

SOLID TUMOUR PANEL REQUISITION

Core Molecular Diagnostic Laboratory (CMDL) (CLIA #99D1042152)
OPTILAB-MUHC Genetics
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Referring Physician:*

Name (Last, First):*

License #:* Institution:*

Address:*

e-mail address:*

Tel:* Fax:*

(Fax # to send results)

Genetic counsellor/Nurse:

Tel: Fax:

I acknowledge that the patient/guardian is aware of the benefits, limitations and risks associated with the requested test(s) and that I have obtained informed consent to perform genetic testing for this patient. I authorize the laboratory to fax results to the number provided above.

Signature:* Date:*/*/

Sample Information:*

FFPE specimen:

10 x 5µm scrolls in 1.5 mL tube (minimum of 5 scrolls)

6 x 5µm unstained slides

Please provide one H&E stained slide.

Please circle the tumour region if the tumour cell count (TCC) is low.

Frozen specimen:

Collection Date - Time:*/*/ at h min

Frozen tissue - Specify :*

5 mm³ frozen fragment (minimum 2 mm³) in a labelled cryogenic tube

10 x 5µm scrolls in a labelled cryogenic tube (only if TCC ≥ 20%)

Please ship the same day on dry ice.

CMDL - Laboratory use only:

Date - Time received:

/ /

h min

SAMPLE LABEL(S) HERE

Sample type and # of tubes:

Patient #:

Name (Last, First):*

Birth date (YYYY-MM-DD):* / /

Father's name:

Mother's name:

Medical Record # (MRN):*

RAMQ # :*

For babies, please provide mother's RAMQ #

Sex: Male Female Unknown

Test Requested:*

Solid tumour panel (*AmpliSeq Focus Panel*)

Targeted analysis - Specify: *

Tumour cell count & type:*

Tumour cell count (TCC ≥ 10%):* %

Cholangiocarcinoma

Colorectal cancer

Gastrointestinal stromal tumour (GIST)

Lung adenocarcinoma

Melanoma

Thyroid cancer

Urothelial carcinoma

Ordering Checklist & Shipping:*

Ordering checklist:

Specimens labelled with at least two identifiers*

Completed test requisition (*this form*)*

1 H&E stained slide labelled with at least two identifiers*

Copy of the pathology/cytology report*

**Required information. Samples will not be processed if information is missing.*

Shipping instructions:

- Slides must be shipped in plastic slide holders or foam transport boxes.

- FFPE specimens can be shipped at room temperature.

- Frozen tissue must be shipped the same day on dry ice.

- Please ship specimens and associated documents to the address mentioned at the top of this page.

See attached information for detailed specimen preparation and test description.

AmpliSeq Focus Panel – Preparation and Shipping Guidelines

The quality of the *AmpliSeq Focus Panel* result depends on the following **critical pre-analytical steps** in order to obtain high-quality nucleic acids. This includes fixation, microtome section preparation, specimen preparation and shipping.

1. Fixation:

Fixation of the specimen with only **10% neutral-buffered formalin**, for a period of 24–72 hours, allows preservation of nucleic acids. Other fixatives may lead to false negative results and are not accepted (e.g. Bouin, B5, AZF, Holland's or strong acid decalcification procedures). Under fixation or over fixation can lead to suboptimal results.

2. Microtome Section Preparation:

Ribonucleases (RNases), which is present ubiquitously (eg. normal human skin), can destroy nucleic acids. Careful preparation and clean material are necessary to avoid cross contamination and RNases. Disposable material and smooth tip tweezers cleaned with an ANTI-RNase product (eg. RNase Away™) are encouraged.

