LABSHOT

PROCEDURE MANUAL

2024 2025

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General Laboratory Information

THE RESPONSIBILITY OF THE LABORATORY PERSONNEL

The laboratory worker is responsible to perform laboratory tests and procedures according to their own capabilities and those of the faculty which they are working. The tests should be precise and accurate to provide the physician with proper information in order to benefit the patients. The laboratory personnel, like the doctor, must regard the information gained from the course of their daily work, as strictly confidential. Only the doctor or his/her authorized representative should receive the test results, this includes the patient themselves.

LABORATORY RULES

1. Always use the proper test methods; do not make up your own.
2. Refer to the manual for procedure questions.
3. Inform co-workers when you don't know something.
4. When in doubt, ask for second opinion.
5. Always confirm irregular results (repeat the test if necessary).
6. Always maintain your equipment.
7. Learn to work quickly and accurately.
8. Keep your work area clean and free from contaminants.
9. Keep your registers neat and simple.
10. Always follow manufacturer's instructions when using equipments, reagents, etc.
11. All reagents should be clearly labeled.
12. When making new reagents, labels should include:
    - Name of reagent
    - Date made
    - Expiration date
    - Your initials
13. Do not disregard expiration dates.
14. ALWAYS wear proper personal protective equipment.
15. Do not eat inside the laboratory.

SAFETY RULES

1. Always add acid to water, slowly.
2. Keep acids and alkalis on the lower shelves of cupboard. Always hold the bottle upright when removing or storing. Your hands should always be dry.
3. Never pipette by mouth.
4. Never heat the bottom of the test tube. Heat the middle of the tube, shaking gently with the mouth of the tube pointed away from you.
5. Never place inflammable liquids near an open flame.
6. Always light the match and hold it to the burner before turning on the gas.
7. Always turn the gas off at the end of the workday or when you are leaving the area for a long period of time.
8. Wash your hands after drawing blood or handling specimens.
9. Be familiar with first aid practices for laboratory accidents.
10. Know where first aid supplies are stored.
11. Label all hazardous chemicals.
12. Work cautiously around an open flame.
13. Always dispose laboratory wastes such as specimen containers, used needles and lancets, gauze, etc., in their proper waste receptacles.
14. Never drink in the laboratory.
15. Keep all chemicals out of reach for children.

FIRST AID FOR THE LABORATORY

Most often, accidents in the laboratory are caused by carelessness, rushing, and untrained personnel. Items that incur injuries are:

- Acids
- Alkalis
- Toxic substances, inhaling toxic fumes, or accidental swallowing by pipetting.
- Heat
- Broken glass
- Electricity

To minimize injury, the following items should always be found in the laboratory.

- Wash bottles containing clean water.
- Fire blanket
- Tincture of iodine
- Adhesive bandages
- Cotton wool and gauze.

These items should readily be available. D**O NOT** lock in a cupboard.

Refer to physician in charge for first aid measures to employ to minimize the damage caused by the accident.

LABELING AND LABORATORY RECORDS

All specimens that come to the lab must have a request form and be given a number or some other form of identification.

- Numbering of the specimens. NOTE: This should be done immediately.
    a. On the specimen
    b. On the request form
    c. On the slide
    d. Any test tubes that will be used.
- Record all results in the appropriate register.

CLEANING GLASSWARES

- Rinse all used glasswares in cold or lukewarm water before residue dries in the glass. Never rinse blood stained tubes in hot water.
- Soak glassware in water mixed with soap (wash powder or liquid detergent) for 2-3 hours. Be sure to clean the insides with a test tube brush.
- Remove individually and rinse thoroughly with tap water. Make sure no soap residue is left that will interfere with testing.
- Place containers on a draining rack to drip dry. Place tubes upside down in a wire basket. Place the basket in a hot air oven at 60 degrees celsius or cover with a clean cloth and place in a sunny place to dry.
- All clean glasswares should be stored in a cupboard to protect it from dust. If possible all beakers and flask tops should be plugged or covered.
- For pipettes:
    - Rinse immediately after use in a stream of cold water.
    - Soak in soapy water.
    - Rinse with tap water to remove soap and then rinse with acetone and blow dry.

