APPENDIX CONTENTS

Specimen Collection & Processing

Page

Appendix A

Specimen Collection General Information

Specimen Collection Tube Guide

Red Top Tube (Plain)

1. Does not contain an additive of any kind
2. Used for serum determinations in Chemistry, Immunology and HLA

Red with Gray Speckled Tube (SST)

1. Contains a clot activator and gel for serum separation SST brand tube for serum determinations in chemistry
2. Invert tube at least 5 times to ensure adequate mixing

Royal Blue Top Tube ("Metal")

1. Contains one of the following (check tube label carefully !!)
   Sodium Heparin
   Sodium EDTA (Na2EDTA)
   None
2. For trace elements, toxicology and nutrient determinations
3. Invert tube at least 8 times to ensure adequate mixing
4. Only Sodium Heparin is acceptable for Cytogenetic Cultures

Green Top Tube (Heparin)

1. Contains one of the following (check tube label carefully !!)
   a. Sodium Heparin (note restrictions below)
   b. Lithium Heparin (note restrictions below)
   c. Ammonium Heparin
2. Only Sodium Heparin is acceptable for Immunology testing. Please check tube label carefully.
3. Only Sodium Heparin is acceptable for Cytogenetic cultures.
4. Lithium Heparin is suitable for most chemistry assays, but NOT lithium drug levels.
5. Invert tube at least 8-times to ensure adequate mixing.
6. Only Sodium Heparin is acceptable for Flow Cytometry testing.

Purple Top Tube (EDTA)

1. Contains one of the following (check tube label carefully !!)
   a. Liquid K_3 EDTA or
   b. Freeze-dried Na_2EDTA
   c. Silicone-coated liquid EDTA
2. Use the Silicone-coated liquid EDTA only for ACTH specimens
3. For whole blood hematology determinations. Use a 3 ml tube. Invert tube at least 8 times to ensure adequate mixing.
4. May be used for Blood Bank testing

Light Blue Top Tube (Citrate)

1. Contains the following (check tube label carefully !!)
   a. .105 M Sodium Citrate (3.2%)
2. For coagulation determinations on plasma specimens
3. Invert tube at least 8-times to ensure adequate mixing. NOTE: It is very important the tubes are allowed to fill to the specified volume.

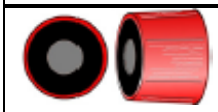
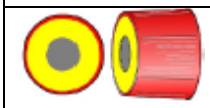
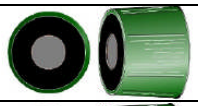
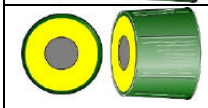
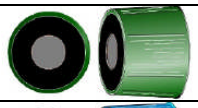
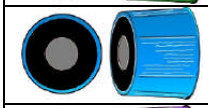
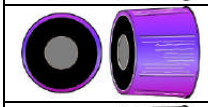
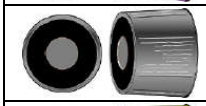
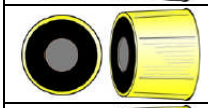
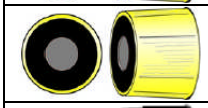
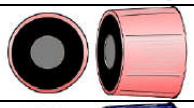
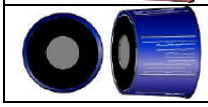
Yellow Top Tubes (ACD-A)

1. Contains Acid Citrate Dextrose solution A
2. For whole blood Immunology determinations.
3. The tube must be filled completely. Invert tube at least 8-times to ensure adequate mixing.
4. Use only when Sodium Heparin tubes are unavailable.
5. Use for HLA (Histocompatibility) testing

Pink Top Tubes (K3 EDTA)

1. Use for Blood Bank testing

TUBE TYPE AND COLOR:

	Anticoagulant	Inner Ring Color	Cap Color
	PLEASE NOTE: All tubes with white inner rings are Pedi draws only !		
	Serum Tube/clot activator	Black	Red
	Serum Tube/gel and clot activator	Yellow	Red
	Lithium Heparin	Black	Green
	Gel and Lithium Heparin	Yellow	Green
	Sodium Heparin	Black	Green
	Sodium Citrate	Black	Light Blue
	EDTA	Black	Lavender
	Sodium Fluoride	Black	Grey
	ACD-B	Black	Yellow
	ACD-A	Black	Yellow
	Sodium Citrate	Black	Black
	EDTA (Blood Bank)	Black	Pink
	Sodium Heparin	Black	Royal Blue

Tube Stopper Color & Type of Additive

Red Stopper	**No Additive (BD)**
Red Black Stopper	**Clot Activator (Greiner)**
Red Yellow Stopper	**Clot Activator/ (Greiner)** **Serum Separator Gel (SST)**
Gray Stopper	**Potassium Oxalate/** **Sodium Fluoride**
Green Stopper	**Sodium or Lithium Heparin**
Light Blue Stopper	**Sodium Citrate**
Lavender Stopper	**Liquid K_2EDTA**
Yellow Stopper	**Acid Citrate Dextrose (BD)**
Royal Blue Stopper	**May have EDTA** **Or No Additive**
Pink Stopper	**EDTA (Greiner)**

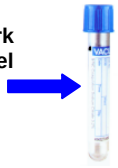
Stopper Colors & Most Common Laboratory Use

Stopper Color	Laboratory Use
 **Red Stopper**	**Chemistry & Serology**
 **Red/Black Stopper**	**Chemistry & Serology**
 **Red/Yellow Stopper**	**Chemistry & Serology**
 **Gray Stopper**	**Lactic Acid**
 **Green Stopper**	**Chemistry or Stat Chemistry**
 **Light Blue Stopper**	**PT, PTT, Coagulation**
 **Lavender Stopper**	**<u>Hematology</u> (CBC & Sed Rate)** **2- three ml tubes** **<u>Blood Bank</u> (3 ml. tube)** **Pediatric Patients** **∗ May also be substituted for pink stopper when drawing adult patients** **<u>Special Chemistry</u> - Hgb A_1C and Electrophoresis**
 **Yellow Stopper**	**DNA, Paternity Testing**
 **Royal Blue with EDTA**	**Lead and Zinc**
 **Royal Blue – No Additive**	**Copper**
 **Pink Stopper**	**Universal Color for Blood Bank**

LAB TUBE TYPES and ORDER OF DRAW

Blue Top: (Citrate) **– tubes must be a full draw**

PT/PTT **2ml and 3 ml**
Protime (PT) **Fill to black mark**
APTT (PTT) **(square) on side of label**
Fibrinogen (FIB)
D-Dimer (D-DI)
Thrombin Time (TT)
Fibrin Monomer (FM)