- 1) Wear gloves and change them frequently, especially between specimens
- 2) Change the microtome blade for each case
- 3) Use clean instruments to transfer the sections (i.e. sterile needle to transfer the scrolls into clean 1.5 ml Eppendorf tubes)
- 4) Use hot plate or a clean water bath (for unstained slides)
- 5) All material (tube, slides) must be adequately labelled (two patient identifiers)

3. Specimen Preparation:

<i>Specimen type</i>	<i>Requirements</i>
Scrolls FFPE*	≥ 10% of tumor cell content (TCC) in the whole cut surface is required. TCC is the number of viable nucleated tumor cells in proportion to the total number of cells. 1) 10 scrolls at 5-µm (minimum 5 scrolls) in a labelled 1.5 ml Eppendorf tube 2) 1 H&E slide
Unstained Slides FFPE	Required for macrodissection when TCC <10% 1) 6 unstained labelled slides at 5-µm thickness, uncharged and unbaked 2) 1 H&E slide , pointed for tumor area if tumor content < 10%
Frozen tissue	- 5 mm ³ frozen fragment (minimum 2 mm ³) in a labelled cryogenic tube. - Or if TCC ≥ 20%, 10 scrolls at 5-µm thickness in a labelled tube. - Collection date and time should be stated. Ship on dry ice.

*FFPE: Formalin-fixed paraffin-embedded. When the tumor cell content (TCC) ≥ 10%, scrolls are preferred.

AmpliSeq Focus Panel – Test Information

Indications for ordering

This test allows the detection of genomic abnormalities with a diagnosis, prognosis or predictive role in diverse solid tumours (e.g. lung, lower GI, melanoma).

Test Methodology

The *AmpliSeq Focus Panel* is a targeted next generation sequencing assay for analysis of hotspot regions in 52 genes with known relevance to solid tumours. The panel enables the simultaneous analysis of genes associated with several most common cancers (e.g. lung, colon). The DNA and RNA analysis can detect multiple alterations including: (i) single nucleotide variants (SNVs), (ii) small insertion / deletions (indels), (iii) copy number gains and (iv) gene fusions in RNA samples. See below for gene list. For more information on the hotspot regions tested, please contact the laboratory.

Gene List

DNA (hotspot variations and *amplifications)				
AKT1	EGFR*	FGFR4*	JAK3	MYCN*
ALK*	ERBB2*	GNA11	KIT*	NRAS
AR*	ERBB3	GNAQ	KRAS*	PDGFRA*
BRAF*	ERBB4	HRAS	MAP2K1	PIK3CA*
CCND1*	ESR1	IDH1	MAP2K2	RAF1
CDK4*	FGFR1*	IDH2	MET*	RET
CDK6*	FGFR2*	JAK1	MTOR	ROS1
CTNNB1	FGFR3*	JAK2	MYC*	SMO
DDR2				

RNA (fusions)				
ABL1	EGFR	ETV5	NTRK1	PPARG
ALK	ERBB2	FGFR1	NTRK2	RAF1
AKT3	ERG	FGFR2	NTRK3	RET
AXL	ETV1	FGFR3	PDGFRA	ROS1
BRAF	ETV4	MET		

Test Interpretation

Only variants with a recognized clinical significance, diagnostic, prognostic or predictive are reported (Tier I and II; PMID: 27993330). Additional results are available upon request.

Note: This test does not detect large deletions.

Type of specimens accepted

- Tumour cell content $\geq 10\%$ is required. This information is mandatory to assess the validity of the test.
- Specimen (cytology or histology), frozen or formalin-fixed paraffin-embedded (FFPE) are accepted.
- For FFPE: only SCROLLS or UNSTAINED SLIDES are accepted.
- Unstained slides: a minimum of 5 sections of 5 μm thickness non-baked and uncharged, with 1 H&E slide (tumour area should be outlined with permanent marker if macrodissection is needed).

The full procedure is detailed in the document Preparation and Shipping Guidelines.

Limitations

Results must be interpreted in the **context of clinical, radiological and histological findings**. If results obtained do not match other clinical or laboratory findings, or if you have novel relevant information, please contact the laboratory as soon as possible for updated interpretation.

Results may be compromised if the recommended procedures (tissue fixation and preparation) have not been followed.

A negative (wild type) result does not fully rule out the presence of an alteration but may be linked with limits of detection of this assay (i.e. insufficient % of tumour cell content or poor fixation).

Rare polymorphisms may be present that could lead to false-negative or false-positive results.

Turnaround time: 10 working days