Department of Clinical Laboratory Genetics
Genome Diagnostics & Cancer Cytogenetics
Somatic Solid Tumour Testing

Toronto General Hospital
Eaton Wing 11-444, 200 Elizabeth Street
Toronto, Ontario M5G 2C4
Phone: (416) 340-4800 x5739
Fax: (416) 340-3596 Cancer Cytogenetics
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Email: Genome.diagnostics@uhn.ca
Email: cancercytogenetics@uhn.ca

Hours of Operation (Mon-Fri) 8:30AM-4:30PM
CAP: 7175217 CLIA: 99D1106115 IQMH: 4204-site 0141

Patient Information or Hospital Stamp Here
Last Name:

First Name:

Date of Birth (DD/MMM/YYYY):

Sex assigned at birth:

Health Card #:

Hospital #:

Instructions:

THIS REQ IS FOR SOMATIC SOLID TUMOUR TESTING ONLY – see link at bottom of page for HEREDITARY or MALIGNANT HEMATOLOGY requisitions

1. Complete all information as requested
2. Send req with specimen to address above
3. If shipping, send same day or next day delivery
4. Label specimen with **Name, DOB, MRN#, Date Taken**

Information For Reporting:
Full Name of Referring Physician:
CPSO#
Hospital/Address:

Phone:

Fax:

Physician Signature:_____

Copy Report To:_____

Specimen Requirements – Genome Diagnostics:

- ❑ **Peripheral blood -** For circulating cell-free tumour DNA (ctDNA) – 27 ml in Cell-Free DNA BCT **STRECK** tubes (tan/mottled tan cap)
- ❑ **Peripheral blood** - DPYD and qEBV – 5 ml in EDTA
- ❑ **Fresh/Fixed Cytology fluid:** as much as possible

Solid Tumour: Keep shipped PE material below 30°C

- ❑ **Tissue block** (**PREFERRED**)
  -Recut H&E slide (slide will not be returned) and copy of the pathology report is required
  -note that a 3mm punch biopsy will be taken from block
- ❑ **Unstained slides** (**only if BLOCK is not available**)
  - Cut **8 unstained sections** @ 7µm thickness on uncoated slides
  - **Air dry (not in oven)** unstained sections at room temperature.
  - For all tissues we require **2 H&E** stained sections, one cut before cutting slides from block and one cut after.
- ❑ **Cell Block or Paraffin Embedded Tissue (curls)** 5x10µm sections in a sterile Eppendorf tube

For Cytology specimens: Please provide ALL fixatives used:
_____

- ❑ **Fresh tissue**: 5mm^3 frozen or in 10 ml sterile medium at room temperature

Please ensure that you are using an updated copy of this requisition available at:
https://www.uhn.ca/UHNReferrals/Cytogenetics-Somatic-Testing.pdf

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Genome Diagnostics – Lymphoma/Leukemia:

- ❑ ^B-cell Clonality
- ❑ ^T-cell Clonality
- ❑ ^MYD88
- ❑ ^Mantle Cell - BTK,PLCG2,TP53
- ❑ CLL/SLL - BTK,PLCG2,TP53

Genome Diagnostics - Other:

- ❑ **DPYD** - Pharmacogenomic Testing for Gene Variants prior to Fluoropyrimidine Treatment (DPYD*2A, DPYD*9B, DPYD*13, HapB3)
- ❑ **^Identity Testing/Specimen Matching** (15 STRs and Amelogenin XY loci):
  -Please provide control specimen, specimen in question and details

Genome Diagnostics - Solid Tumour

Adrenal Cortical Carcinoma

- ❑ MLH1 Promotor Methylation

Breast Cancer – Advanced/Metastatic

(where PIK3CA directed therapy is being considered)

- ❑ Comprehensive Sequencing (NGS) – AKT1, PIK3CA, PTEN, ESR1

NTRK only - Breast Cancer (Secretory) - Advanced/Metastatic

(where NTRK directed therapy is being considered)

- ❑ Comprehensive Sequencing (NGS) - NTRK1, NTRK2, NTRK3 (Fusions)

Cholangiocarcinoma (Hepatobilary) - Advanced/Metastatic

- ❑ Comprehensive Sequencing (NGS) - FGFR2 (fusions only)
- ❑ Canadian Cholangiocarcinoma Collaborative (C3) – Please follow https://www.cholangio.ca/professionals/getmoleculartesting for study specific requisition, patient attestation form as well as testing details and directions for submitting sample for testing.

