

# Cytogenetics Laboratory POLICY

CURRENT AS OF: 7/09

---

**SUBJECT: Cytogenetics Laboratory**

The Cytogenetics Laboratory is open from 7:00 a.m. to 5:00 p.m. Monday through Friday. The Lab is open 8:00 a.m. to 4:30 p.m. Saturday but has limited staffing. The Lab is located in the University of Kentucky Hospital, room HL423. The Laboratory performs chromosome analysis and FISH testing on peripheral blood, fetal and cord blood, amniotic fluid, chorionic villi, skin and other non-neoplastic tissues, abortus products, bone marrow aspirates, lymph nodes and some solid tumors. A list of FISH tests available is provided at the end of this document.

Lab phone (859) 257-3736  
Lab FAX (859) 257-6838

Shipping address: Cytogenetics Laboratory  
HA619  
University of Kentucky Hospital  
800 Rose Street  
Lexington, KY 40536-0293

## **Required information**

All specimens must be labeled with the patient's name, birth date and medical record number and must be accompanied by a University of Kentucky Cytogenetics Lab requisition form.

The requisition form must contain the patient's name, medical record number, sex, date of birth, type of specimen, source of specimen (if applicable), date of specimen collection, and the attending physician's name. Pertinent clinical information should also be included on the form. This information is critical in determining how the specimen is initiated into culture and what analytic techniques will be performed.

Lack of necessary information will delay specimen set up and can impact turn around time. Any specimens not meeting these requirements cannot be accepted.

If urgent or STAT processing is needed on a specimen, call to discuss this with the Laboratory Supervisor. All urgent or STAT processing requires Laboratory approval. Urgent and STAT specimens require hand delivery to the laboratory.

## **Requisition forms**

Specific electronic cytogenetic requisition forms are available through SCM for UKMC inpatients and in some outpatient clinics.

Order chromosome analysis (and FISH testing) according to specimen type. The requisition forms are the following:

- Chromosome Analysis Amniotic Fluid
- Chromosome Analysis Blood
- Chromosome Analysis Blood (leukemia)
- Chromosome Analysis Bone Marrow
- Chromosome Analysis Chorionic Villi
- Chromosome Analysis Cord Blood

Chromosome Analysis Non-Neoplastic Tissue  
Chromosome Analysis Skin  
Chromosome Analysis Solid Tumor  
Chromosome Analysis Stem Cells

Additional special purpose requisition forms are:

Freeze Fibroblast Cells Viably  
Grow Cells for Additional Testing  
Mitomycin C Breakage Study  
Thaw and Grow Fibroblast Cells

Specific paper requisition forms are available for UKMC outpatients and for specimens from outside clients. They are the following:

Cytogenetics Requisition – Medical Genetics (11/05) is for peripheral blood, skin, other non-neoplastic tissue, and abortus specimens  
Cytogenetics Requisition – Prenatal (11/05) is for amniotic fluid, CVS, and cord blood specimens  
Cytogenetics Requisition – Oncology J530 (2/06) is for bone marrow, peripheral blood for leukemia/lymphoma evaluation, lymph node, and tumor specimens

### **Specimen collection, transport and shipping**

All specimens must be collected in such a way as to insure viability and sterility of the sample.

Transport specimens at room (ambient) temperature. Do not heat specimen or allow to freeze. Specimens may be refrigerated overnight if transport to the lab will be delayed.

Specimens from the University of Kentucky Hospital and clinics should be transported to the Laboratory as soon as possible after collection.

Specimens from outside clients should be shipped to arrive within 24-48 hours of collection.

### **Specimen Requirements for Chromosome Analysis**

Peripheral blood:

Children and adults: 3-5 mL in sodium heparin

Neonates: 1-2 mL in sodium heparin

Cord (fetal) blood: 1-2 mL in sodium heparin

Bone marrow aspirates:

1-3 mL in sodium heparin

The first or second draw of bone marrow from the patient is preferred for chromosome analysis as the first two draws are more concentrated with blasts than subsequent draws.

