**UW Medicine - Pathology**

400-06-01-01

Cytogenetics Testing Workup Guidelines Procedure

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| Adopted Date: 09/1991  Review Date: 06/2006  Revision Date: 02/05/2013 |

PURPOSE

### To understand case priority and appropriate requirements of each sample type.

PROCEDURE

**Table 1. Target Turnaround Times**

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| TABLE 1. TARGET TURNAROUND TIMES | | |
| UNIVERSITY OF WASHINGTON MEDICAL CENTER  CYTOGENETICS LABORATORY SERVICES AND TURN AROUND TIMES | | |
|  | TURN AROUND TIME | |
| **Specimen Type** | **Final diagnosis (Goal)** | **Preliminary diagnosis** |
| Fetal Blood | 2-7 days | 24 - 48 hr |
| Peripheral Blood Cytogenetics Analysis | 4-14 days |  |
| Family Follow-Up on Peripheral Blood | 3-14 days | 72hr (if requested) |
| Breakage Study on Peripheral Blood | 10-28 days |  |
| Stat Bone Marrow Cytogenetics Analysis | 2-3 days | 24 hr |
| Amniotic Fluid Cytogenetics Analysis | 7-10 days |  |
| Chorionic Villi Cytogenetics Analysis | 7-10 days | 24 - 48 hr |
| Solid Tissue Cytogenetics Analysis | 9-21 days |  |
| Neoplastic Bone Marrow or Blood Cytogenetics Analysis | 7-10 days | 48 hr |
| Solid Tumor Cytogenetic Analysis | 8-28 day |  |
| FISH Studies, PB, ST, AF | 2-4 days |  |
| Interphase FISH for Prenatal Diagnosis | 1-2 days |  |
| Interphase FISH for Neoplasia Routine | 2-4 days |  |
| Interphase FISH for Neoplasia STAT | 1-2 days |  |
| Molecular (MC) studies including array CGH | 14-21 days |  |

### 1. Notes to General Guidance for Cytogenetics Work-Ups and Case Signout

1. When necessary cases are discussed with the requesting physician.
2. Analysis from at least 2 different cultures, whenever possible.
3. If abnormal clone is found in a prenatal case, counts are extended to 30 additional cells.
4. 50 cells are counted if mosaicism is indicated, or if clinically relevant aneuploidy is seen in first 20 cells.
5. A panel needs to include a minimum of 5 pairs of chromosomes that are not the same as those in the karyotypes. Panels can be requested by the Laboratory Director or used if a technologist is questioning an abnormality and would like the Director to review more closely.
6. If first 20 cells are normal and mosaicism is indicated or if clinically relevant abnormality is seen in the first 20 cells, extend analysis by looking for the abnormality in another 30 cells.

**Prioritization of Cytogenetics cases:**

**Cases are organized by due date and then within the due date cases are prioritized by case type and physician request. Cases are prioritized once at the beginning of the day and then several times throughout the day. Cases should be taken as they are in the queue. Technologists should always take the next case in line. If a physician calls to request results, that case may then take priority of others.**

1. **STAT** cases such as **APL** and newborn babies

2. Prenatal cases

3. Baby bloods or blood from a pregnant woman not requested by physician as STAT

4. Neoplasia

5. Routine Blood

6. Solid Tissue and Tumor

**Guidelines for GCS case sign-out:**

1. Log onto GCS and choose chromosome icon

2. Go to the results icon and enter case number

3. Click the microscopy icon

A. fill out the cell count chart

B. go to exception icon and enter exceptions if appropriate (skip if no exceptions). Once entered, close the door

C. enter the quality score (**morphology** based)

D. enter the staining (ie: G-bands)

E. enter the band level

F. choose the appropriate growth of the case

a. less than 20 cells = **poor** (AF’s less than 15 = poor)

b. 3 cells or less = **no growth**

c. All other cases should be **good** (do not choose adequate)

*Note: If necessary, make notes about the case in the specimen details icon under comments. This should be used to say things like “all material exhausted” or all cells very poor, most unanalyzable”. This section should include notes that are important for the directors to note in their report.*

G. Close door back to the results screen

4. In the results screen

A. Enter Karyocode

B. Enter Micro by and log out case

C. Close door

5. Select print routine, select your case and print the lab sheet

### Table 2. Band Levels

VANCOUVER GENERAL HOSPITAL

CYTOGENETICS LABORATORY

Table 3. Record Sheet

Patient # \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Technologist \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Chromosome # Tech Supervisor Cytogeneticist