CNS Tumours (Primary) – Advanced/Metastatic

(where NTRK directed therapy is being considered)

- ❑ Comprehensive Sequencing (NGS) - NTRK1, NTRK2, NTRK3 (Fusions)

Colorectal/Small Bowel Carcinoma

- ❑ Comprehensive Sequencing (NGS) - BRAF, ERBB2, KRAS, NRAS, PIK3CA, PTEN
- ❑ MLH1 Promoter Methylation
- ❑ Query Lynch Syndrome - BRAF single gene testing only –
  - ❑ If negative, reflex to MHL1 methylation
- ❑ ^MSI - Only performed for cases with equivocal IHC MMR results - requires normal reference (normal tissue or blood)

Somatic Lynch Syndrome

- ❑ Comprehensive Sequencing (NGS) - EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, POLD1, POLE

REQUIRED - Please include both mismatch repair immunohistochemistry (MMR IHC) and germline test results. Germline testing should include the complete list of genes assessed by Somatic Testing for Lynch Syndrome panel above to allow for appropriate correlation.

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Genome Diagnostics Tests – Solid Tumour

Endometrial Carcinoma (Invasive)

- ❑ Comprehensive Sequencing (NGS) - CTNNB1, CCNE1, FGFR2, KRAS, PIK3CA, POLD1, POLE, PTEN, TP53
- ❑ MLH1 Promotor Methylation
- ❑ ^MSI – Only performed for cases with equivocal IHC MMR results - requires normal reference (normal tissue or peripheral blood)

Esophageal/Gastroesophageal/Gastric Adenocarcinoma

- ❑ MLH1 Promotor Methylation

Gastrointestinal Stromal Tumour

- ❑ Comprehensive Sequencing (NGS) –BRAF, KIT, PDGFRA

Glioma

- ❑ MGMT Promotor Methylation
- ❑ Comprehensive Sequencing (NGS) - ALK, ATRX, BRAF, CDK4, CDK6, CDKN2A, CTNNB1, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, H3F3A, HIST1H3B, HRAS, IDH1, IDH2, KRAS, MDM4, MET, MYB, MYBL1, MYC, NF1, NRAS, NRG1, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, POLE, PTEN, RAF1, RB1, TERT Promoter, TP53

Lung Adenocarcinoma

- ❑ Comprehensive Sequencing (NGS) - ALK, ATM, BRAF, CTNNB1, EGFR, ERBB2, FGFR1, KRAS, MET, NRG1, NTRK1, NTRK2, NTRK3, PIK3CA, RB1, RET, ROS1, SMARCA4, STK11, TP53
- ❑ EGFR – p.T790M mutation only (solid tumour/cell block or cytology fluid)
- ❑ **^***EGFR – p.T790M mutation only (Circulating tumour DNA in blood) ****peripheral blood in STRECK tube required (see pg. 1 for specimen requirements)**
- ❑ **CATALYST** (Circulating tumour DNA testing)– funded by AZ until Dec 31, 2025 – ***peripheral blood in STRECK tube required (see pg. 1 for specimen requirements)**

Eligibility Criteria:

- ❑ Patients with advanced or metastatic NSCLC (confirmed by biopsy) where molecular biomarker testing on tissue failed or tissue was insufficient for testing (would otherwise require another biopsy/repeat NGS).
- ❑ Patients with suspected advanced or metastatic lung cancer who are unsuitable for or unable to get a tissue biopsy.
- ❑ Patients with a high-risk for deterioration who cannot wait for NGS tissue results.

Melanoma - Cutaneous

- ❑ Comprehensive Sequencing (NGS) - ALK, BAP1, BRAF, CCND1, CDK4, CDK6, CDKN2A, EIF1AX, ETV6, GNA11 , GNAQ, HRAS, KIT, MAML2, MAP3K3, MAP3K8, MET, MYB, NF1, NFIB, NRAS, NTRK1, NTRK2, NTRK3, NUTM1, RET, ROS1, SF3B1, TERT, TRIM11, YAP1

Melanoma - Uveal

- ❑ Comprehensive Sequencing (NGS) - ALK, BAP1, BRAF, CCND1, CDK4, CDK6, CDKN2A, EIF1AX, GNA11, GNAQ, HRAS, KIT, MBD4, MET, NF1, NRAS, RET, ROS1, SF3B1, TERT

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Mammary Analogue Secretory Carcinoma - Advanced/Metastatic (where NTRK directed therapy is being considered)

- ❑ Comprehensive Sequencing (NGS) - NTRK1, NTRK2, NTRK3 (Fusions)

Nasopharyngeal Carcinoma

- ❑ ^ Quantitative EBV detection from blood plasma

Ovarian Carcinoma

- ❑ **High Grade Epithelial Carcinoma (Ovarian/Fallopian/Peritoneal)** - Ccomprehensive Sequencing (NGS) – BRCA1, BRCA2
- ❑ **Epithelial and low malignant potential ovarian tumours** - MLH1 Promotor Methylation
- ❑ **Sex-Cord Stromal Tumour** - Comprehensive Sequencing (NGS) - APC, CTNNB1, DICER1, FOXL2, STK11, VHL
- ❑ **Small cell Carcinoma (SCCOHT)** - Comprehensive Sequencing (NGS) - SMARCA4