Peripheral blood for evaluation of leukemia/lymphoma

If a bone marrow aspirate cannot be obtained, 7-10 mL peripheral blood in sodium heparin can be substituted for bone marrow if the following criteria are met:

peripheral WBC is  $\geq 15,000$  and the blast count exceeds 10%

Lymph nodes

Minimum 5 mm x 5 mm x 5mm obtained under sterile conditions

Place the specimen in a sterile container with RPMI media. Sterile containers with appropriate media can be obtained from the Lab.

Tumors

Minimum 5 mm x 5 mm x 5 mm obtained under sterile conditions

A 10 mm x 10mm x10mm is more optimal if clinically feasible

Place the specimen in a sterile container with RPMI media. Sterile containers with appropriate media can be obtained from the Lab.

**Amniotic fluid:**

20-30 mL divided among three sterile 10 mL tubes. Also send the "first draw" of 1-3 mL in a separate tube. Make sure to send a completed AFP (alpha-fetoprotein) requisition form if AFP and/or ACHE testing is desired. If an aneuploidy FISH screen is ordered as an adjunct to chromosome testing, the minimum volume is 20 mL of non-bloody fluid. Specimens should be protected from light during transport.

**Chorionic villi:**

15-30 mg of tissue is optimal. 10 mg is the minimum acceptable sample. More than 10 mg is required if FISH, DNA or biochemical testing is ordered in addition to chromosome analysis. The Laboratory should be notified 24 hours in advance of all planned CVS procedures. This will insure a technologist will be available to immediately assess specimen adequacy upon delivery to the Lab. Specimens should be hand carried to the Lab. CVS procedures should be scheduled to insure delivery to the Lab before 3:00 PM Monday – Thursday. Specimens received after 3:00 PM Thursday, Friday-Sunday or on a day prior to a holiday will not have results from a direct preparation.

**Skin:**

A 4 mm x 4 mm punch biopsy is preferred. The punch biopsy should be deep enough to include dermis. If Betadine is used to sterilize the skin surface, remove all traces of Betadine on the skin with isopropyl alcohol prior to the biopsy. (Betadine inhibits cell growth.) Place the specimen in a sterile container with RPMI media. Sterile containers with appropriate media can be obtained from the Lab.

**Abortus and autopsy specimens:**

Less than 12 weeks gestation: Placenta (chorionic villi) and any identifiable fetal parts.

Greater than 12 weeks gestation:

1. 2-3 mL unclotted cord or cardiac blood in sodium heparin
2. 10 x 10 x 10 mm placenta\* Sample fetal side near the site of the cord insertion.
3. Lung\*
4. Kidney\*
5. Skin\* Obtain from a central location (eg. buttock or upper thigh) rather than from a distal location (eg. foot) to enhance cell viability and likelihood of results

\*Specimens should be 5 mm x 5 mm x 5 mm or as large as feasible given fetal/patient size. Place each specimen in a separate sterile container with RPMI media and label with source of specimen. Containers with media can be obtained from the Lab. In the absence of media, place specimen in a sterile container with a small amount of sterile saline. Never place samples in formalin or other fixative.

If fetal demise occurred more than 48-72 hours prior to retrieval of tissue and delivery to the Lab, fetal tissue is unlikely to grow. In the case of prior fetal demise, placenta is most likely to grow.

**Fluorescence *in situ* Hybridization (FISH)**

One to two FISH tests can often be performed on the same specimen as for chromosome analysis. Call the Lab to verify specimen requirements if more than 2 FISH tests are desired on a specimen. Some tests always require additional specimen and are noted below by an \*.