DISPOSAL OF SPECIMENS

All specimens that are brought to the lab should be considered infectious. Specimens may be:

a) Burned (incinerated)
b) Buried
c) Boiled

Incineration is the easier and most effective method. Here are the steps:

1. Use and old metal drum with removable lid.
2. Fix a metal grate about 1/3 the way up to the drum.
3. Cut a vent in the drum below the grate.

The incinerator should be closed (lid and vent) when not in use.

Burial:

- Dig a pit 4-5 meters deep and 1-2 meters wide.
- Make a lid that fits tightly over the pit.
- Strengthen the upper rim using bricks or stones.
- Throw the infected material into the pit and replace the lid.
- At least once per week, cover the refuse with a layer of dried leaves or quicklime.

NON-DISPOSABLE CONTAINERS

Stool

- Fill the containers with 5% phenol.
- Leave for 24 hours.
- Empty into the lavatory (toilet).
- Clean with soap and water.

Sputum

- Pour into each container 10ml of 10% formaline solution or 5ml of 5% phenol.
- Leave for 24 hours.
- Boil in detergent.
- Keep a pan only for this purpose.
- Boil for 30 minutes.
- Water should be a strong solution of detergent.

Urine

- Empty bottles in the lavatory.
- Fill with 10% solution of bleach.
- Leave for 24 hours.

NOTE: Disposable containers require less work and are easier to get rid of.

BACTERIAL CULTURE FOR SENSITIVITY TESTING

For Microbiology Section

PRINCIPLE OF BACTERIAL CULTURE FOR SENSITIVITY TESTING

A bacterial culture is a test that identifies the type of bacteria or fungus causing an infection. A sample of blood, urine, stool, skin, mucus, or spinal fluid is sent to a lab, where the bacteria or fungus is grown in a solution and spread onto a culture plate. The type of germ is identified using a microscope or chemical tests.A sensitivity test, also known as an Antimicrobial Susceptibility Test (AST), this test determines which medicine, such as an antibiotic, will work best to treat the infection. Culture and sensitivity testing is done to help diagnose an infection. It may also help your health care provider decide which medicines to use in treating your infection.

SPECIMEN REQUIREMENTS

Types of Specimen

A culture can be performed on a variety of samples from the body, such as:

- Blood
- Urine
- Lung secretions (sputum)
- Wound, skin, and soft tissue
- Genital tract
- Throat and nares (nostrils)

Sample Collection Procedure

- Samples should be collected from the suspected infection site before antibiotic therapy begins, as prior treatment can suppress bacterial growth and affect the results.
- Proper hand hygiene and the use of sterile equipment, such as swabs, syringes, or collection containers, are essential to prevent contamination.
- The sample should be of sufficient quantity and handled appropriately, depending on the type of specimen (e.g., blood, urine, sputum, or wound exudate).

Handling and Storage Requirements/ Transport

- Samples should be transported to the laboratory as soon as possible, ideally within 2 hours of collection, to prevent degradation or overgrowth of non-target organisms.
- If immediate transport is not possible, samples should be stored at an appropriate temperature: swabs for aerobic culture should be kept at room temperature, while samples requiring anaerobic culture should be stored in an anaerobic transport medium and kept refrigerated.
- Certain samples, such as urine or sputum, should be refrigerated at 4°C if delayed in transport, while blood cultures must be kept at room temperature in sterile, well-sealed containers to avoid contamination.
- For prolonged delays (more than 24 hours), transport media like Amies or Stuart medium are often used to preserve the sample's integrity.

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

Reagents/ Media

1. Culture Media:

- Nutrient Agar
- Blood Agar
- MacConkey Agar: Sabouraud Agar
- Mueller-Hinton Agar

2. Selective Media:

- CNA Agar (Colistin-Nalidixic Acid Agar)
- Eosin Methylene Blue (EMB) Agar

3. Anaerobic Media:

- Thioglycollate Broth
- Anaerobic Transport Medium

4. Biochemical Reagents:

- Catalase Reagent
- Oxidase Reagent
- Coagulase Reagent
- Antibiotics including amoxicillin, ciprofloxacin, and gentamicin.

