Pathology quality control

Tissue passed pathology QC

The primary task of the Pathology Quality Control (QC) stage is to determine the presence of sufficient tumor DNA and RNA in the sample and assess the degree of tissue damage. This helps exclude samples that cannot be successfully sequenced from proceeding to the expensive Next-Generation Sequencing (NGS) stage and saves time.

Samples that pass the Pathology QC stage have the following:

✔ large numbers of tumor cells
✔ satisfactory tissue quality
✔ high tumor purity

Blocks

![FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE)]

- 1 FFPE block (resections, core-needle biopsies, cell-blocks)
- Each FFPE block should be prepared according to industry standards (e.g., fixed in 10% neutral-buffered formalin for 6–72 hours).
- Each FFPE sample should be <5 years old, preferably from the most recent procedure.

Unstained slides

![NEXT-GENERATION SEQUENCING (NGS)]

- 10 unstained slides
- 1 H&E (hematoxylin and eosin) slide
- Slides should be <6 years old, preferably from the most recent procedure.
- Slides should have a thickness of 4 μm on positively charged glass.
- Make sequential sections; label all slides with the slice number (№1 from the top, etc.) and ID of the sectioned block.

![IMMUNOHISTOCHEMISTRY (IHC)]

- 1 additional unstained slide for PD-L1
- 4 additional unstained slides for dMMR

Tumor content ≥20%

*When the tumor content is <20%, in certain cases we can perform macrodissection in BostonGene’s laboratory to try and achieve a higher tumor content.

REQUAED NUMBER OF CELLS

For NGS: The number of cells required is 40,000 tumor cells per specimen (in total, from all unstained slides).

For IHC: The number of cells required is 100 viable tumor cells.

Sample size (NGS)

![OPTIMAL 25 mm²](image)

![MINIMAL 5 mm²](image)

For small (<25 mm²) or impure samples, additional unstained slides may be needed to extract sufficient DNA for testing.
Special cases

- Cell blocks (and slides sectioned from them) and liquid-based cytology slides (preferably ThinPrep and SurePath methods) are accepted for NGS testing. Cell blocks are not accepted for IHC testing.
- Bone samples that are decalcified with EDTA are accepted.
- Samples from patients who have received hematopoietic stem cell transplantation (HSCT / BMT / UCBT) are not accepted.

BostonGene dissection may increase tumor content

If tumor cells are dispersed among normal tissue, their concentration is too low for extraction. A dissection procedure may exclude normal cells and increase tumor content to a satisfactory level.

The figures above demonstrate how dissection can increase the tumor content of a surgical sample that initially had a large amount of muscle tissue and squamous epithelium. The yellow border represents the region of interest for dissection. Low magnification (left), higher magnification (right).

Examples of cases that passed pathology QC

- Figure shows a core biopsy that consisted predominantly of tumor cells (hepatocellular carcinoma).
- Figure shows that fragments from the surgical resection of a gastrointestinal stromal tumor have a high tumor purity.
Sample rejection

**No tissue in the block**

FFPE block preparations from small biopsies may result in limited (less than 5 mm$^2$) or no tissue.

**No tumor cells in the tissue**

The tissue sample does not contain tumor cells.

**Low tumor purity**

If tumor cells are widely dispersed among normal cells, their concentration is very low. If dissection is unable to increase tumor purity, the sample is rejected.

✔ **Dissection is used to increase tumor purity**

If tumor cells are dispersed among normal tissue, their concentration is too low for extraction. For such cases, our experienced laboratory technicians perform macrodissection based on the annotations made by our pathologist analyst. A dissection procedure may exclude normal cells and increase tumor purity to a satisfactory level. This method enables performing the Tumor Portrait™ test for 92.9% of samples that would have otherwise been rejected (see figure).
Insufficient quantity of tumor cells

If the tumor tissue area in the biopsy is smaller than 5 mm², or if the number of tumor cells is insufficient, the extraction is inadequate.

- Tumor cells (low tumor purity, small tumor area, 0.5 mm²)
- Tumor border

Cytological samples with a low number of tumor cells

Cell blocks often contain minimal amounts of tumor cells, leading to the rejection of the sample.

- Tumor cells (poor quality cytology specimen)
- Tumor border

![Figure shows a cell block sample of poor quality that is not suitable for extraction.](image)
Hemorrhages and necrosis

If the sample contains extensive necrosis and/or hemorrhages, tumor cells are dispersed among such areas (see figures below). In that case, the desired purity is unreachable with dissection and the sample is rejected.

Inappropriate laboratory procedures (decalcification)

Decalcification with strong acids, such as hydrochloric acid, may damage tumor tissue, impacting nucleic acid quality and quantity.

Figures show damaged tissue caused by HCl decalcification.
**Shipping**

01
Place the FFPE block or slides in the provided plastic container.

02
Label containers with at least 2 patient identifiers (name, DOB, MRN) and the date of collection using the provided stickers.

03
Place the containers back in the provided cardboard box then place into the biohazard bag with absorbent paper.

04
Check that all required fields in the provided forms have been completed.

05
Insert supporting documentation* in the side pocket of the provided plastic bag. Place it in the box.

06
Put the kit box in the provided FedEx bag with the prepaid shipping label.

07
Ship the specimens via FedEx to the BostonGene Lab at 100 Beaver St, Waltham, MA, 02453.

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*PLEASE PROVIDE THE FOLLOWING DOCUMENTS:

- Pathology report
- Gross description of the FFPE block specifying anatomical features
- Microscopic descriptions for surgical and biopsy specimen(s)
- Descriptions and results of additional tests (IHC, flow cytometry, etc.)