**Available FISH tests for Constitutional Disorders**

1p36 deletion  
7q11.2 duplication

15q11-13 duplication

22q11.2 duplication

\*Aneuploidy screen (CVS, amniotic fluid only)

Amniotic fluid – requires an additional 5 mL of non-bloody fluid. Minimum volume for chromosome analysis and FISH testing is 20 mL

CVS – requires an additional 10 mg aspirate. Minimum weight for chromosome analysis and FISH testing is 25-30 mg villi (15 mg cleaned villi)

Angelman syndrome

Aniridia-Wilm's tumor association

Autism (duplication 15q)

Cri du Chat syndrome

DiGeorge syndrome I /Velo-Cardio-Facial syndrome (22q11.2)

DiGeorge syndrome II (10p)

Kallmann syndrome

Miller-Dieker syndrome

Neurofibromatosis 1

Prader-Willi syndrome

Retinoblastoma

Sex chromosome complement

SHOX

Smith-Magenis syndrome

Sotos syndrome

Steroid sulfatase deficiency

SRY

\*Subtelomere panel - requires an additional 3-5 mL peripheral blood

Neonates: minimum volume 4 mL peripheral blood in sodium heparin for chromosome analysis and subtelomere FISH panel

Children and adults: minimum volume is 7-8 mL peripheral blood in sodium heparin for chromosome analysis and subtelomere FISH panel

Williams syndrome

Wolf-Hirschhorn syndrome

### **Available FISH Tests for Neoplastic Disorders**

#### Myeloid Disorders

<sup>1</sup> BCR/ABL1	t(9;22)	CML, AML, ALL
D8Z1, EGR1/D5S721, D7Z1/D7S486/D20S108	+8, del 5q, -5, del 7q, -7, del 20q	AML, MDS
D8Z1	+8	AML, MDS
EGR1/D5S721	del 5q, -5	AML, MDS
D7Z1/D7S486	del 7q, -7	AML, MDS
D20S108	del 20q	AML, MDS, MPD
CBFB	inv(16), t(16;16)	AML
MLL	t(11)(q23)	ALL, AML, MDS
ETO/AML1	t(8;21)	AML
PDGFRB	t(5;12) and variants	CMML with eosinophilia
PDGFRA/FIP1L1	del 4q12 (cryptic)	CEL
PML/RARA	t(15;17)	APL

#### Lymphoid Disorders

TEL/AML1	t(12;21) (cryptic)	ALL
D4Z1, D6Z1, D10Z1, D17Z1	hyperdiploidy	ALL
p16	del 9p	ALL
hyperdiploidy, BCR/ABL1, TEL/AML1, MLL	hyperdiploidy, t(9;22), t(12;21), t(11)(q23)	pediatric ALL
<sup>2</sup> D12Z3, ATM, D13S319, 13q34, p53	+12, del 11q, del 13q, del 17p	CLL/SLL
ALK	t(2;5) and variants	Anaplastic large cell lymphoma
MYC, IGH/MYC	t(8;14) and variants	Burkitt lymphoma, L3 ALL

BCL2, IGH/BCL2 API2/MALT1 BCL6 IGH/CCND1	t(14;18) and variants t(11;18) 3q27 rearrangements t(11;14)	Follicular lymphoma MALT lymphoma Diffuse large cell/follicular lymphoma Mantle cell lymphoma
---------------------------------------------------	----------------------------------------------------------------------	--------------------------------------------------------------------------------------------------------

Multiple myeloma

IGH/CCND1, IGH/FGFR3, IGH/MAF, D13S319, p53	t(11;14), t(4;14), t(14;16), del1 3q/-13, del 17p	Multiple myeloma
------------------------------------------------	------------------------------------------------------	------------------

Solid tumors

CHOP EWSR FKHR NMYC SYT RB1	12q16 rearrangements t(11;22) and variants t(2;13) and variants NMYC amplification t(X;18) and variants del(13q)	Myxoid/round cell liposarcoma Ewing's sarcoma/PNET Alveolar rhabdomyosarcoma Neuroblastoma Synovial sarcoma Retinoblastoma
--------------------------------------------	---------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------------------------------------------------

Miscellaneous

DXZ1/DYZ1	engraftment status	Post cross sex BMT
-----------	--------------------	--------------------

<sup>1</sup> BCR/ABL1 FISH for evaluation for CML can be performed on peripheral blood (5 mL in sodium heparin)

<sup>2</sup> The CLL FISH panel can be performed on peripheral blood (5 mL in sodium heparin)

---

Approved by: Neysa Kiser, Supervisor, Cytogenetics Laboratory  
 Authorized by: Anjana Pettigrew, MD, Director, Cytogenetics Laboratory