection is requested, send H&E with circled areas (using current drop-off process in CHS A7 215A).

Clinician Order: Ordered by clinical team

- MDL will monitor for these orders, which go to a molecular in-basket.
- MDL will contact the surgical pathologist who signed out the case. If the surgical pathologist is not available or a surgical case was not specified in the order, MDL will contact the sub-specialty pathologist on sign-out service.
- The surgical pathologist or sub-specialty pathologist will place an order for 'Pathologist Order' workflow (see above).
- There is no need to cancel the original order. MDL staff will cancel the original Solid Tumor Molecular order so there are no duplicates.

Outreach Order: Requested by outside institution

- Outreach will place the solid tumor molecular order.
- Outreach will order a molecular H&E slide and the standard 10 unstained molecular slides. The unstained slides will be sent directly to MDL.
- The molecular H&E slide will be delivered to Outreach.
- Outreach will send the molecular H&E slide with a paper req to the surgical pathologist who signed out the case to provide an assessment. If the surgical pathologist is not available, Outreach will contact the sub-specialty pathologist on sign-out service.
- The surgical pathologist will assess an H&E slide and fill in the assessment fields in the Discrete Results section on the corresponding Solid Tumor Molecular case. (Molecular case number will be on the Outreach Req accompanying the slides)
- Enter the Pathologists Name in the 'Reviewing Surgical Pathologist' result section on the same Solid Tumor Molecular case.
- If needed, the surgical pathologist will order additional unstained slides. The unstained slides will be sent directly to MDL.

Discrete Results										
Component	Value	Units	!	▲	L	IE	R	PV	Chart	
Macrodissection										
Estimated % Tumor (Low End)		%								
Estimated % Tumor (High End)		%								
2000 or More Cells Per Slide (or in circled area if macrodissection)?										
Number of Slides Ordered										
Specimen Adequate (enter comment if Unable to determine or No)										
1 Request Repeat?										
1 Completed Repeat?										
2 Request Repeat?										
2 Completed Repeat?										

Items to be sent to MDL by surgical pathologist (For Outreach Orders Only):

- If macrodissection is not requested, send paper req (using current drop-off process in CHS A7-215A).
- If macrodissection is requested, send paper req and H&E with circled areas (using current drop-off process in CHS A7-215A).

Slide Ordering:

- Outreach will place the solid tumor molecular order.
- Outreach will order a molecular H&E slide and the standard 10 unstained molecular slides. The unstained slides will be sent directly to MDL.

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
(This order will automatically pull any unstained slides if they exist, or new unstained slides will be cut if they don't)

This includes the workflow when ordering Therascreen on Lung Cancer cases.

If additional unstained slides are needed for adequacy (see adequacy information below), please add on the following task protocol in Care Connect.

- 2) 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.

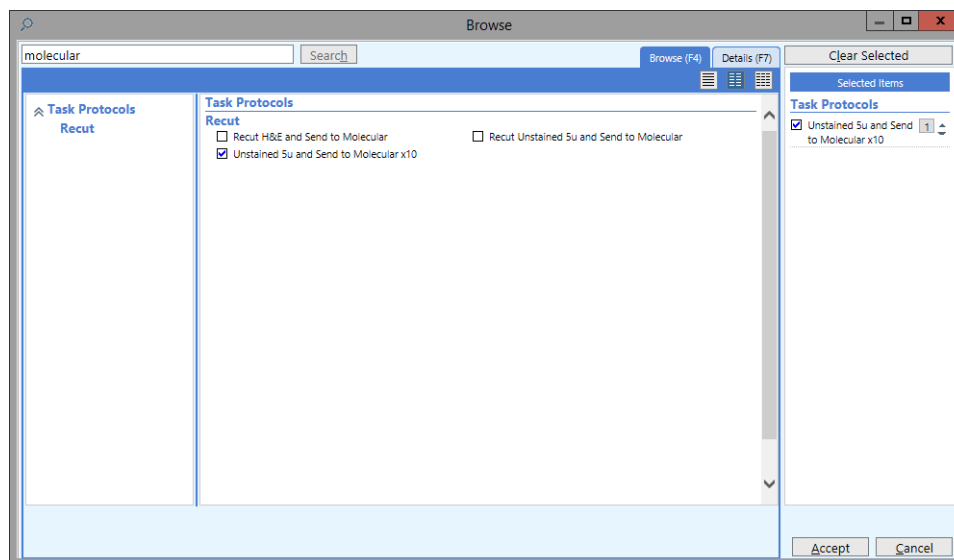


Figure 1. Example of order for total of 10 unstained slides.