Supplies

- Sterile Swabs
- Sterile Containers
- Transport Media
- Requisition Forms

Equipment

- Incubators
- Autoclave
- Laminar Flow Hoods
- Centrifuge
- Microscope
- Bunsen Burner
- Petri Dishes and Inoculation Loops
- Sensitivity Testing Equipment:
  - Antibiotic Disks
  - Automated Systems

CALIBRATION

Incubator Calibration:

- Use a certified thermometer to measure the internal temperature.
- Compare the observed temperature with the set value.
- Adjust the incubator settings if there is a discrepancy.

Spectrophotometer Calibration

- Calibrate the spectrophotometer using a blank (e.g., sterile saline or water).
- Measure the absorbance of a standard McFarland solution (e.g., 0.5 McFarland corresponds to an optical density of 0.08–0.1 at 600 nm).

Automated System Calibration

- Perform regular quality control using reference strains with known susceptibility patterns (e.g., *E. coli* ATCC 25922).
- Check machine alerts for calibration needs and follow manufacturer guidelines.

QUALITY CONTROL

PRE-ASSAY

- Use sterile equipment to avoid contamination.
- Verify that the culture media is correctly prepared and sterilized. Pre-test media for sterility and expected bacterial growth by using control strains.
- Ensure the potency and proper storage of antibiotic discs. Use quality control strains to test the effectiveness of each antibiotic disc batch.
- Ensure incubators, autoclaves, and other essential equipment are calibrated and functioning properly before testing. This includes confirming the incubation temperature is 37°C for optimal bacterial growth.

INTERNAL CONTROLS

Control Strains:

- Use known bacterial strains with well-established susceptibility profiles (e.g., *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923) during each testing procedure to validate the performance of both culture media and antibiotic discs.

- Control strains should show expected susceptibility or resistance to the selected antibiotics, ensuring the accuracy of the susceptibility testing.

Growth and Inoculation:

- Monitor bacterial growth at each stage (e.g., after incubation). Ensure there is visible bacterial growth on the agar and check that the zones of inhibition are clearly defined and reproducible.

Antimicrobial Testing:

- Use appropriate quality control strains to confirm the antibiotic susceptibility results. Measure zones of inhibition or MIC values against known control data for each antibiotic tested.

POST-ASSAY

Interpretation of Results:

- Measure the zones of inhibition accurately for disk diffusion methods. Compare the results to standardized tables (e.g., CLSI or EUCAST guidelines) to determine susceptibility (S), intermediate (I), or resistance (R).

- After performing broth dilution, confirm the MIC value and compare it with established breakpoints for clinical interpretation.

Reporting and Documentation:

- Ensure that all findings are documented accurately in patient records, including the species identified and its antimicrobial susceptibility profile.

- Report the results to the attending physician with clear recommendations for treatment, including a list of effective antibiotics based on susceptibility.

Post-Assay Troubleshooting:

- Review and investigate any unexpected results or discrepancies, such as no bacterial growth, unusual antibiotic resistance patterns, or inconsistent control strain results.

- Re-test or repeat assays if any post-assay checks reveal issues that may have impacted the accuracy of the results.

STEP-BY-STEP INSTRUCTIONS

1. Collect a representative specimen from the patient using sterile swabs, needles, or containers, depending on the type of sample (e.g., urine, blood, sputum, wound swabs). Properly label the sample with patient details and transport it promptly to the laboratory in appropriate transport media to maintain bacterial viability.

2. Prepare suitable culture media, selecting specific media such as Nutrient agar, MacConkey agar, or Blood agar, based on the suspected pathogen. Sterilize the media using an autoclave, then pour it into sterile Petri dishes to solidify before use.

3. Inoculate the prepared media with the sample using aseptic techniques to prevent contamination. Use an inoculating loop to streak the sample onto the agar surface or inoculate liquid media, and then incubate the cultures at 37°C for 18-48 hours.

4. Examine the bacterial growth on the agar plates, noting colony morphology and any hemolysis patterns on Blood agar. Perform Gram staining to differentiate between Gram-positive and Gram-negative bacteria, followed by biochemical tests (such as catalase or oxidase) to identify the bacterial species.

