SPECIMEN COLLECTION AND HANDLING

Specimen Preparation in Accurate Laboratory Analysis
Effective physical separation of the blood’s cellular fraction from the serum fraction is essential for reliable analysis of many serum-based analytes. A variety of tests can be affected by prolonged contact with red blood cells (RBC’s). This occurs from a combination of continued metabolic activity of RBC’s, leakage of intracellular components into the serum and degradation or lysis of RBC’s resulting in hemolysis and mixing of components normally removed with the cells from the serum fraction. The magnitude of the effect of RBC contamination will be translated into artificially elevated analyte concentrations in several components of the typical blood chemistry profile.

The gel barrier tube was developed to assist in the process of separating RBC’s from serum. A properly formed barrier between the cellular and serum fractions will preserve serum integrity prior to testing. Unfortunately improper handling and centrifugation procedures often result in inadequate gel barrier formation. Transport of specimens can exacerbate these pre-analytical problems. In these instances if the serum is not promptly transferred to separate pour-off containers, RBC contamination and the adverse affects associated with this pre-analytical problem will affect test results. To ensure the most accurate and consistent patient test results, and to avoid potentially erroneous results, PBL recommends that serum specimens be poured off to transport tubes prior to shipment to the laboratory.

To alert the physician that a pre-analytical problem may affect the chemistry test results for a patient, PBL includes a remark on the report indicating “RBC’s in contact with serum.” Individual analytes that may be artificially elevated will have remarks indicating “test may be affected by pre-analytical handling.” Specimens that have measurable amounts of hemolysis will have serum appearance results that indicate the degree of hemolysis: slight, moderate or severe. Various chemistry tests can be affected on the blood profile depending on the degree of interfering hemolysis in that sample. In severely hemolyzed samples some tests may be unreportable and will have a result of “specimen unsuitable for analysis.” Specimens that are received without centrifugation will have a remark on the report indicating: “Specimen received unspun.”

**Bicarbonate (CO₂) and Ionized Calcium** determinations, unlike other serum based assays, should be centrifuged but not opened or poured off prior to transport to the laboratory. A partial list of chemistry tests that may be affected by prolonged contact with RBC’s includes:

- **Potentially Increased:**
  - Potassium
  - Creatinine
  - Lactate Dehydrogenase (LDH)
- **Potentially Decreased:**
  - Glucose*
  - Chloride
  - Phosphate
Sodium fluoride preserved samples for glucose analysis are stable without the removal of RBC’s from the sample.

**Specimen Handling Requirements and Rejection Criteria**
The accuracy of test results depends on the quality of the specimen tested. The specimen collection, preparation and transport instructions should be followed carefully to ensure accurate results. Individual test requirements may vary. See the Test Listing for test-specific information.

**Specimen Volume Requirements**
The specimen volumes indicated in the alphabetized test listing are optimal volumes, with minimal acceptable volume in parentheses. The optimal volume is the volume sufficient to perform repeat runs, if necessary. Certain collection tubes require a minimum volume of specimen to ensure the proper anticoagulant-to-specimen ratio. Refer to the tube manufacturer’s literature for minimum requirements.

**Specimen Labeling**
*Note:* Each specimen container must be labeled with the patient’s name and date.

**Specimen Rejection Criteria**
Specimens will be rejected for the following reasons:
1. Improperly collected specimen (improper tube or anticoagulant, improper temperature).
2. Unlabeled or mislabeled specimen for which a positive identification cannot be made.
3. Containers that are not properly sealed (e.g., a leaky container or a swab that has fallen out of its holder).
4. Insufficient specimen quantity.
5. Specimens submitted with needle (e.g., body fluid).

**Requirements for Frozen Specimens**
1. When submitting frozen specimens, allow enough space in the transport container for specimen expansion.
2. Always use plastic containers for frozen specimens. Do not use glass; it will break during specimen expansion.
3. Freeze the specimen immediately after collection.
4. If more than one test is ordered on a specimen that needs to be frozen, send a separate specimen for each test. Make sure each specimen is labeled with the patient’s name. Indicate type of frozen specimen (e.g., serum, EDTA plasma) and write FROZEN directly on each bag.
5. Transport the frozen specimen with dry ice. The specimen must remain frozen throughout the shipping process. Make sure the dry ice does not come in contact with other (non-frozen) specimens.
6. In case of nonavailability of dry ice place the frozen sample in the insulated frozen transpak Bottle provided by the Laboratory.
SPECIMEN REJECTION CRITERIA FORM

The accuracy of test results depends on the quality of the specimen tested. The specimen collection, preparation and transport instructions should be followed carefully to ensure accurate results.