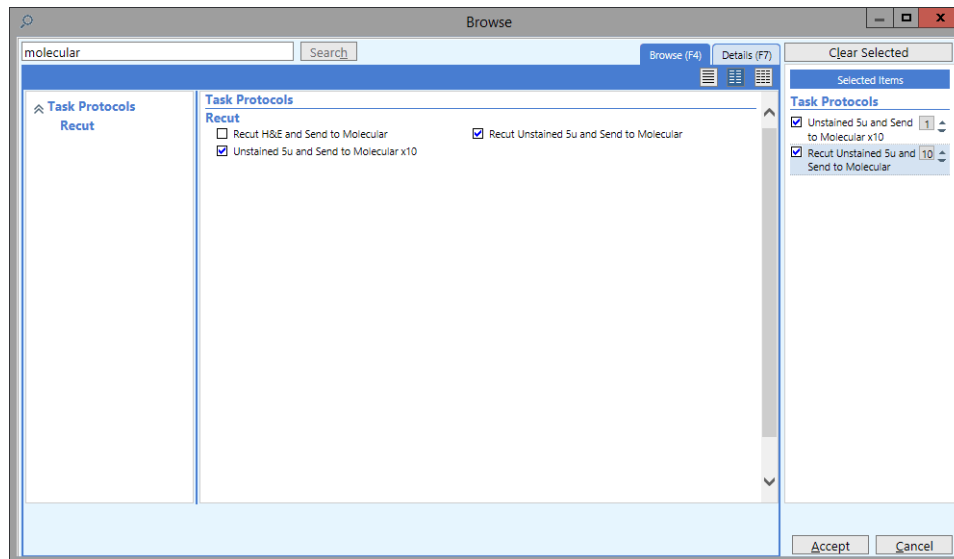


Figure 2. Example of order for total of 20 unstained slides.

Slide Assessment:

Macrodissection:

- 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. Macrodissection 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. Macrodissection should be considered for large specimens with necrosis if focal areas of viable tissue are present.

Slide Assessment: (cont'd)

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 variably 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.

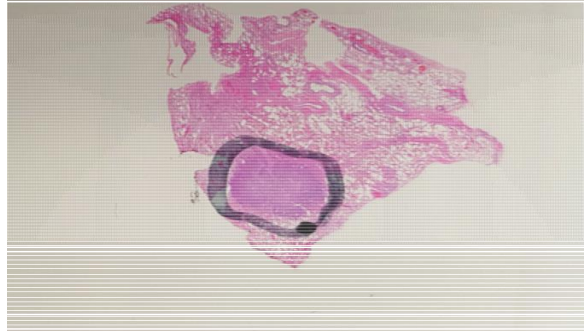


	<p>a. The circled area containing tumor is adequate for macrodissection and should be straightforward to identify and scrape from the unstained slides.</p>
	<p>b. The two circled areas containing tumor are adequate for macrodissection, but more challenging to identify and scrape due to the irregular and focally narrow shapes.</p>
	<p>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.</p> <p><u>Recommendations:</u></p> <ol style="list-style-type: none"> 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.