FSP or FDP

SST: (no anticoagulant) **– 8 cc**

Most Chemistries **Pregnancy**
Glucose **TSH**
BUN Lipid Profile
Creatinine TIBC/Iron
Comprehensive Metabolic Profile (CMP)
E-Group (Electrolytes)

Drug Levels :
Acetaminophen Ethanol Phenobarbital Theophylline
Carbamazepine Gentamicin Phenytoin Tobramycin Digoxin
Lithium Salicylate Vancomycin

Green Top:(Lithium Heparin)	**OR**	***(Sodium Heparin)***
(green top with yellow +gel)		(green top with black-no gel)
Ammonia (on ice)		
Cardiac Enzymes		Immune Survey
CKMB		LAP
Troponin I		Chromosome studies

Purple Top: (EDTA) **– 3 cc**

CBC Retic Count Platelets
Sed Rate **Sickle Cell Screen**
BNP

Pink Top: (EDTA) **– 6 cc**

Type and Cross **Type and Screen**
***1st tube filled in Trauma Alert/Red Blanket**
****Red top tube or SST tube unacceptable**

Gray Top: *(sodium fluoride/potassium oxalate)* – 2 cc
Lactic Acid (on ice)

➔ To prevent clotting, tubes must be gently inverted 8 to 10 times immediately after being drawn. After 4 to 5 inversions the SST tube should begin to clot.

NOTE: If a tube is NOT a full draw, add-ons MAY NOT be possible!

Revised: 22 May 2008

Order of Draw

NOTE: When a butterfly is used, the 1st tube will under fill. Therefore, a discard tube is filled 1st, then the rest of the tubes in the series.

Tube 1 - Blood Culture bottles/Isolator tubes
Tube 2 - Light blue top tube (coagulation testing)
Tube 3 - Serum tube with or without clot activator, with or without gel
Tube 4 - Green top tube (Heparin)
Tube 5 - Lavender top tube (EDTA)
Tube 6 - Gray top tube (Oxalate/Fluoride)
Tube 7 - Yellow tube (ACD)

Appendix B

Anatomic Pathology

Biopsy Specimens for Special Techniques

Please call and alert the Anatomic Pathology Laboratory (724-2435) or Electron Microscopy Lab (724-3697 or 724-4236), when sending in all biopsy specimens for the following techniques:

Renal (Kidney) Biopsy

Contact Anatomic Pathology for special instructions and media. The following guidelines apply:

Specimen

A kidney biopsy may be obtained by percutaneous needle biopsy or open surgery. It should then be placed immediately in a petri dish with distilled water, gauze moistened solution to prevent drying.

Open Surgery:

The tissue from open surgery should be divided into three parts. When dividing the specimen, each part must have glomeruli. These specimens should be submitted for:

1. Light Microscopy
2. Immunofluorescence, and
3. Electron Microscopy

Percutaneous Needle Biopsy:

Ideally, more than one percutaneous needle biopsy specimen can be obtained. Specimens should be labeled for:

1. Immunofluorescence
2. Light Microscopy, and
3. Electron Microscopy

If only one tissue core from a biopsy is obtained, it should be divided as illustrated below. When dividing the specimen, each part must have glomeruli. A hand lens or a dissecting scope can be useful in recognizing the difference between renal cortex and medulla. The glomeruli in the cortex appears as red dots, the cortex is darker than the medulla and is proximal-within the needle used for the biopsy.

Specimens should be labeled for:

1. Immunofluorescence
2. Light Microscopy, and
3. Electron Microscopy.

Specimen Processing

1. Immunofluorescence

Place tissue in Michaels solution available commercially from Zeus Laboratories, or the Anatomic pathology Lab. Call 724-4236 to obtain a vial of this fixative.

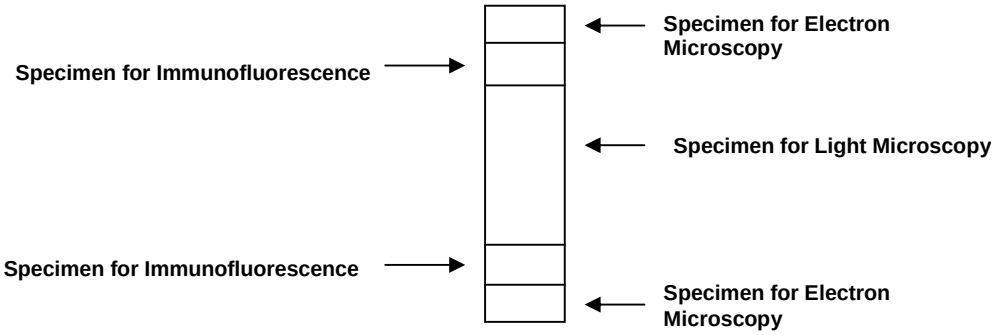
2. Light Microscopy

Kidney biopsies should be fixed in 10% neutral buffered formalin solution.

3. Electron Microscopy

Tissues should be glutaraldehyde fixed and should be more than 1 mm in diameter. Please ship at room temperature. Previously frozen tissue is acceptable. Tissue fixed in formalin or from paraffin blocks give POOR results. Please submit appropriate clinical history.

Preferred Division of Kidney Needle Biopsy



Skin Biopsy
for Immunofluorescence

Specimen Transport

The skin biopsy specimen should be placed in a vial of Michaels solution for transport. Michaels solution is available commercially from Zeus laboratories or the Scott and White Reference Laboratory. Call 724-4236 to obtain a vial of this fixative. Do not use 10% formalin as the fixative.

Properly label the vial with the appropriate patient information and transport to the laboratory.

Appendix C

BLOOD BANK

Specimen Collection and Labeling

Collection

Collect a 6ml EDTA (Lavender/purple/pink top tube)

Labeling

Labeling of all Blood Bank Specimens is highly regulated by federal agencies and specimens for Blood Bank testing without proper labeling will be rejected and a redraw of the patient requested.