Specimen will be rejected for the following reasons

1. _______Improperly-collected specimen (improper tube or improper temperature.)
2. _______Unlabelled or Mislabeled specimen for which a positive identification cannot be made.
3. _______Name and I.D on the specimen and the requisition not matching.
4. _______Leak during transit (containers that are not properly sealed e.g. a leaky container or swab that has fallen out of its holder).
5. _______Insufficient specimen quantity.
6. _______Specimen submitted with needle e.g. body fluids.
7. _______Broken tubes or containers.

Reviewed by: ________________    Sample Account#: ________________

Date: ________________
Specimen Collection and Handling

Always verify the patient’s identity by asking their full name and date of birth. Please include this information on all specimens submitted to the laboratory.

COLLECTION OF BLOOD, PLASMA and SERUM

Serum
Serum is obtained by drawing blood into Red Top Tube or Serum Separator Tube (SST).

Serum Separator Tube
(Tiger or Red Top, with or without gel)
Tests requiring serum should be drawn in gel or serum separator tubes unless indicated otherwise in the alphabetized test listing.

Draw blood into evacuated tube without anticoagulants or preservatives: When using a serum separator tube (SST), follow these instructions:
1. One full tube is recommended for every 3-4 mL of serum
2. Perform venipuncture as with any other blood collection device. Venous Stasis (Tourniquet Application) should be minimal. Venipuncture should be clean and atraumatic.
3. Invert the tube gently no more than five times. Further inversion may cause alterations in sample integrity.
4. Allow blood to clot for 30 minutes (no longer than one hour).
5. Do not remove the stopper at any time. Allow the blood to clot in an upright position for at least 30 minutes but not longer than 1 hour. Do not centrifuge immediately after drawing blood.
6. **Centrifuge at 2200-2500 RPM for at least 15 minutes.** Improper centrifugation will interfere with Electrolytes results. Serum should be clear of red cells.
7. Transfer the clear serum to a plastic vial for transport to the laboratory, if provided.
8. Label transfer tube with patient’s name and date.

PLASMA
(Lavender, Light blue, Green, Gray, PPT)
Plasma contains fibrinogen and other clotting factors when separated from the red blood cells. Evacuated tubes used to collect plasma specimens contain anticoagulant and frequently, a preservative. The additive in each tube is specified on the label and tube stoppers are color coded according to the additive present. Consult the individual test
specimen requirement to determine the correct additive/tube to use. Indicate that the specimen is plasma on the plastic vial for transport and test requisition. If a syringe is used, immediately transfer the blood to a tube containing anticoagulant. Do not over fill the tube.

Draw blood into evacuated tube containing the proper anticoagulant.
1. Invert tube gently *8-10 times*.
2. Centrifuge for 10 minutes at 1100 RCF.
3. Remove the stopper and transfer the plasma to a plastic transfer tube with a disposable pipette.

**Label the tube “plasma.” Indicate the anticoagulant.**
4. Label the transfer tube with the patient’s name and date.
5. PPT (Plasma preparation tube) White top tube is used in Molecular Diagnostic tests where an undiluted plasma specimen is required.

**WHOLE BLOOD**
The following are some of the collection tubes used for whole blood:

1. **Blue Top** – This tube should be filled to the required volume to maintain appropriate concentration.
2. Purple / Lavender Top
3. Green Top
4. Yellow Top
5. Navy Blue Top
6. Grey Top

- Invert tube gently *8-10 times*.
- Do not centrifuge or separate the specimen.
- Label tube with patient’s barcode and name.

Maintain the specimen at ambient temperature before shipping to our laboratory unless instructed otherwise by the specimen requirements. Never freeze whole blood unless specifically instructed in the specimen requirements.
**Order of Blood Draw**

Draw multiple blood tubes in the following order:

Blood culture or sterile tubes should be drawn prior to any other tube and than use following order of draw.

1\textsuperscript{st} - Red Stopper/Red & Black stopper/Pink stopper  
• tubes containing no anticoagulant

2\textsuperscript{nd} - Lavender Stopper  
• tubes containing EDTA

3\textsuperscript{rd} - Blue stopper*  
• tubes containing sodium citrate

4\textsuperscript{th} - all other tubes  
  o When they need only a blue stopper, please draw a smaller red stopper tube first and discard the red stopper.

Tubes with powdered anticoagulant should be tapped near the stopper to dislodge any powder adhered to the stopper. Tubes with liquid anticoagulant should be filled to the exhaustion of the vacuum to ensure the proper ratio of anticoagulant to blood.