Figure 3. Macrodissection Examples

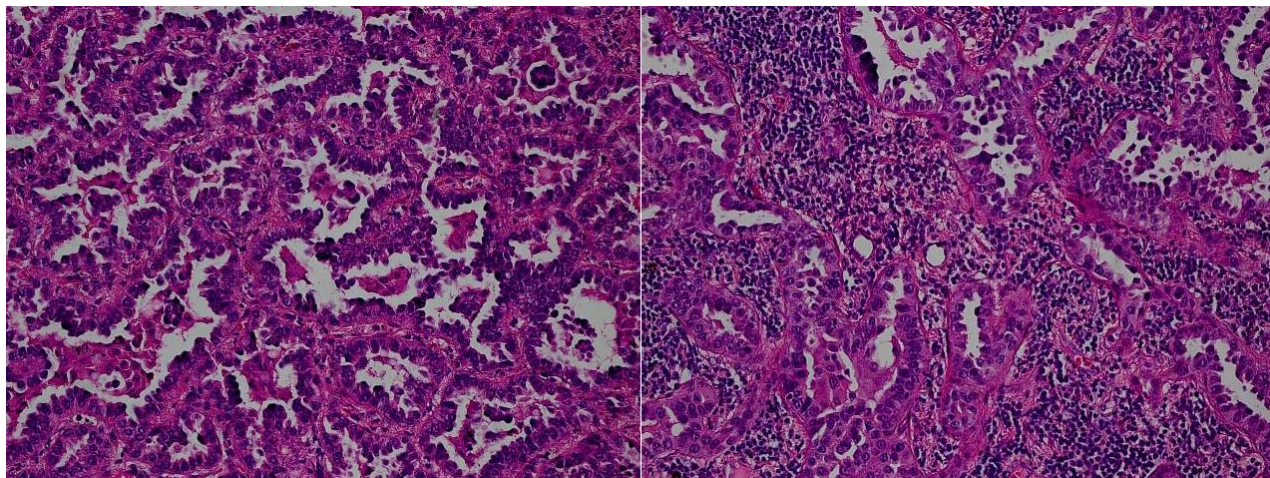


Figure 4: The left field shows high % tumor. The right field shows a smaller but somewhat similar number of tumor cells, but significantly lower % tumor due to the presence of many lymphocytes. Although small, each lymphocyte is assumed to contribute the same amount of DNA as a larger cell.