1p31-32

10

11p

12q

X

Total

Band Level

Total = Bands Band Level

18 350

21 400

28 450

34 500

40 550

47 650

54 750

60 850

### Peripheral Blood (Always verify setup with Director, Supervisor, or Specialist if unsure)

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| --- | --- | --- | --- |
| Type of Analysis | Indication | Cultures | Work-Up/Action |
| STAT Newborn | For all types, including multiple congenital anomalies (MCA), Down syndrome (DS), Turner syndrome (TS), etc | A--48-hr  B--48-hr  C--72-hr | 20 Metaphase cells with at least 10 from the 72 hr culture. 24 hour cultures should not be used unless necessary. IFISH can be performed on a direct harvest if enough material present. |
| Routine and High Resolution  See also FISH Analysis for PB | For all types, including Down syndrome, mental retardation, autism, fragile X (separate DNA test done by Genetics lab), developmental delay (DD), Turner syndrome (TS), Klinefelter syndrome (KS), gonadal dysgenesis, short stature, habitual abortion (HAB) and HAB partner, infertility, | A--72-hr  B,C--72-hr Thy | Analyze 20 metaphase cells from 2 different cultures (A,B or A,C), including 3 karyotypes and 2 metaphases.  If indication is for infertility or sex chromosome abnormality, scan 30 extra cells from A culture.  If abnormal:  --Structural: Make panel for the abnormal chromosome. If, necessary proceed to NOR, C-banding, or Q-banding  --Mosaicism: Scan 30 extra cells from A culture  --FISH: Follow Director's instructions.  --Satellite: NOR banding |
| Breakage Study Only | Fanconi anemia | Set up E and G for both Patient and Control:  E--72-hr DEB culture  G--76-hr MMC culture | Use Breakage analysis sheet:  Patient: Analyze 50 cells each for E and G cultures  Control: Analyze 50 cells each for E and G cultures |
| Routine and Breakage Study | Fanconi Anemia | Routine as above  Plus G and E cultures for Patient and Control as above | See Routine plus Breakage Study Analysis |
| BrdU Staining | X-Inactivation | C,D (BrdU Cultures) | Screen 20 cells to identify abnormal X chromosome.  Capture 5-10 metaphase cells.  Confirm X chromosome by FISH or G-banding.  If G-banding necessary, analyze 20 metaphase with 2 karyotypes and 3 metaphases |
| Sister Chromatid Exchange | Blooms syndrome |  | Analyze 20 metaphase cells each from Patient and Control  Score exchanges in all metaphase cells in control. Calculate Average SCE.  For positive SCE, score the SCE in 5 metaphases with high number of SCE. Calculate Average SCE. |
| Family Follow UP | Parental blood for abnormal amnio, etc. | A--72 hr culture  B--72 hr Thy culture | Analyze 5 metaphase cells, including 1 karyotype  If necessary, scan 30-50 cells to rule out mosaicism, or scan for markers.  Make a panel for abnormal translocations, markers, etc. |
| Research | For all types | A--72 hr RT  B--72 hr Thy | Analyze 5 metaphase cells, including 1 karyotype |

### Neoplasia

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| --- | --- | --- | --- |
| Type of Analysis | Indications | Cultures | Work-Up/Action\* |
| Preliminary diagnosis | For all types |  | 5 cells analyzed (including 3 drawings or photo analysis). If the 5 cells are normal, extend to 10 cells. |
| Routine neoplasia analysis  (See also IFISH for Neoplasia Studies) | B-Cell Leukemia, pancytopenia, anemia, lymphoma, MDS, MPD, AML, ALL | Unstimulated culture  A--24-hr harvest  B--24-hr harvest with methotrexate  C--48-hr harvest | 1. Analyze 10 cells in one harvest harvest and 5 cells from each of the other two harvests. If abnormal cells are found in the first harvest, all 20 cells can be from that harvest.  2. If typical abnormality relevant to the disease (e.g., Ph' in CML) is found in the first 5 cells, partially analyze 15 more cells for this anomaly unless the indication is tumor progression. In that case, completely analyze 20 cells.  3. Include in the workup a minimum of 2 karyotypes from each different stemline or sideline. |
| Neoplasia cases requiring additional scoring |  |  | Score as above, plus 30 additional cells, when the following is found:  ---one cell with a structural abnormality  ---one cell with a trisomy  ---one cell with monosomy 5 or 7 in MDS and AML  ---one cell with monosomy 13 in multiple myeloma  ---two cells with monosomy 5, 7, 12, 13, 17, 18, 20, X, Y for all indications, except multiple myeloma  ---two cells with monosomy for the same chromosome (any chromosome) in multiple myeloma  ---do not disregard cells with hypodiploidy or hyperdiploidy, especially in multiple myeloma |
| T-Cell Leukemia |  | As routine, plus  D--72-hr PHA-stimulated harvest | As routine, plus analyze 20 cells from PHA-stimulated harvest, with additional analysis of 3 photos and 2 karyotypes |
| Chronic Lymphocytic Leukemia (CLL), Small lymphocytic Leukemia (SLL) |  | As routine, plus  H culture-72-hr IL-2/DSP-30 | Analyze 20 cells from the IL-2/DSP-30 stimulated harvests, if abnormal, stop here, including analysis of 3 photos and 2 karyotypes. Continue with routine cultures if stimulated are normal |
| Plasma cell dyscrasia; plasma cell disorder | Myeloma, Monoclonal Gammopathy (MGUS), Waldenstrom's | As routine, except  B--48-hr harvest with methotrexate  H--72-hr harvest with IL-2/DSP-30 | As routine |