**Centrifugation Criteria for Blood Collection Tubes**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Color of Top (minutes)</th>
<th>Anticoagulant (RCF)</th>
<th>Centrifuge Time</th>
<th>Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>red without gel</td>
<td>None</td>
<td>15</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>red with gel</td>
<td>None</td>
<td>15</td>
<td>1100</td>
</tr>
<tr>
<td>Plasma</td>
<td>light blue</td>
<td>citrate</td>
<td>10</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>green</td>
<td>heparin</td>
<td>10</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>dark blue</td>
<td>EDTA or heparin</td>
<td>10</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>lavender</td>
<td>EDTA</td>
<td>10</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>gray</td>
<td>Fluoride yellow</td>
<td>10</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACD</td>
<td>10</td>
<td>1100</td>
</tr>
</tbody>
</table>

RCF is related to centrifuge speed setting (rpm) using either of the following equations (where “r,” expressed in cm, is the radial distance from the center of the centrifuge head to the bottom of the tube):

\[ \text{rpm} = \sqrt{RCFC} \times 10 \text{’ or approximately… rpm } = 10,000 \]

\[ 1.12r \quad \sqrt{r} \]
To calculate the radius: measure the centerline of rotation to the bottom of the tube in the centrifuge bucket.

The following table relates radius of centrifuge to required speed, in order to obtain the appropriate g-force:

**Centrifuge Radius / Speed Table**

<table>
<thead>
<tr>
<th>RADIUS (cm)</th>
<th>SPEED (rpm)</th>
<th>RADIUS (cm)</th>
<th>SPEED (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>3750</td>
<td>17</td>
<td>2400</td>
</tr>
<tr>
<td>8</td>
<td>3500</td>
<td>18</td>
<td>2350</td>
</tr>
<tr>
<td>9</td>
<td>3300</td>
<td>19</td>
<td>2280</td>
</tr>
<tr>
<td>10</td>
<td>3150</td>
<td>20</td>
<td>2200</td>
</tr>
<tr>
<td>11</td>
<td>3000</td>
<td>21</td>
<td>2160</td>
</tr>
<tr>
<td>12</td>
<td>2900</td>
<td>22</td>
<td>2100</td>
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<tr>
<td>13</td>
<td>2750</td>
<td>23</td>
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<td>14</td>
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<tr>
<td>15</td>
<td>2550</td>
<td>25</td>
<td>2000</td>
</tr>
<tr>
<td>16</td>
<td>2500</td>
<td>26</td>
<td>1950</td>
</tr>
</tbody>
</table>

Caution!
Do not centrifuge glass tubes at forces above 2200 RCF in a horizontal head (swinging bucket) centrifuge, as breakage may occur. Glass tubes may break if centrifuged above 1300 RCF in fixed angle centrifuge heads.

**Skin Puncture Procedures**
1. Avoid a finger that is cold, cyanotic (blue), swollen or inflamed.
2. If possible, have the patient wash his or her hands in warm water.
3. With your thumb and index finger, grasp either the patient’s long or ring finger about three inches from the tip of the finger.
4. With your other hand, hold the sides of the patient’s finger.
5. Moving your supporting hand toward the tip of the patient’s finger, apply a massaging motion to the fleshy portion of the finger.
6. Repeat this process five or six times.
7. Cleanse the patient’s finger with an alcohol pad.
8. Dry the tip of the finger with a gauze pad.
9. With one hand, firmly grasp the lancet.
10. With the other hand, firmly grasp the patient’s finger.
11. Appropriate puncture: 2.2 to 2.5 mm deep for adults; 1.0 - 2.0 mm deep for children over 20 pounds.
12. With a quick motion, make a cut on the side of the ball of the finger. The cut should be across the fingerprint.
13. Wipe the first drop of blood from the patient’s finger with a dry gauze pad.
14. Best blood flow occurs with the patient’s arm held downward, with the hand resting below heart level.
15. If necessary, apply gentle pressure to the finger to stimulate blood flow.
16. The blood is now ready to collect in micro-collection devices.
17. Always collect the hematology specimen first, followed by the chemistry and blood-bank specimens. This is the opposite of the order of draw by venipuncture and minimizes the effects of platelet clumping.
18. Follow the specimen collection procedures specified by the manufacturer of the device.
19. Place the filled micro collection device in an unused red-top tube for shipment to the laboratory.