All Blood Bank Tubes **MUST** be labeled with the following:

1. The patients full name, (First and last name)
2. A unique identifier (MRN that does not change or a Social Security number- the R# from a client request form does not meet this criteria as it is not used every time a patient has work done in the Blood Bank)
3. Date of Birth
4. Date of Collection
5. Time of Collection
6. Initials of the person collecting the sample

Tests

The tests performed by the Blood Bank include the following tests:

1. ABO/Rh
2. Antibody Screen (Indirect Coombs)
3. Antibody Identification
4. Antibody Titers
5. Direct Coombs (DAT)
6. Crossmatch
7. Type and Screen
8. Type and Crossmatch
9. Rh Immune Globulin Workup
10. Fetal Bleed Screen
11. Kleihauer Betke Stain
12. Red Cell Antigen Typing

Appendix D

Chemistry
Specimen Collection & Processing

I: 24-Hour Urine Collection

General Information:

1. Proper collection technique includes voiding first specimen of the morning; discard this urine sample. Have the patient record this time as the START TIME. This will be the patient's FINISH TIME at the end of the 24-hour (timed) collection period. The patient must place all subsequent urine collections for a 24-hour period in the proper collection container. This includes the first morning specimen on the following morning. The patient should completely empty their bladder as close to the FINISH TIME as possible and add this urine to the container. If required, the container can be kept cool in a refrigerator, cooler with ice or basin with ice during collection. The patient should then transport the specimen to the lab as soon as possible.

2. The total volume must be recorded on the request ticket if complete specimen is not submitted. The contents of the container should be thoroughly mixed prior to aliquoting. If two or more containers with the same preservative are collected, the contents of all containers should be combined and thoroughly mixed prior to aliquoting.

3. Collection containers may contain strong acid as a preservative - handle with care !

The following listing is of tests than can be performed on 24-hour urine specimens. The first preservative listed after the test name is the preservative of choice, followed by other acceptable preservatives. All unlisted preservatives are not satisfactory for that test.

IN HOUSE

Test	Final pH	Adjust pH with	24 Hour (Timed) Preferred Preservative	Preferred Aliquot Volume for lab	Perferred Storage Temperature	Notes	Acceptable Preservative
In-House Urine Testing							
Beta 2 Microblobulin			No Preservative	1mL	Refrigerated	*1E	
Drug Screen - Medical	5-8	*1A	No Preservative	20 mls	Refrigerated		
Electrophoresis			No Preservative	50 mls	Refrigerated		
HCG - Qualitative			No Preservative	1ml	Refrigerated		
Immunofixation			No Preservative	50 mls	Refrigerated		
Microalbumin			No Preservative	1 ml	Refrigerated		
Osmoality			No Preservative	1 ml	Refrigerated		
Urine Chemistries							
Amylase	7	*1B	No Preservative	1 ml	Ambient		
Calcium	<3	6N HCL	(10-20 mls) 6 N HCL	1 ml	Refrigerated		
Chloride			No Preservative	1 ml	Refrigerated		
Creatinine			No Preservative	1 ml	Refrigerated		HCL, Acetic Acid and Boric Acid Acceptable
Glucose			No Preservative	1 ml	Refrigerated		
Phos	<3	6N HCL	(10-20 mls) 6 N HCL	1 ml	Refrigerated		
K			No Preservative	1 ml	Refrigerated		
Mg	<3	6N HCL	(10-20 mls) 6 N HCL	1 ml	Refrigerated		
Na			No Preservative	1 ml	Refrigerated		
T Protein			No Preservative	1 ml	Refrigerated		
Urea			No Preservative	1 ml	Refrigerated		
Uric Acid	8-9		(10 mls) 5% NaOH	1 ml	Refrigerated	*1D	

*1A pH between 5-8 adjust with 1N HCL or 1N NaOH (Do NOT use Boric Acid)
*1B pH urine to 7.0 with 1N HCL or 1N NaOH after collection then refrigerate
*1D 5%NaOH (5 gm NaOH/100 mL distilled water)
*1E If testing not performed within 24 hours, adjust pH between 5.5-7.5 with 0.5N NaOH or ammonium hydroxide and FREEZE sample.

Random urines should also be adjusted to the correct pH with appropriate preservative if (pH) listed.

Urine Preservatives

Test	Final pH	Adjust pH with	24 Hour (Timed) Preferred Preservative	Preferred Aliquot Volume	Perferred Storage Temperature	Notes	Acceptable Preservative
Referral Mailout 24 Hour Urines							
5HIAA	2-4		(25 mls) 50% Acetic	5 mls	Refrigerated	*8	6N HCL, NA2CO3, Toluene, 6N HNO3, Boric Acid, Thymol
17 Ketosteroids Frac			(25 mls) 50% Acetic	11 mls	Refrigerated		6N HCL, NA2CO3, Toluene, 6N HNO3, Boric Acid, Thymol
ALA	2-4		(25 mls) 50% Acetic	10 mls	Ambient	*8,*2	6N HCL
Aldosterone			1 gram boric/100 mls urin	5 mls	Frozen		6N HCL. 50%Acetic Acid
Arsenic			None	10 mls	Refrigerated	*6	6N HCL, 50%Acetic Acid, Toluene, 6N HNO3
Catecholamines	2-4		(25 mls) 50% Acetic	10 mls	Refrigerated	*1,*7, *8, *12	6N HCL, 6N HNO3, Boric Acid, Thymol
Chromium			No Preservative	10 mls	Refrigerated		
Citrate			10 gm Boric Acid	5 mls	Refrigerated	*10	6N HCL, Acetic, Toluene, 6N HNO3, Thymol
Copper			No Preservative	10 mls	Refrigerated		6N HCL
Cortisol			(10 g) Boric	10 mls	Refrigerated		50%Acetic Acid, Na_2CO_3, Toluene, Thymol
Cystine			(20 mls) Toluene	5 mls	Frozen	*5	
Heavy Metals			No Preservative	10 mls	Refrigerated	*6	6N HCL, 50%Acetic Acid, Toluene, 6N HNO_3
Histamine	2-4		(25 mls) 50% Acetic	1 ml	Refrigerated	*8	6N HCL, Na_2CO_3, Toluene, 6N HNO_3, Boric Acid
HVA	2-4		(25 mls) 50% Acetic	10 mls	Refrigerated	*4, *8	6N HCL (pH 2-4)
Iron			No Preservative	10 mls	Refrigerated		6N HCL, 50%Acetic Acid, Toluene, 6N HNO_3, Boric Acid
Lead			No Preservative	10 mls	Refrigerated		6N HCL, 50%Acetic Acid, Toluene, 6N HNO_3
Lysozyme			No Preservative	12 mls	Frozen		NONE
Magnesium			No Preservative	10 mls	Refrigerated		6N HCL, 50%Acetic Acid, Toluene, 6N HNO_3
Mercury			No Preservative	10 mls	Refrigerated		6N HCL, 50%Acetic Acid, Toluene, 6N HNO_3
Metanephrine	<7		(25 mls) 50% Acetic	10 mls	Refrigerated	*8, *12	6N HCL, NA2CO3, Toluene, 6N HNO3, Boric Acid, Thymol
Oxalate	2.5-3.0		(30 mls) 6N HCl	5 mls	Refrigerated	*3	50%Acetic Acid, Toluene, 6N HNO_3, Boric Acid, Thymol
Porphyrins	>7		(5 g) Na_2CO_3	30 mls	Frozen	*2,*9, *12	
Urorisk			special 24 hr collection jug provided by Specialty			*11	
Thallium			No Preservative	10 mls	Refrigerated		6N HCL, 50%Acetic Acid, Toluene, 6N HNO_3
VMA	1-5		(25 mls) 50% Acetic	5 mls	Refrigerated	*8	6N HCL, 6N HNO_3
Zinc			No Preservative	10 mls	Refrigerated		6N HCL, 50%Acetic Acid, Toluene, 6N HNO_3, Thymol