Pancreatic Carcinoma - Invasive

- ❑ MLH1 Promotor Methylation

Prostate Carcinoma – Advanced/Metastatic

- ❑ Comprehensive Sequencing (NGS) - ATM, BRCA1, BRCA2, MLH1, MSH2, MSH6, PALB2, PMS2

Salivary Carcinoma - Advanced/Metastatic (where NTRK directed therapy is being considered)

- ❑ Comprehensive Sequencing (NGS) - NTRK1, NTRK2, NTRK3 (Fusions)

Sebaceous Carcinoma/Neoplasms (Skin or Ocular)

- ❑ MLH1 Promotor Methylation

Sinonasal Carcinoma

- ❑ Comprehensive Sequencing (NGS) – AFF2, DEK, EGFR, EWSR1, FLI1, IDH2, NUTM1, PAX3, PAX7
- ❑ MLH1 Promotor Methylation

Soft Tissue Sarcoma - Advanced/Metastatic (where NTRK directed therapy is being considered)

- ❑ Comprehensive Sequencing (NGS) - NTRK1, NTRK2, NTRK3 (Fusions)

Thyroid Carcinoma

- ❑ **Metastatic Sporadic Medullary Thyroid Carcinoma**
- ❑ **Metastatic Follicular Cell-Derived Thyroid Carcinoma (including Anaplastic, Papillary, Follicular and poorly differentiated Thyroid Carcinoma)**
- ❑ **Anaplastic, Poorly differentiated, High Grade Differentiated (High Grade Papillary, High Grade Follicular, High Grade Oncocytic), or Iodide Refractory Differentiated Thyroid Carcinoma**

Comprehensive Sequencing (NGS) - ALK, BRAF, DICER1, HRAS, KRAS, NRAS, NTRK1, NTRK2, NTRK3, PAX8, PPARG, RET, TERT Promoter

Urothelial/Urinary Tract Carcinoma - Advanced/Metastatic

- ❑ Comprehensive Sequencing (NGS) – ERBB2, FGFR1, FGFR2, FGFR3, FGFR4

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A Pathology report must accompany or be sent (fax/email) for all bone marrow samples and solid tumour testing. Samples will be banked and testing delayed until this information is received. Decalcified specimens cannot be tested.

Clinical Diagnosis/Reason for Referral:

- ❑ **Diagnosis/Reason for Referral:** _____
- ❑ **Follow up testing (reason):**_____
- ❑ **Other:** _____

Cytogenetics Specimen Requirements – Fluorescence *in situ* Hybridization (FISH):

❑ **Paraffin-Embedded Tissue Block**
- H&E slide, with 12mm area for FISH circled (slide will not be returned)

❑ **Unstained slides (FFPE)**
- Cut **three (3) unstained sections** per probe at 4 µm thickness on positively charged slides

❑ **Cytology Specimens**
- Air-dried smear/touch prep (1-2 per test)
- Cytospin slide (1-2 per test)
- Please indicate fixative(s) used

General Considerations for sending FFPE Tissues
- For all tissues we require **an H&E** slide, cut one level above or below the slides sent for testing with tumour area of interest **circled** (12mm) where FISH is to be performed (slide will not be returned)*.
- Copy of pathology report is required
- **Air dry** unstained sections at room temperature
- **(DO NOT BAKE)**

Lymphoid Disorders:

Large B-Cell Lymphoma Panel
- ❑ ^Reflex Panel (BCL2 and BCL6 only when MYC Positive)

Burkitt Lymphoma
- ❑ ^MYC ONLY

Follicular lymphoma / Diffuse Large B-Cell Lymphoma
- ❑ ^IGH/BCL2 *t(14;18)(q32;q21)*
- ❑ ^BCL6

Anaplastic large cell lymphoma
- ❑ ^ALK

MALT lymphoma
- ❑ ^MALT1

Mantle cell lymphoma
- ❑ ^CCND1/IGH t(11;14)(q13;q32)

Solid Tumour:

HER2 Amplification (indicate tumour primary)
- ❑ Breast
- ❑ Gastric
- ❑ Endometrial

Brain Cancer: Gliomas
- ❑ 1p/19q
- ❑ CDKN2A FISH (IDH Mut)
- ❑ EGFR + PTEN FISH (IDH WT)

Sarcoma and Carcinoma FISH
- ❑ ^EWSR1 - EWS-Family Tumours
- ❑ ^FUS – Low Grade Fibromyxoid Sarcoma
- ❑ ^SS18 – Synovial Sarcoma
- ❑ ^MAML2 – Mucoepidermoid Carcinoma
- ❑ ^ETV6 – Secretory Carcinoma
- ❑ ^CDKN2A (p16) – Malignant Mesothelioma

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For all cytogenetics inquiries please contact cancercytogenetics@uhn.ca