Urine Collection
Urinalysis
Specimens for urinalysis should be clean catch, first morning urine in a clean (non-sterile) container with a tight, leak-proof lid.
1. Give patient a non-sterile urine collection cup and an antiseptic towel.
2. Provide the patient with instructions to obtain a clean-catch specimen (see instructions).
3. Label a urine aliquot tube (if available) with the patient’s name and date.
4. Pour the specimen into the urine aliquot tube and secure cap for transport. Refrigerate prior to shipment.

If a urine aliquot tube is not available, you may send the urine collection cup to the laboratory. Be sure to label the collection cup with the patient’s name and date. Tighten the lid securely to avoid leakage

Timed Urine Collection

Some urine chemistries require a timed urine collection. If you need supplies for timed collections, call the PBL Client Services Department at 412-678-1628. PBL can supply you with 24-hour collection containers, preservatives (with chemical hazard labels) and 50 mL conical-bottomed tubes.
Some of the timed collections require a preservative to be added to the collection container (see table 3.3). PBL’s preservatives are pre-measured into 50 mL conical-bottom tubes. Save these tubes, as they should be used to send an aliquot of the 24-hour collection to the laboratory. For collections that do not require a preservative, use a clean 50 mL conical-bottomed tube.

Prior to Collection...
1. Mark the 24-hour collection container with the patient’s name and the test(s) required.
2. Refer to table 3.3 to determine the appropriate preservative.
3. Add the entire contents of the preservative vial (50 mL conical tube) to the 24-hour collection container. *Add the preservative prior to collection.*

4. Save the 50 mL conical tube. Mark it with the patient’s name and bar-coded ID label.

5. Affix the chemical hazard label to the 24-hour collection container. Provide the patient with instructions for obtaining a 24-hour specimen (instructions included).

**After Collection...**

1. Invert the container 5-6 times to mix.

2. Read the markings on the side of the collection container to measure the total volume. Record the total volume and the duration of the collection on the requisition form. If required, record the patient’s height and weight. Mix before aliquotting.

3. Fill the 50 mL conical tube with urine from the 24-hour collection container. Be sure the conical tube is labeled with the patient’s name and bar-coded label. Secure lid tightly to avoid leakage.

4. Wrap the tube with aluminum foil if the test requires protection from light.

5. Return the 50 mL aliquot to PBL. Discard the remaining urine.

Note: Multiple timed urine tests may only be requested on the same 24 hour urine container if all tests can use the same type of preservative. Example: Timed calcium and timed magnesium may be requested together when using a 24 hour container with no preservative. Only 1 aliquot is necessary.

Multiple timed urine tests may NOT be requested on the same 24 hour urine container if the tests requested require different preservatives. Example: Timed magnesium and a timed citrate require different preservatives, so 2 separate 24 hour collections with an aliquot from each will be required. When more than one 24-hour collection is required, please send the aliquot specimens from each container to the lab on separate requisitions.

**Patient Instructions for 24-Hour Urine Collection**

1. Discard the first morning specimen. Make note of the time on the 24-hour urine container, as this is the start of the collection.

2. Collect all urine voided for the next 24 hours. Collect urine in a separate container (a clean paper cup will do) and *immediately* transfer it to the 24-hour receptacle. (The 24-hour receptacle may or may not contain a preservative.)

3. Cover and refrigerate the 24-hour receptacle during the collection period, unless otherwise instructed.

4. The final collection will be the first morning void of the next day. This void should be 24 hours from the start of collection.
Example: Wake up at 7 AM. and urinate; discard this first specimen. Collect urine all day. Wake up the next day, at or around 7 AM. urinate, and add urine to the container. The collection is now over.

The collection does not have to start with the morning specimen. It the same collection procedure is followed, you may start at anytime during the day. For example, void and discard a specimen at 5 P.M., then collect all day and night, collecting the last specimen at 5 P.M. the next day.

Preservative Requirements for 24-Hour Urine Collections

Note: Multiple timed urine tests may only be requested on the same 24 hour urine container if all tests can use the same type of preservative. Example: A timed calcium and timed magnesium may be requested together when using a 24 hour container with no preservative. Only 1 aliquot is necessary.

Multiple timed urine tests may NOT be requested on the same 24 hour urine container if the tests requested require different preservatives. Example: A timed magnesium and a timed citrate require different preservatives, so 2 separate 24 hour collections with an aliquot from each will be required.

When more than one 24-hour collection is required, please send the aliquot specimens from each container to the lab on separate requisitions.