*1: This assay is of greatest value when the specimen is collected during a hypertensive episode.
*2: Protect from light
*3: Avoid taking larges doses of Vitamin C during collection, Add preservative (30 mls 6N HCL) at end of collection.
*4: Discontinue Levodopa 24 hrs prior to & during collection
*5: Collect before IVP
*6: Patient should not eat seafood for 48 hrs prior to collection
*7: Discontinue epinephrine, norepinephrine or dopamine injections 12 hrs before collection and discontinue drugs that release or hinder metabolism of epinephrine, norepinephrine or dopamine 1 week prior to collecting specimen.
*8: Add 15 mL Acetic Acid for children less than 5
*9: Abstain from alcohol for 24 hrs prior to and during collection. Include list of current medications patient is taking.
*10: Any drug that causes alkalemia or acidemia maybe expected to alter citrate excreation and should be avoided, if possible.
*11: Paperwork: aliquot containers & outer box are retained at 1D until jug is returned. Specimen must be aliquoted into provided containers before sending to Ref Lab with paperwork.
*12: Preservative must be added to container before collection.
*****Preservative added after end of collection must be added within 4 hrs of completion unless otherwise noted.
***** All urines should be sent in a plastic aliquot container with no metal lid or glued insert.

Updated 3-02-200

Appendix E

COAGULATION
Specimen Collection and Processing

Introduction

To obtain reliable results in coagulation testing, it is imperative to begin with proper sample collection. The four primary factors necessary for a good quality sample are a trauma free collection, free flow of blood, immediate and proper mixing of blood with the anticoagulant, and gentle handling of sample after collection. A fifth factor, patient's predisposition, also is an important but is outside the control of a phlebotomist (blood collector). If one or more of the primary collection factors fail to meet ideal standards, the sample may be compromised. Test results are a direct reflection of sample integrity.

CAUSES FOR SAMPLE REJECTION: Specimens that are clotted, hemolyzed, contaminated with heparin or I.V. fluid, sample exceeded sample stability time limit, tubes under-filled or over-filled, or inappropriate anticoagulant.

Centrifugation: Double Centrifuge Technique

1. Purpose: For ALL FROZEN SAMPLES. This procedure ensures that the plasma submitted is platelet-free-plasma (<10,000 platelets/mm^3). Assays associated with lupus coagulation, hypercoagulation profile and other platelet sensitive assays require platelet free plasma.
2. Select a centrifuge with a centrifugal force of 2000-2800g.
3. Label <u>three</u> non-polystyrene, plastic aliquot tubes (e.g., polyurethane, polypropylene, or polyethylene). **<u>DO NOT USE GLASS TUBES.</u>**
4. Centrifuge the primary collection tube for 10-15 minutes.
5. Without disturbing the buffy coat layer of platelets and WBCs, transfer about ¾ of plasma to the first labeled aliquot tube.
6. Seal aliquot tube with parafilm or tube cap
7. Re-centrifuge specimen at same settings
8. While not disturbing the button, transfer equal amounts of plasma into <u>two</u> labeled aliquot tubes.
9. Identify the contents of the aliquot tubes as "FROZEN CITRATE PLASMA.
10. Double seal the aliquot tube by tightly securing tube cap and then wrapping parafilm around the cap
11. Immediately, place aliquot tubes in a freezer after processing. **<u>DO NOT FREEZE PLASMA IN GLASS TUBES.</u>**
12. DO NOT transport until sample completely frozen (i.e., frozen solid for at least 1 hours prior to shipping). DO NOT thaw sample after freezing.

Sodium citrate samples

1. **WHOLE BLOOD**: If a protime (PT) and INR is ordered as the only coagulation assay and it will arrive in the laboratory within 24-hours of collection, send the sample unopened (STOPPER seal unbroken). Store at room temperature and transport cool. Avoid refrigeration when possible. If a PT is ordered in conjunction with other coagulation tests, process as plasma.
2. **FROZEN PLASMA**: For coagulation tests requiring plasma, process samples immediately upon collection. Process samples by centrifuging the sample twice for 10-15 minutes with a centrifugal force of 2000-2800g (see Double Centrifuging Technique). Separate sample into two aliquot tubes. Freeze immediately. Transport frozen. DO NOT transport until sample completely frozen (i.e., frozen solid for at least 1 hours prior to shipping). DO NOT thaw sample after freezing.
3. **SODIUM CITRATE CONCENTRATION**: Collect all coagulation samples requiring sodium citrate with a concentration of 3.2% (0.105 M)

All coagulation tests that require serum should be drawn in plain red top collection tubes !

Primary Collection Tubes

Serum Citrate Tubes - Filled with a buffered tri-sodium citrate solution with a concentration of 3.2% (0.105 M) which mixes with blood after the tubes are gently inverted 5 - 8 times.

Serum Tubes - Do not contain an anticoagulant, but are coated with micronized silica particles that help activate clotting when the tubes are gently inverted 5 -8 times.

Coagulation Samples

Introduction - It is imperative to begin with proper sample collection to obtain reliable results in coagulation testing. The four primary factors necessary for a good quality sample are:

1. trauma free collection
2. free flow of blood
3. immediate and proper mixing of the blood with the anticoagulant
4. gentle handling of the sample after the collection

If one or more of the collection factors fails to meet the ideal standards, the sample may be compromised. Test results are a direct reflection of sample integrity.

Causes for Sample Rejection - Samples that are clotted, hemolyzed, contaminated with heparin, or IV fluid, sample exceeded the stability time limits, tubes that re over or under filled or inappropriate anticoagulant.