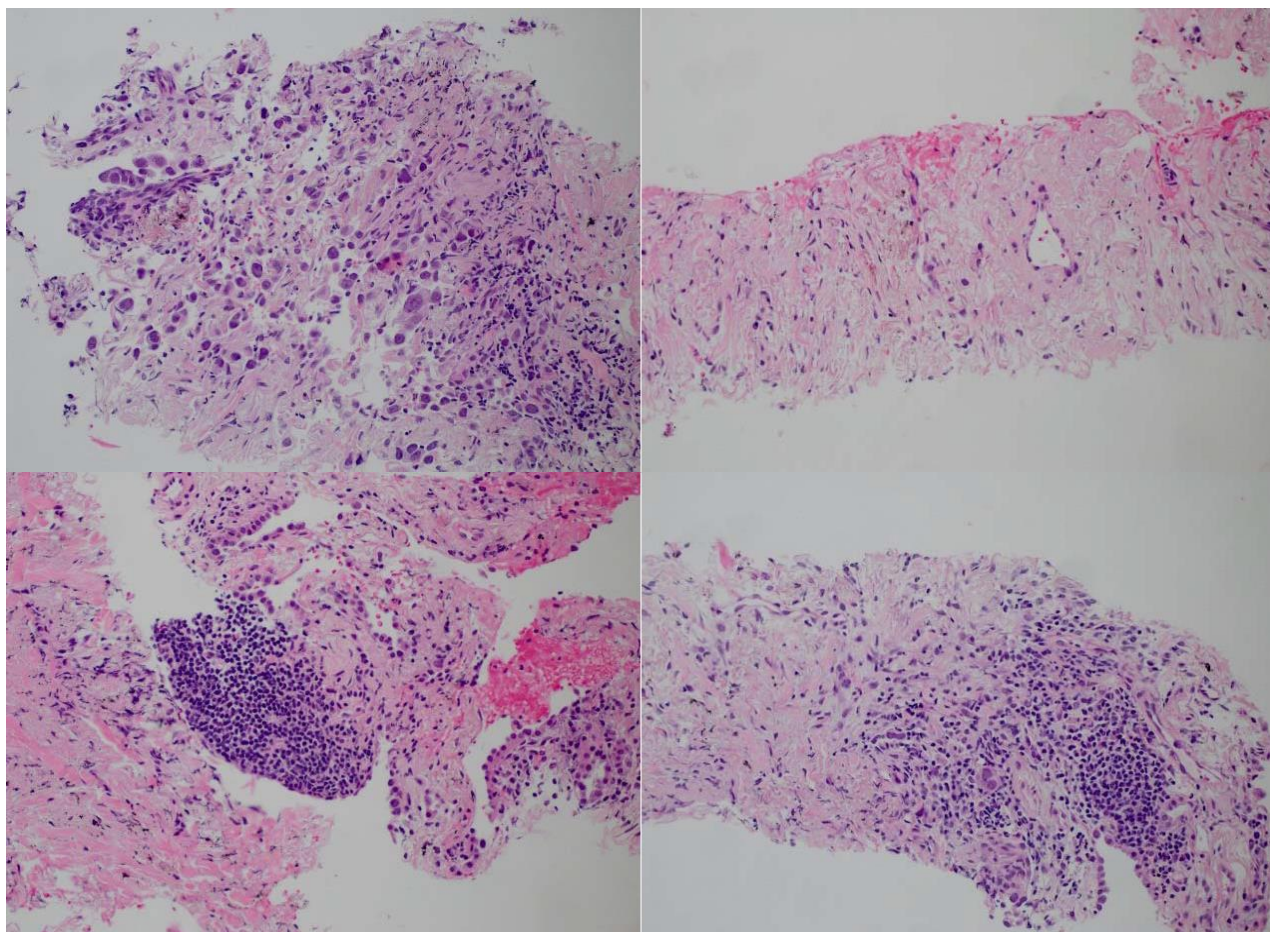


Figure 5: Averaging over multiple representative fields resulted in an overall estimated % tumor of 5- 10%. This lung biopsy specimen was submitted without macrodissection.

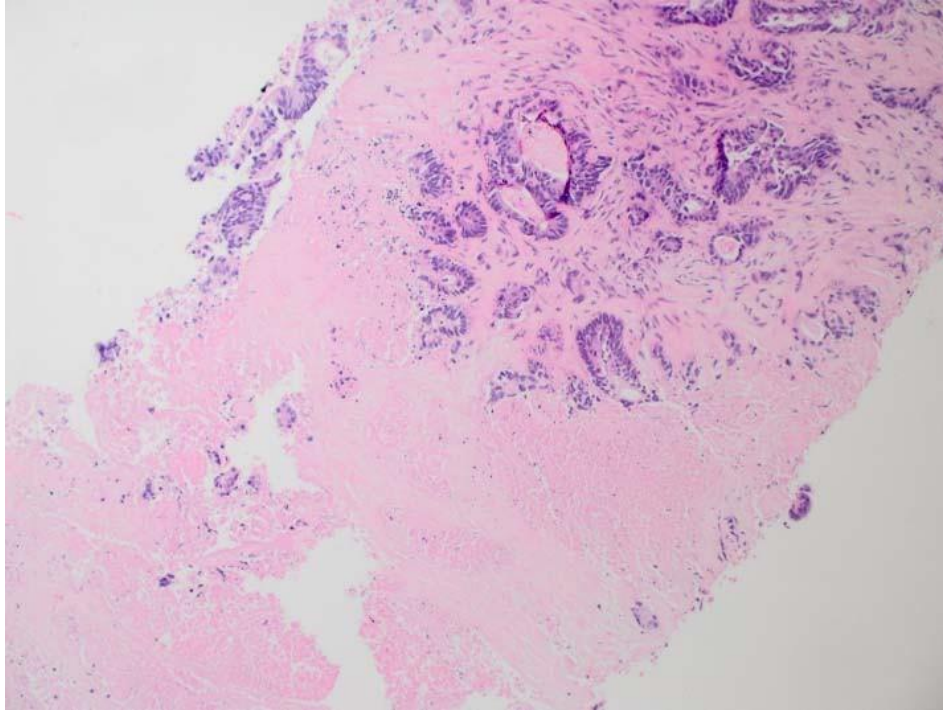


Figure 6: DNA integrity may be reduced with necrosis. In cases with necrosis, consider ordering more slides for small biopsies and requesting macrodissection for large specimens.