Urine Drugs of abuse (DAU):
Submit a specimen in a regular urine cup and tightly secure the cap and put a paper seal over the cap. The donor providing the urine for DAU must be advised regarding the purpose of the specimen collection. The donor must sign the chain of custody request form. Collection of specimen should be done in the presence of collection site personnel and sealing of the specimen must be done in the presence of the donor. The donor must be asked to initial the seal.

Glucose Testing
Serum glucose tests do not have to be specifically ordered when they are part of a panel. Draw whole blood or plasma specimens into the gray-top tubes only.

Fasting Glucose
1. The patient should not have anything to eat or drink except water for 8-to-12 hours before the specimen is collected.
2. Draw one gray-top tube

Postprandial Glucose (Two-Hour Specimen)
1. The specimen should be drawn two hours after the consumption of a meal.
2. Draw one gray-top tube.

Gestational Screen
I. This test is for pregnant women only. Fasting is not required. No urine is collected.
2. Administer 50-gram oral glucose load (Glucola) to the patient (unless otherwise instructed by the physician).
3. Wait one hour.
4. Draw one gray-top tube.
5. Label tube with patient’s name, gestation, and time drawn.

**Glucose Tolerance Tests**
The glucose tolerance test involves collection of a fasting (baseline) specimen, followed by the administration of an oral glucose load. Specimens are then drawn at time intervals specified by the physician. The patient should be inactive during the entire procedure.

1. The patient should not have anything to eat or drink except water for 8-to-12 hours before the specimen is collected.
2. Draw the fasting glucose into a gray-top tube.
3. **Label tube with patient’s name, date and time. Note “Fasting” on the tube.**
4. Give the patient the appropriate dosage of Glucola (unless otherwise specified by the physician).

Gestational Tolerance (pregnant women only): 100 grams
Non-Gestational Tolerance (not pregnant): 75 grams
5. Draw one gray-top tube at each of the required time intervals as specified by the physician.
6. **Label each tube with the patient’s barcode and name. Note the time interval (“one hour,” “two hours,” etc.) on each tube.**

If urines are requested with the GTT, collect the specimens just prior to each blood draw. Label each urine specimen in the same manner as the blood specimens (name and time interval).

**Microbiology Collection**
Deliver specimens to the laboratory as soon as possible to ensure recovery of all clinically significant organisms. Label all collection containers with the patient’s name and date.
PBL’s microbiology specimen collection supplies and procedures support sample viability for a minimum of 48 hours except where indicated.

**Cultures**
The test requisition should state the specific source of culture and the collection time. If multiple specimens from different sites or for different cultures are required, please submit a separate request form for each.

If a particular organism is suspected, please record this information so that special culture techniques may be employed if necessary. Susceptibility testing will be performed on potential pathogens isolated from cultures when requested, except anaerobic cultures and Group A Beta Streptococci.
General considerations for collection and transport of clinical specimens for culture:

- Use sterile technique and transport to the laboratory as soon as possible.
- Close collection containers securely to prevent leaking of sample during transport.
- Whenever possible obtain specimens prior to the administration of antibiotics.
- Do not use expired tubes or media.
- Please write the patient’s name on each specimen container.
- Send specimens in one of the transport systems.

**Swabs with transport media: can be used for Bacterial culture from**

- Eye
- Ear
- Nose
- Stool
- Throat
- Wounds
- Strep screen
- Genital

Store and transport at room temperature or refrigerate. Do not refrigerate Genital Culture.

Non sterile container: for collection of sputum

Sterile containers: Body fluids (Except Blood and Urine)

**Acid-Fast Bacilli (AFB) Culture**

Respiratory secretions (sputum, bronchial washing, transtracheal aspirates, bronchoalveolar lavage, bronchial brushings), urine, stool: collect in a sterile leak-proof container. Ship to laboratory at ambient temperature.

Body fluids, CSF: collect in a sterile leak-proof container. Ship to laboratory at ambient temperature.

Whole blood: Collect 7-10 mL in SPS (yellow top) tube. Do not use yellow top ACD tubes. Send to the laboratory at ambient temperature.

Swab specimens are not acceptable.

**Anaerobe Culture**

Fluid aspirates: Submit in a capped syringe (without the needle). Send to the laboratory at ambient temperature.

Tissues: Submit in a sterile container with sterile saline. Send to the laboratory at ambient temperature.

Swabs: Collect on a swab with transport media. Send to the laboratory at ambient temperature.