### Solid Tissue and Tumors

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| Solid Tissue Analysis | | |
| Analysis | Indication | Work-Up/Action |
| Routine | Most Indications | Analysis (Standard)  --20 cells analyzed, including 2 karyotypes and 3 metaphase images to analyze by hand  --Additional scoring of 30 more cells is indicated when the following are found (to rule out mosaicism of 6% or more at 95% confidence level)  ---one cell with a structural abnormality  ---one cell with a trisomy  ---two cells with a monosomy for the same chromosome  If abnormal:  --same as for Peripheral Blood **[page: 4]** |
| Mosaicism |  | 50 cells analyzed (Extended Analysis)  --if no mosaicism: 2 karyotypes and 3 metaphase images  --if mosaic: 1 karyotype from each cell line, 1 - 2 metaphase images from each cell line |
| Molecular test Request |  | --grow appropriate number and sizes of flasks for send out  --keep T25 flask growing as backup until molecular test is complete |
| Save Cells for Future Studies |  | --grow two T-75 flasks  --freeze, log, and store in liquid N2 |
| Research |  | Abbreviated study --5 cells analyzed, 1 karyotype or 1 of each cell line observed  Complete Analysis--Standard 20 cells, 2 karyotypes, 3 drawings  Extended Analysis--50 cells |

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| Tumor Analysis | | |
| Analysis | Indication | Work-Up/Action |
| Routine | All | Same as for Solid Tissues (see above) |
| Mosaicism |  | N/A |

### Amniotic Fluid and Chorionic Villi

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| Common indications | Abbreviation | Work-Up/Action |
| 1. Advanced maternal age | AMA | Standard workup\* |
| 2. Prenatal risk profile  Also Triple screen, Quad screen | PRP | Standard workup\* |
| 3. Integrated screen |  | Standard workup\* |
| 4. Abnormal ultrasound | ABUS | Standard workup\* |
| 5. Increased nuchal thickening | ↑NT | Standard workup\* |
| 6. Heart defect  Velocardiofacial syndrome | --  VCF | Standard workup\*  +FISH with VCF/DiGeorge FISH probe  (20 metaphase cells + 100 interphase nuclei) |
| 7.↑ or ↓ alpha fetoprotein | AFP | Standard workup\* |
| 8. Diseases that have molecular tests available, such as cystic fibrosis, sickle cell anemia, muscular dystrophy, thalassemia |  | Standard workup\* + cultured cells in flasks of required number and size + one backup flask retained until molecular testing is completed |
| 9. If request form asks for AFP or acetylcholinesterase testing | AFP/ACHE | Forward to SPS (NW-220):  0.5 ml fluid or supernatant for AFP  1.0 ml fluid or supernatant for ACHE |

\*Standard Workup: Analyze 15 clones or 20 cells from the mass culture, including 2 drawings, 1 analyzed photo and 3 karyotypes. Band level for the karyotypes should be two with 550 or greater and one with 450-550 bands.

### FISH/IFISH Workup by Indication

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| FISH for PB, AF, ST, etc |  |  |
| **Microdeletion syndromes** | **FISH locus** | **Work-Up/Action** |
|  | Various | 10 metaphase cells/probe, to r/o duplication (if indicated) score 100 interphase cells each from patient and control if used |
| Subtelomere | 1-15 mix | 5 metaphase cells with 2 pictures each |
| Whole Chromosome Paint (WCP) | 1-22, X, Y | 10 metaphase cells |
| Centromeric Probe (CEP) | 1-22, X, Y | 10 metaphase cells with 2 pictures |

**FISH:**

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| Amniotic IFISH--Aneuvysion | Aneuploidy of 13, 18, 21, X, Y | 50 cells for each of 2 sets of probes (18,X,Y/13,21) |
| Urovysion | Bladder Cancer | 50 cells for Patient |
| Marker identification | Any | 10 metaphases with 2 pictures |

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| **Routine IFISH for Neoplasia Studies:**  *Note: capture 2 images if abnormal, 1 if normal.*  ~Score 200 cells total, 100 from two readers. Counts can be extended if clinically indicated.  ~A 3rd reader must be asked to read if there is a discrepancy between reader one and two  See FISH protocol section of lab manual for detailed information regarding NF IFISH. |

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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Cytogenetic Supervisor