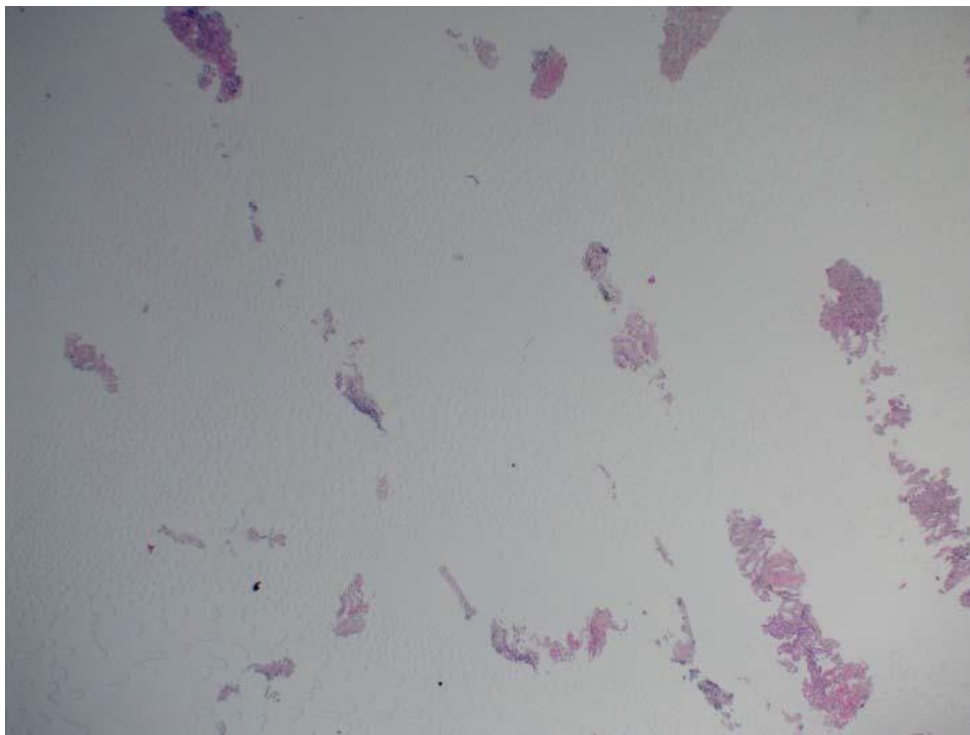


Figure 7: For biopsies showing scanty tissue, order additional slides even if 2000 or more cells are present on the H&E, as the tissue may disappear on deeper slides.

MDL Solid Tumor Slide Assessment Form

Case Number: _____

Patient MRN: _____

	Please fill in all fields	Notes
Macrodissection (Answer Yes/No)		<p>If answer is "No," the entire surfaces of the unstained slides will be scraped off and submitted for processing. Do not send H&E slide to MDL when the answer is "No".</p> <p>If answer is "Yes," only areas on the unstained slides corresponding to circled areas on the H&E slide will be scraped off and submitted for processing. Send the H&E slide with circled areas to MDL when the answer is "Yes".</p>
Estimated % Tumor (Low End)		<p>Low end of tumor percentage estimate (e.g. for an approximate tumor percentage of 10-20%, this would be 10%).</p> <p>If no macrodissection is requested, tumor percentage = # tumor cells / (# normal cells + # tumor cells) across entire H&E slide.</p> <p>If macrodissection is requested, tumor percentage = # tumor cells / (# normal cells + # tumor cells) in circled areas of H&E slide.</p> <p>Exclude necrotic cells when calculating estimate.</p>
Estimated % Tumor (High End)		<p>High end of tumor percentage estimate (e.g. for an approximate tumor percentage of 10-20%, this would be 20%).</p>
2000 or More Cells Per Slide, or in Circled Areas if Macrodissection (Answer Yes/No)		<p>If no macrodissection is requested, are there 2000 or more cells (including both normal and tumor cells) present on the H&E slide?</p> <p>If macrodissection is requested, are there 2000 or more cells (including both normal and tumor cells) present in the circled areas of the H&E slide?</p> <p>Exclude necrotic cells from count.</p>
Total Unstained Slides Ordered		<p>Enter total number of unstained slides that have been ordered and are being sent to MDL for processing.</p> <p>If answer is "Yes" to 2000 or More Cells question, order standard 10 unstained 5 micron thick slides for molecular.</p> <p>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.</p> <ul style="list-style-type: none"> 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.