If both aerobic and anaerobic cultures are needed from the same source, please submit two swabs: one for aerobes and one for anaerobes.
**Blood Culture**
Submit in blood culture bottles (adult or pediatric). For adults, inject at least 10 mL into each bottle (TSB & Thio/Columbia broth). For pediatrics, inject 2-5 mL into pediatric bottle (BHI broth). Wipe the venipuncture site with 70 percent alcohol, then apply two percent iodine to the site. Allow the site to dry 1-to-2 minutes. Before introducing the blood into the bottles, remove the caps from the bottle tops and wipe the stopper with two percent iodine. Allow to dry. Do not vent bottles before transporting to the lab. Send to lab at ambient temperature within 24 hours of collection.

**Body Fluid Culture**
Clean the body site with an iodophor prior to aspiration; appropriate sources include peritoneal, pericardial, synovial and cerebrospinal fluids. Aspirate fluids and promptly inject into a sterile container. Do not submit in EDTA or heparinized tubes. Send to lab at ambient temperature.

**Bordetella pertussis DFA**
Submit two heat-fixed slides from a nasopharyngeal swab. Submit slides in a slide holder at ambient temperature.

**Chlamydia Culture**
Collect an endocervical, male urethral, conjunctival or rectal mucosal specimen on a sterile swab. For endocervical specimens, remove excess mucus from the cervical os and surrounding mucosa using a sterile swab and discard the swab. Insert a second swab into the endocervical canal to obtain the specimen.

Immerse swabs into Bartels Flextrans medium immediately after collection. Send to the laboratory on a cold pack (2-8°C) within 24 hours of collection.

**Diphtheria Culture**
Submit a nasopharyngeal specimen on a culturette swab. Bronchial or nasopharyngeal secretions and transtracheal aspirates are also acceptable. Send to laboratory at ambient temperature within 24 hours of collection.

**Environmental Culture**
Submit specimen in a sterile container or in a regular Culturette Swab. Appropriate sources include water or swabs of surfaces. Send to the laboratory at ambient temperature.

**Fungus Culture**
Collect specimen in a sterile, leak-proof container or on a culturette swab; appropriate sources are respiratory tract fluids (sputum, bronchial washing, transtracheal aspirates, bronchoalveolar lavage, bronchial brushings), urine, CSF, exudates, abscess contents, vaginal material, skin, nails, hair or tissue.

Whole blood: collect 7-10 mL in SPS (yellow top) or heparin tube. Do not use yellow top ACD tubes. Send specimens to the laboratory at ambient temperature.
GC (Neisseria gonorrhoeae) Culture
Collect specimen on a regular culturette swab. Appropriate sources are cervical, rectal, throat, vaginal and penile. Send specimen to the laboratory at ambient temperature.

Genital Culture
Collect specimen on a culturette swab; appropriate sources are cervical, vaginal and penile. Send specimen to the laboratory at ambient temperature.

Mycoplasma pneumoniae Culture
Swabs: collect nasopharyngeal or throat specimen on a sterile swab. Do not use swabs with wooden-shafts. Place swab in transport vial containing tryptic soy broth (TSB). Send to the laboratory on a cold pack (2-8°C) within 24 hours of collection.

Respiratory specimens (sputum, washing, aspirate): collect specimen in a sterile container. Inject approximately one mL of specimen into a transport vial containing tryptic soy broth (TSB) with bovine albumin. Send to the laboratory on a cold pack (2-8°C) within 24 hours of collection.

Respiratory Culture
Collect sputum, bronchial washing or tracheal aspirate in a sterile, leak-proof container. Do not send in formalin. Send to the laboratory on a cold pack (2 to 8 degrees C). Submit 2-3 specimens on separate days to increase the probability of isolating a pathogen.

Ureaplasma / Mycoplasma Culture
Acceptable specimens are urethral, vaginal or cervical swabs, urine, abscess content, prostatic secretions, semen, tissue, and respiratory specimens (sputum, washing or aspirates).

Collect on a sterile swab or in a sterile container. Do not use swabs with wooden shafts. Place the swab or collected material into a transport vial containing tryptic soy broth (TSB) Send to the laboratory on a cold pack (2 to 8 degree C ) within 24 hours of collection.

For urine, collect in a sterile leak-proof container. Centrifuge urine at 2000 rpm and discard supernatant. Resuspend the pellet in a transport vial containing tryptic soy broth (TSB). with bovine albumin. Send to the laboratory on a cold pack (2-8°C) within 24 hours of collection.

Urine Cultures
Use a Urine Culture and Sensitivity Transport Kit (Vacutainer brand #4949) when submitting urine cultures. The transport medium is essential for the recovery of urinary tract pathogens.
1. Use a sterile urine container to obtain a clean-catch specimen (see instructions).
2. Fill a gray-top Urine Culture tube (see instructions to the right).
3. If a urinalysis is requested with the urine culture, submit a urine aliquot tube in addition to the culture tube.