Whole Blood - If a Protime (PT) and INR is ordered as the only coagulation assay and it will arrive at the laboratory within 24-hours of collection, the sample may be sent unopened (Stopper seal unbroken). Transport and maintain room temperature. Avoid refrigeration. If PT is ordered in conjunction with other tests, process as frozen plasma.

Frozen Plasma - Process samples immediately upon collection. Coagulation testing requires platelet poor plasma for accurate testing. Centrifuge the specimen with a g-force of 2000 - 2900 g for 10 to 15 minutes. Separate the plasma into plastic tubes and re-spin the samples as above. Transfer the plasma into two labeled aliquots, again in plastic tubes. **DO not use glass tubes.** Freeze the tubes immediately and transport frozen. Do not allow sample to thaw after freezing.

Serum - Coagulation assays requiring serum should be transported frozen. Using a plain red top tube allow the blood to clot completely for at least 30 minutes. Centrifuge for 10 - 15 minutes. Transfer the serum to a plastic aliquot tube and freeze immediately. Transport frozen and do not allow the sample to thaw.

Hematocrit Below 20% or Above 55% - If a patient's hematocrit is below 20% or above 55%, the volume of anticoagulant to plasma will not fall into the proper 1:9 ratio. Low hematocrits shorten a clot time and patients with high hematocrits falsely prolong clot times. If a special collection tube is needed call the Special Coagulation Laboratory at (254) 724-2426.

Appendix F

Cytology[14]

Specimen Collection and Processing

Gynecological Cytology Specimens (Pap smear)

ThinPrep Pap test

Principle

A gynecological cytology specimen (Pap smear) is an evaluation for the presence of abnormal cells, which may be indicative of malignancy or other conditions requiring treatment. It is important to sample the cervix or vagina well with minimal artifact and obscuring materials.

Precautions

1. Gloves should be worn when collecting and handling the specimen.
2. Specimens should be taken before pelvic examination.
3. The patient should not douche or use vaginal medication for 24 hours before the specimen is obtained. This should not, however, prevent obtaining a specimen. Inform the patient that the test may be unsatisfactory so that she will not unduly be alarmed if a repeat PAP is later required.
4. Do not use lubricant. If necessary, the speculum may be moistened with normal saline. Avoid using water, which is hypotonic and will produce cellular distortion.
5. Avoid, if possible, taking specimen during normal menses. However, if there is abnormal bleeding, obtain routine PAP and consider direct endometrial specimen.

Materials

1. Cytyc Preservcyt solution vial. Preservcyt solution is a methanol-based buffered preservative solution. Store the vials at 15º – 30º C. (59º – 86º F.)
2. Plastic spatula
3. Endocervical brush
4. GYN cytology requisition form number 245 and Client Request Form.

These supplies can be obtained from the Scott & White Reference Lab:

- PreserCyt Solution
- Medescand Cytobrush Plus GT (endocervical brush & plastic spatula)

NOTE: Cotton tip swabs and wooden spatulas should not be used to obtain specimens for ThinPrep PAP test.

Collecting the Specimen

1. Write the patient's name and medical record number on the vial or place the patient's identification label on the vial. This is essential to prevent a mix-up of the specimens during processing.
2. Expose cervix with the speculum. The cervical surface should not be wiped; wiping it will remove the cell-rich adherent cervical mucus.

Cervical Scrape

Scrape the external os 360º with the plastic spatula and as quickly as possible place the spatula into the preservcyt solution vial, swirling the spatula vigorously in the vial 10 times. Never induce bleeding by scraping the cervix vigorously.

Endocervical Brush

Insert the brush into the cervical os and rotate gently. It is recommended that the brush be rotated only 180º. More rotation may cause excessive bleeding. Rinse the brush as quickly as possible in the preservcyt solution vial by rotating the device in the solution 10 times while pushing against the preservcyt vial wall. Swirl the brush vigorously to further release material. For thick mucoid specimens collected using the brush, to further release endocervical cells that might be entrapped in mucous; use the concave side of the spatula and scrape down the brush bristles a few times and on different sides of the brush. This can be done while holding the brush in the vial with the left hand and using the spatula to scrape with the right hand.

Vaginal Scrape:

For specifically desired hormonal evaluation (maturation index), gently scrape lateral wall of upper third of vagina. Rinse the spatula as quickly as possible in the preservcyt solution vial by swirling the spatula vigorously in the vial 10 times.

1. Tighten the cap so that the line on the cap and the line on the vial meet.
2. For best results, please follow these preparation steps diligently.

Method for Submitting Specimen to the Laboratory

Clients will submit a request form and a form 245 with the patient's name, medical record number, and the appropriate clinical and billing information. This included proper diagnostic codes, specimen source and other clinical data such as last menstrual period (LMP), previous treatment, previous abnormals, colposcopic findings, hormonal status, etc. Fill in the date and time the specimen is obtained. The specimen vial should be placed in a biohazard bag and the completed requisition form placed in the side pocket of the bag. Specimens collected after 5:00 PM, on weekends or holidays should be held until the following workday.

Non-Gynecological Cytology Specimens

Principle

It is important that high quality diagnostic material is provided for cytopathologic examination.

Precautions:

Gloves should be worn at all times when handling unfixed specimens in accordance with the Department of Pathology Bloodborne Pathogen Policy. All NON-Gyn cytology specimens must be handled using face protection.

Procedure for Specimen Collection

All specimen containers and slides should be properly identified and labeled with the patient's name and medical record number.

A completed requisition form that matches the specimen identification should be submitted with the specimen. The form should bear patient identification data, date and time of collection, physician/resident name, source of specimen and pertinent clinical information.

Failure to properly identify specimens, or mismatches between specimens and requisition forms will result in delay of processing or rejection of the specimen.

LUNG

Sputum

The patient must rinse his/her mouth with water, bend horizontally to the waist and press his hands against the abdomen (just below rib traction of diaphragm) and expectorate directly into the container.

Sputum specimens are collected in the fresh state in sputum cups and transported to the laboratory inside biohazard plastic bags. The requisition form containing the demographic data, clinical information, date and time of collection must be attached. Specimens received during the night and weekends are to be placed in the refrigerator and delivered to cytology the next working day. Specimens not processed within 18 hours should be fixed with 50% to 70% alcohol.

Bronchial Washing

The bronchial was specimens are collected in the fresh state in a container and transported in a biohazard plastic bag. The completed requisition form must be attached. Reference to the specific site of washing should be included (e.g. right upper lobe). Requests for special studies (e.g. GMS stains, flow cytometry, etc.) should be indicated.

Bronchial Brush

The smear is made at the time of endoscopy by rolling the brush on a totally frosted slide or slides which are immediately immersed in a Coplin jar with 95% alcohol.