4. Label all tubes with the patient’s name and date.

Patient Instructions: Clean-Catch Urine Specimen

<table>
<thead>
<tr>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Wash hands thoroughly with soap.</td>
<td>Wash hands thoroughly with soap.</td>
</tr>
<tr>
<td>Holding the labia apart, wash the entire area with an antiseptic towel, wiping from front to back.</td>
<td>Retract foreskin, if present. Completely wash and clean penis using an antiseptic towel.</td>
</tr>
<tr>
<td>3. Continue to spread the labia and start to urinate directly into the toilet.</td>
<td>Start to urinate directly into the toilet.</td>
</tr>
<tr>
<td>Stop and position the container. Begin urinating again into the container. Do not touch the container to the genital area.</td>
<td>Stop and position the container. Begin urinating again into the container. Do not touch the container to the genital area.</td>
</tr>
<tr>
<td>5. Do not fill the cup completely to the top</td>
<td>Do not fill the cup completely to the top</td>
</tr>
</tbody>
</table>

Instructions for Urine Culture and Sensitivity Transport Kit

1. Submerge the tip of the transfer device to the bottom of the collection cup.
2. Place the evacuated tube all the way into the holder portion of the transfer device.
3. Hold in position until urine stops flowing into the tube.
4. Remove tube from transfer device and set aside.
5. Lift transfer device, allowing urine to drain from the tip. Put the device in the pouch and discard.

Vibrio Culture
Place fresh stool in a stool culture transport vial until the level of fluid reaches the “fill” line. Send to the laboratory at ambient temperature. Specimen may also be submitted in a sterile container on a cold pack (2-8°C). Do not submit 0 & P vials, frozen stool or stool that contains barium.

Virus Culture (HSV, CMV, etc.)
Blood: collect 8 mL whole blood in a heparin tube.

Fluids: collect 2-10 mL of fluid into a sterile container with no viral transport medium.
**Tissue:** collect in a sterile container with viral transport medium.

**Swabs:** collect on a sterile swab with Bartels Flextrans transport medium.

Send specimens to the laboratory on a cold Pack (2 to 8 degrees C) within 24 hours of collection.

**Requirements for Fecal Tests.**

**Clostridium difficile Toxin**
Collect 1-10 g (mL) fresh stool with no preservative in a sterile, leak-proof plastic container. Freeze immediately. The specimen must be stored frozen throughout the shipping process. Specimens collected in formalin or PVA or on swabs are not acceptable.

**Cryptosporidium Stain**
Submit fresh stool in a sterile container (2 to 8 degrees C) or in a 10 percent formalin vial from an 0 & P transport kit (ambient temperature). Do not submit stool that contains barium or stool in PVA or MIF preservative.

**Fecal Fat, Qualitative**
Submit fresh stool in a dry, sterile container. Send to the laboratory at ambient temperature. Do not submit 24-, 48- or 72-hour collections or stool specimens containing barium.

**Fecal Leukocytes**
Submit fresh stool in a dry, sterile container or in a PVA vial from an 0 & P transport kit. Send to the laboratory at ambient temperature. Do not submit stool in MIF preservative or stool specimens containing barium.

**Fecal Meat Fibers**
Submit fresh stool in a dry, sterile container. Send to the laboratory at ambient temperature. Do not submit swab or stool specimens containing barium.

**Fecal Reducing Substances**
Submit fresh stool in a clean, dry container with no preservatives. Send to the laboratory at ambient temperature.

**Isospora Stain**
Submit fresh stool in a sterile container (2 to 8 degrees C) or in a 10% formalin vial from an 0 & P transport kit (ambient temperature). Do not submit stool that contains barium or stool in PVA or MIF preservative.
Occult Blood Stool
Submit a Seracult card at ambient temperature. Follow the instructions provided in the Seracult Kit.

Ova & Parasites
Follow the instructions provided in the 0 & P transport kit (ParaPack Kit). Submit both the 10 percent formalin and PVA vials to the laboratory at ambient temperature. Indicate consistency of stool and suspected parasite. Do not submit a stool culture transport vial or stool that contains barium. If a series of three specimens is indicated, they should be submitted on separate days (preferably every other day). Should not be refrigerated for more than three hours without proper fixation.

Cytology Directions

Relevant clinical information such as L.M.P, prior diagnosis, etc should be noted in each space provided.

FNA
A high percentage of smears are difficult and sometimes difficult to accurately diagnose. This difficulty is due to poorly preserved cellular material or a lack of adequate cellular material. Please follow the standard techniques for aspiration and slide preparation for cellular material and send them to Princeton Biomedical laboratories.

Routine Cervical smear
1. Do not use lubricating Gel
2. Do not use Q-tip to obtain endocervical cells use endocervical brush.
3. Do not obtain during menstruation
4. Obtain a direct smear of cervix preferably at the junction between exocervix and endocervix.
5. In order to comply with the standards established by Bethesda system, following guidelines will be followed

The pap smear will be reported as unsatisfactory with the following conditions.
   a. Smears unlabelled
   b. Scant cellularity
   c. Poor fixation or preservation
   d. Slide broken beyond repair.

**Thin Prep**
1. Obtain an adequate sampling from the cervix using broom like device.
2. Rinse the broom into the PreserveCyt. Solution vial by pushing the broom into the bottom of the vial 10 times forcing the bristles a part. As final step, swirl the broom vigorously to further release material.
3. Tighten the cap
4. Record the patient name and date and ID on the vial.
5. Send it to PBL in a specimen bag at room temperature.

**Urine for Cytology**
1. Specimen can be randomly collected anytime.
2. Female patients should be instructed to wash their genitalia with soap and water prior to collection.
3. Void directly into the container with 50% alcohol.
4. Send immediately to lab in securely closed container.
Miscellaneous Tests requirements

GC/Chlamydia Amplified DNA analysis
GC/Chlamydia by DNA probe can be performed using either urine samples or male urethral or endocervical swabs.
Specimen collection swabs in the form of endocervical and male urethral specimen swab collection kit are supplied by the laboratory.

Endocervical swab collection procedure
1. Remove excess mucus from the cervical OS with large tipped cleaning swab provided in the kit and discard.
2. Insert the endocervical collection and dry transport swab into the cervix canal and rotate for 15—30 seconds.
3. Withdraw the swab carefully and place the swab into transport tube and label the tube.

Male urethral swab collection procedure
1. Insert the male urethral collection swab 2-4 cm into the urethra and rotate 3-5 seconds.
2. Withdraw the swab and place it into the transport tube.

Urine samples for Amplified DNA analysis
1. Patient should not have urinated at least one hour prior to collection.
2. Collect specimen in a sterile, plastic, preservative free specimen collection cup.
3. The patient should collect the first 15-29 ml of voided urine.

After the collection the specimens can stored and transported to the laboratory at 2-27°C within 4-6 days.

Gram Stain
Carefully collect material from the infected area on a sterile swab or in a sterile container. Send to the laboratory at ambient temperature.

KOH Prep
Collect nail or hair clippings or skin scrapings in a dry, sterile container. Send to the laboratory at ambient temperature.

Malaria Smear
Submit two smears, one thin and one thick, from blood drawn in an EDTA tube. Prepare the smears within one hour of collection. Submission of the original EDTA tube is recommended.
Prepare the thin smear as you would for a differential WBC count; visually, this film should be rounded, feathered, and progressively thinner toward the middle of the slide. Allow the slide to air dry.
The thick smear should be round to oval and approximately two cm across; you should barely be able to read newsprint through the wet or dry film. Allow the slide to air dry. Do not place in a fixative.
If the smears cannot be prepared, submit the EDTA tube to the laboratory at ambient temperature as soon as possible.

**Pinworm Prep**
Collect specimen on a pinworm paddle before patient arises and prior to defecation or bathing. Pat the perinatal area with the sticky side of the clear plastic paddle. Do not insert the paddle into the anus. Do not cover the paddle with stool. Replace the paddle into the tube and send to the laboratory at ambient temperature.

**Strep Screen (Rapid)**
Run a dry swab firmly over the back of the throat, both tonsils or tonsillar fossa and any inflamed area; avoid touching the cheeks and tongue. Send to the laboratory at ambient temperature.
If a culture is required when the Strep screen is negative, submit an additional charcoal swab at ambient temperature.

**Erythrocyte Sedimentation Rate (ESR)**
Whole blood is viable for up to 24 hours. If the laboratory will not receive the specimen within 24 hours.

**Prothrombin Time (PT) or Partial Thromboplastin Time (PTT)**
Draw blue top tube at least 2/3 full, If specimen will not be delivered to the lab within an acceptable time frame (48 hours for PT, 24 hours for PTT), centrifuge specimen at 1100 RCF for 15 minutes, separate plasma from cells and freeze plasma. Ship with dry ice