FLUID FROM SEROUS CAVITY

All effusions submitted for cytologic evaluation must be heparinized at the time of collection to prevent coagulation. The recommended quantity of heparin (1:10,000) is 1 ml heparin to 300 ml body cavity fluid. If the patient has been bed ridden, it is advisable to gently rotate him prior to tapping the fluid filled area; this is necessary to re-suspend those cells, which have settled within the body cavity due to their heavy cellular density.

The specimen should be brought fresh to the cytology lab immediately following the procedure during the day. Should the procedure need to be performed at night or on weekends, the fluid should be placed in a refrigerator. Pleur-evac containers must not be submitted to the cytology lab.

CEREBROSPINAL FLUIDS

Spinal fluid for cytologic examination obtained during working hours (M-F from 5:00 AM to 6:00 PM) should be immediately delivered to the cytology lab to be processed. Specimens obtained after 6:00 PM or on weekends/holidays should be mixed with an equal volume of 50% or 70% ethyl alcohol and placed in the refrigerator in the microbiology lab to be delivered the following working day.

URINE

All voided specimens should be collected as a mid-stream clean catch. The first morning specimen should be discarded.

To the urine specimen, add an equal part of 50 to 70% alcohol. The patient's name should be placed on the urine container. The clinical data should include whether the urine is voided or instrumented. The presence or absence of a previous tumor or previous treatment should also be noted and the cytoscopy findings indicated. A form that included the requested clinical data has been attached to this page and can be duplicated. It should be attached to all requisition forms of urine specimens.

ENDOSCOPIC BRUSHINGS

Gastric, esophageal, duodenal, bile duct or colonic brushings are done on completely frosted slides. The smears are prepared quickly and placed immediately in 95% ethyl alcohol. Air-drying should be avoided.

FINE NEEDLE ASPIRATION

Direct smears made at the time of aspiration are immersed immediately in Carnoy's fixative (provided by the cytology lab) for approximately 5 minutes then transferred into 95% ethanol. Caution should be taken not to leave the slides in the Carnoy's fixative for more than 5 minutes, because this will result in cellular distortion and artifacts. The purpose of this short immersion in Carnoy's fixative is to lyse the red blood cells and prevent obscuring of cellular detail by blood.

Specimens that are not directly smeared, are to be collected in 20 ml tube of a 1:1 solution of 50% ethyl alcohol and Ringer's solution (5 ml each). The sample is flushed into the container and sent with the appropriate cytology form to the lab for processing. Aspirates from cystic lesions can be forwarded to the lab in the syringe (after discarding the needle) if they are sent immediately after the procedure is completed. If that is not possible, they should be flushed into the above-mentioned fixative solutions.

BREAST FLUID SECRETION

The area of greatest accumulation of secretion is found immediately below the nipple and the areolar area. A breast pump may be used, although material collected for cytologic evaluation is more frequently obtained through spontaneous secretions. Care should be taken not to manipulate the breast unnecessarily. The material for cytologic evaluation is collected by placing a frosted-tip slide against the nipple and smearing the fluid quickly over the slide. Immediate fixation may be accomplished if the patient (or an assistant) is allowed to hold the open bottle of 95% ethanol in front of the breast so that the slide can be immediately dropped into the fixative. Air-drying should be avoided as it may render the specimen non-diagnostic.

Appendix G

Hematology & Urinalysis

Specimen Collection and Processing

Hematology Samples

Introduction

In general, the quality of hematology blood samples is dependent upon good blood collection techniques. The four primary factors necessary for a good quality sample are a trauma free collection, free flow of blood, immediate and proper mixing of blood with the anticoagulant, and gentle handling of sample after collection. A fifth factor, patient's predisposition, also is an important but is outside the control of a phlebotomist (blood collector). If one or more of the primary collection factors fail to meet ideal standards, the sample may be compromised. Common interferences that can compromise the results a hematology sample are platelet clumps, fibrin strands, clots (fibrin mess) and hemolysis.

Urinalysis Samples

Introduction

Proper sample collection and immediately delivery or correct storage are essential factors in providing reliable and truly representative urinalysis report.

It is important to realize that the results of a routine urinalysis can be seriously affected by testing delays and improper storage. The following 10 changes may occur in a urine specimen allowed to remain unpreserved at room temperature for longer than 1-hour.

URINE CHANGES AFTER 1-HOURS WHEN STORED AT ROOM TEMPERATURE	
PH	Increased pH from the breakdown of urea to ammonia by urase-producing bacteria
Glucose	Decreased glucose due to glycolysis and bacterial utilization
Ketones	Decreased ketones because they readily evaporate into the atmosphere
Bilirubin	Decreased bilirubin from exposure to light
Urobilinogen	Decreased urobilinogen as a result of its oxidation to urobilin
Nitrite	Increased nitrite due to bacterial reduction of nitrate
Bacteria Yeast	Increased bacteria and/or yeast
Turbidity	Increased turbidity caused by bacterial growth and possible precipitation of amorphous material
Red Blood Cells (RBC's)	Disintegration of RBC's, particularly in dilute alkaline urine
Casts	Disintegration of casts, particularly in dilute alkaline urine
Color	Changes in color due to oxidation or reduction of metabolites

Appendix H

Histocompatibility (HLA)
Specimen Collection & Processing

TESTING

HLA Typing

HLA Class I (ABC) and HLA Class II (DR, DQ)

HLA Single Antigen testing either Class I or Class II (specify antigen)
- HLA B27 screen
- Narcolepsy associated antigen screen (DQB1*0602)
- Celiac Associated HLA-DQ Type

HLA DNA Tests: HLA Class I and/or HLA Class II, either low resolution or high resolution.

Serum antibody screening

HLA-Antibody screens for Panel reactive antibody (Class I and/or Class II) screen.

HLA-Antibody Identification (Class I and/or Class II)

HLA-Platelet antibody screen

HLA Serum crossmatch: donor and recipient, or autologous

SPECIMEN

1. Anticoagulated sterile blood samples are required for HLA-ABC, HLA-DR, and crossmatching. Appropriate anticoagulants include ACD (yellow top vacutainers) and sodium heparin (green top vacutainers).
   a. Samples should be as fresh as possible, and ideally not more than 48 hours old.
   b. ACD (solution A is preferable to B) is the anticoagulant of choice. An acceptable alternative is sodium heparin at 25-50 units of Na heparin **(no preservatives)** per ml of blood
   c. Samples collected in ACD may contain viable lymphocytes up to 96 hours. Testing will be done based on minimum pretest viability greater than 80%.
   d. ACD is the anticoagulant of choice for extended transport of samples.
   e. All samples must remain at room temperature, and be kept from temperature extremes for transport and storage.
   f. DO NOT SPIN TUBES
2. Serum samples for antibody screening and crossmatching are drawn in a dry (red top) tube (no anticoagulant). The sample should remain at room temperature or may be refrigerated for transport. Frozen serum, separated from the clot, should remain frozen during transport.
3. Lymph nodes and spleen are alternative sources for lymphocytes from deceased donors. Samples should be collected aseptically and suspended in RPMI 1640 with antibiotics or other support media, and kept at 4º C. Saline should not be used for tissue collection or as transport media.
4. Requirements for histocompatibility testing
   a. HLA typing by lymphocytoxity
      - HLA-ABC: 10-20 ml of anticoagulated blood (ACD or heparin)
      - HLA-DR: 10-20 ml of anticoagulated blood (ACD or heparin)
      - HLA-ABC & DR: 30 - 50 ml anticoagulated blood (ACD or heparin)

      Minimum requirements:
      1. Pediatric: 3 - 5 ml
      2. Adult: 10 ml
      3. A patient who is lymphopenic or undergoing chemotherapy may require 60 ml peripheral blood drawn to provide an adequate cell number for testing. A minimal WBC of 1.0 required.

   b. Crossmatches
      - **Each donor**: 30 - 40 ml anticoagulated blood (ACD or heparin)
      - Deceased donor: 60 - 80 ml anticoagulated blood (ACD or heparin)
      - If donor has been transfused with >2 units of blood, pre-mortem nodes should be requested.
      - **Recipient:** 5 - 10 ml of clotted blood (serum dry clot tube)

c. HLA Antibody screening (any method)
   - 5 - 10 ml of clotted blood (serum dry clot tube)
   - Minimum: 1 ml of serum

d. HLA typing by DNA
   - 10 - 20 ml of ACD or EDTA (lavender tube) anticoagulated blood
   - Heparinized blood is known to interfere with the PCR process

e. Platelet antibody screening
   - 1 ml of plasma or serum

Specimen should be examined to determine that they were appropriately collected and maintained.

Unacceptable specimens include:

- Unlabeled specimens (no name or name and no date)
- Refrigerated green (heparinized) or yellow (ACD) top tubes
- Cracked or leaking tubes
- Heparinized specimens >72 hours old for crossmatch. (Testing will be based on viability, if it cannot be redrawn)
- Specimens drawn in Lithium heparin
- Grossly hemolyzed tubes
- Unclotted clot tubes
- Mismatched labels, label on tube does not match the form label, either in ID number or name.

Appendix I

Immunology
Specimen Collection & Processing

Serum Specimens

Specimen must not be grossly hemolyzed, lipemic or bacterially contaminated or it will be rejected. Specimens should be frozen if not received in the lab within 24 hours unless specifically addressed below.

Plasma Specimens

Specimen must not be grossly hemolyzed, lipemic or bacterially contaminated or it will be rejected. Specimens should be frozen if not received in the lab within 24 hours unless specifically addressed below.

Check the test listing to determine if EDTA or Sodium Heparin anticoagulant should be used. Lithium heparin anticoagulant should NOT be used for Immunology testing. Therefore, the green top collection tube should be carefully checked and identified as sodium heparin (Na Heparin).

Cerebrospinal Fluid

Spinal fluid must not be visibly contaminated or grossly bloody.

Procedures needing acute & convalescent sera

Two serum specimens must be sent together. The first should be drawn during the onset of the illness (the acute phase). This specimen should be clearly marked “acute” and the date drawn listed on the specimen container. The second specimen should be drawn 10 to 14 days later during the patient’s convalescent stage. This specimen should be clearly marked “convalescent” and the date drawn on the specimen container. These paired sera will then be tested simultaneously.

Immunology procedures requiring special handling

COLD AGGLUTINATION:

10 ml red top (plain). Maintain temperature of the tube at 37° C until delivered to the Immunology laboratory (use heel warmer).

If this is not possible, place the tube in a 37° C water bath or incubator, immediately after the specimen is drawn. Allow to clot for 15 minutes. Spin specimen for 5 minutes; remove serum and place in a separate plastic tube. Transport **both** the separated serum and remaining clot to the Immunology laboratory. Transport serum and cells at room temperature. **DO NOT FREEZE!**

COMPLEMENT – TOTAL HEMOLYTIC (CH50)

One red top tube of whole blood. Serum MUST be separated from the red cells **and frozen** within 2 hours of collection. Place serum into two plastic tubes. Freeze immediately at -10° C or colder. Ship frozen on dry ice.

CRYOGLOBULINS

Two 10.0 ml red top (plain) tubes. Maintain temperature at 37° C until delivered to the Immunology laboratory. If this is not possible, place the tube in a 37° C water bath or incubator immediately after the specimen is drawn. Allow to clot for 15 minutes. Spin specimen for 5 minutes; remove serum and place in a separate tube. Transport the separated serum at room temperature. **DO NOT FREEZE!**

CRYPTOCOCCAL ANTIGEN

1.0 ml serum or spinal fluid (NO plasma); specimens must be free of white blood cells, platelets, fibrin, mucus or contaminates.

FLOW CYTOMETRY ANALYSIS

(Specimen requirements for ALL flow cytometry analysis: Immune profiles, leukemia or lymphoma panels).

Peripheral Blood

Specimen drawn in sodium heparin (green top) preferred; ACD Solution A (yellow top) or EDTA (purple top) also acceptable. **NO lithium heparin.**

Specimen should be held at room temperature and transported to the Immunology laboratory as quickly as possible. DO NOT SPIN!

Minimum requirements: Child – 3.0 ml
Adult – 10.0 ml

Bone Marrow

Specimens should be drawn into a sterile preservative-free liquid sodium heparin tube (available from the Immunology Laboratory). Specimen should be held at room temperature and transported to the Immunology Laboratory as soon as possible. The cap must be securely fastened. Identify site of bone marrow on requisition and/or tube.

Lymph Node or other Tissue

Specimen should be stored in holding media (RPMI-1640/gentamicin/fetal calf serum) available from the Immunology Laboratory. Specimen should be held at room temperature and transported to the Immunology Laboratory as soon as possible. Refrigerated if tissue is held for > 24 hrs. **Saline should not be used as a collection or transport media.**

Rejection Criteria for Flow Cytometry

ALL (Peripheral Blood, Bone Marrow, or Tissue)

1. Specimen must be in the recommended anticoagulant, and properly filled. Partial draws should be noted on the request form.
2. Specimen should be less than 48 hours old.
3. Specimen should be free from hemolysis.
4. Specimen should not be clotted.
5. Specimen should be held and transported at room temperature ONLY. Frozen specimens will not be accepted.

Bone Marrow

1. Specimen must be collected in the correct media (sodium heparin) and transported at room temperature.
2. Specimen must be less than 48 hours old.
3. Specimen tube cap must be securely fastened. Specimens that leak or spill into the transport bag will not be accepted.

Tissue

1. Specimen must be collected in the correct holding media and transported at room temperature.
2. Specimen must be less than 48 hours old.

HEPATITIS ANTIBODY TESTING

Serum or Plasma may be used, but specimen should not contain sodium azide.

***** Hepatitis B antibody is serum only.

Scott & White Flow Cytometry Information Sheet

All specimens must be labeled with Date and Time of Collection and collector's initials

Leukemia/Lymphoma Panels

Peripheral Blood

1. Acceptable Anticoagulants in order of preference
    a. Sodium Heparin - viable for 48 hours
    b. ACDA - viable for 48 hours
    c. EDTA - must be received within 24 hours of draw time
2. Specimens cannot be hemolyzed, clotted or drawn in Lithium Heparin
3. Specimens must be kept at room temperature (20-24º C) and not spun.
4. Minimum volume requirements
    a. Adults - 10 mL
    b. Children - 3 mL

Bone Marrow

1. Collect Heparin tubes provided by the Flow lab. The specimen is viable for 48 hours
    a. If no Immunology tubes are available, it is acceptable to collect the bone marrow in Sodium Heparin (48 hrs), ACDA (48 hrs) or EDTA (24 hrs) tubes. The specimen must be labeled as BONE MARROW
2. Specimens must be kept at room temperature (20 - 24º C) and transported to the Flow Lab as quickly as possible.

Lymph Nodes or other Tissues (including FNA)

1. Specimen should be stored in RPMI holding media provided by Flow Lab
2. Specimen must be kept at room temperature (20 - 24º C) and transported to the Flow lab as quickly as possible.
3. Specimens should be less than 48 hours old.

Body Fluids (pleural, pericardial, peritoneal or CSF)

1. If anticoagulants are used, Sodium Heparin is preferred. ACDA or EDTA is also acceptable.
2. Specimen cannot be clotted and must be less than 24 hours old.
3. Specimen must be kept at room temperature (20 - 24º C).
4. Call Flow lab for specimen volume requirements.

Appendix J

Paraffin Immunohistochemistry

1. When paraffin section immunohistochemistry is requested a paraffin block should be sent rather than unstained slides. An H&E section will be made from the block if one is not received with the request. The block will be returned when the studies are completed.
2. The routine panels for selected diagnoses are listed below. The stains in bold face will be done routinely with other stains added as needed.
3. All cases will be reviewed prior to reporting results and an interpretation of the stains will be issued. This may suggest additional stains for adequate confirmation of the suspected diagnosis. Communications with the requesting pathologist will be initiated in these cases.

Key for Antibodies

Hematopoietic Markers

CD1a
CD3
CD4
CD5
CD7
CD8
CD10
CD15
CD20
CD21
CD23
CD30
CD31
CD35
ALK -1P
CD43
CD45

CD56
CD57
CD68
CD79a
CD99
CD117
Kappa
Lambda
Cyclin D1
bcl-2
bcl-6
HBME-1
EMA
EBV-LMP
Myeloperoxidase
Mast cell tryptase
TdT
Ki-67 (MIB-1)

Non-Hematopoietic Markers

Epithelial Markers
MNF 116
AE1/AE3
Keratin 7
Keratin 20
34BE-12
AE-1
CAM 5, 2
MOC-31
CEA
EMA
TTF-1
E-cadherin
RCC
CD10
Hepatocyte

Breast CA
ER/PR
GCDFP-15
HER-2
E-cadherin
CD99

Prostate CA
PSA
PSAP
AMACR-Racemase

Myoepithelial Layers (In-SITU vs Invasive)
Smooth muscle myosin
Calponin
P63

Vascular Markers
Von Willebrand factor
CD31
CD34
Thrombomodulin

Melanoma
S100
HMB45
Mel-A
MART-1
Microophthalmia
NGFR-p75

Epithelial Mesothelioma vs Carcinoma
BER-EP4
CEA
CD15
Thrombomodulin
HBME-1
CK5/6
Calretinin
WT-1
TTF-1

Mesenchymal Markers
Vimentin
S100
HMB45
CD34
CD57
Desmin
Muscle-specif actin (HHF35)
Caldesmon
Smooth muscle actin
Smooth muscle myosin
Calponin
Factor XIIIa
CD117
CD10

Small Round Cell Tumors
CD45
Desmin
HHF35
Myf4
NSE
Synaptophysin
Neurofilaments
CD99
NCAM(CD56)

CNS
GFAP
S100
Neurofilaments
NSE
Synaptophysin
AE1/3
EMA
Neu N

Neuroendocrine Markers
NSE
Synaptophysin
Chromogranin A
Parathormone
Thyroglobulin

Islet Cell Tumor
Chromogranin A

Adrenal Cortical Tumors
Mel-A

Germ Cell Tumors
CD30
Alpha-fetoprotein
HCG
Placental alkaline phosphotase

Infection
SV40 T antigen
CMV
H. pylori

Oncogene Products
HER-2
P53

Others
Alpha-1-antitrypsin
MLH1, MSH2, MSH6
CDX2

Glossary of Terms

Units

cm	**=**	**centimeter**
d	**=**	**day**
dL	**=**	**deciliter**
fL	**=**	**femtoliter**
g	**=**	**gram**
IU	**=**	**international unit**
kg	**=**	**kilogram**
L	**=**	**liter**
ug	**=**	**microgram**
µL	**=**	**microliter**
umol	**=**	**micromole**
mEq	**=**	**millequivalent**
mg	**=**	**milligram**
mL	**=**	**milliliter**
mm	**=**	**millimeter**
mmol	**=**	**millimole**
mOsm	**=**	**milliosmole**
min	**=**	**minute**
mol	**=**	**mole**
ng	**=**	**nanogram**
nmol	**=**	**nanomole**
O.D.	**=**	**Optical Density**
ppm	**=**	**parts per million**
%	**=**	**percent**
pg	**=**	**picogram**
RFU	**=**	**Rheumatoid Factor unit**
sec	**=**	**seconds**
SD	**=**	**Standard Deviation**
U	**=**	**unit**