5. Conduct antimicrobial susceptibility testing using either the disk diffusion method or broth dilution method. For disk diffusion, inoculate the plate with the bacterial suspension, place antibiotic discs, and measure the zones of inhibition after incubation. In the broth dilution method, determine the Minimum Inhibitory Concentration (MIC) by testing various antibiotic concentrations.

6. Interpret the susceptibility results by comparing the zones of inhibition from disk diffusion or the MIC values from broth dilution with standard reference values.

Classify the bacteria as susceptible, resistant, or intermediate to each antibiotic tested.

7. Prepare and report the findings, including the bacterial species identified and the susceptibility profile to guide appropriate antimicrobial therapy. The report should include recommendations for treatment based on the susceptibility testing results.

REPORTING RESULTS

- Verify Results
- Document Findings
- Communicate with Supervisors or Specialists
- Prepare a Comprehensive Report: Include the following in the report:
  - Details of the specimen (type, source, and collection method).
  - Identified organism(s) and their clinical significance.
  - Antibiotic susceptibility results categorized as susceptible, intermediate, or resistant.
  - Any critical findings (e.g., ESBL or carbapenemase production).
- Maintain Records

Component	**Reference Range**
Culture Results	Positive: Growth of pathogenic bacteria. Negative: No growth observed after incubation.
Quantitative Results	- Blood culture: Any growth is significant. - Urine culture: ≥100,000 CFU/mL indicates significant bacteriuria. - Sputum culture: Pathogen count varies based on clinical

	context and contaminant.
Antibiotic Sensitivity	- **S (Susceptible)**: Likely effective in treatment. - **I (Intermediate)**: May require higher dose or not fully reliable. - **R (Resistant)**: Unlikely to respond to therapy.
Zone of Inhibition (Disk Test)	Ranges depend on antibiotic-organism pair (e.g., *S. aureus* & Penicillin: ≥29 mm).
MIC (Minimum Inhibitory Concentration)	Breakpoints based on CLSI or EUCAST guidelines vary by organism and antibiotic.
Resistance Mechanisms	- Detected: Indicates specific resistance (e.g., ESBLs, carbapenemases). - Not detected: No evidence of resistance mechanisms identified.

LIMITATION OF METHODS

- Non-Culturable Bacteria
- False-Negative Results
- Contamination Risk

- Limited Spectrum of Antibiotics Tested

TROUBLESHOOTING

1. Problem: No Growth on Culture Media

Troubleshooting Steps:

- Verify that the sample was collected properly and transported under the correct conditions (e.g., using a sterile container and maintaining appropriate temperature).
- Ensure that the incubator is functioning correctly and set to the appropriate temperature (e.g., 35–37°C for most bacteria).
- Use fresh, correctly prepared, and quality-assured media that matches the suspected organism's growth requirements.

2. ***Problem: Contaminated Culture***

Troubleshooting Steps:

- Reinforce the use of strict aseptic techniques during all stages of testing.
- Ensure proper sterilization of tools, work surfaces, and media.
- Evaluate the sample collection process and train staff to minimize contamination (e.g., proper disinfection of the skin before sample collection).

3. Problem: Unexpected Antibiotic Susceptibility Results

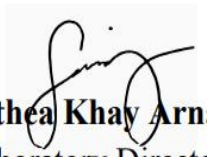
Troubleshooting Steps:

- Check the storage conditions and expiry dates of antibiotic discs, and ensure they were applied correctly on the agar plate.
- Repeat the test using a control strain (e.g., *E. coli* ATCC 25922) to validate the results.

- Re-measure the zones of inhibition or MIC values and compare them to CLSI or EUCAST guidelines.

EFFECTIVE DATE

October 6, 2024



Althea Khay Arnaiz
Laboratory Director

BIOPSY EXAMINATION

For Histopathology Section

PRINCIPLE OF BIOPSY EXAMINATION

The principle of biopsy processing is to preserve and visualize tissue morphology, enabling detailed microscopic examination. This involves steps of fixation, embedding, sectioning, and staining to prepare tissue samples for evaluation.

- **Fixation**: Prevents tissue degradation by stabilizing proteins and cellular structures. Formalin is the most widely used fixative, as it maintains tissue morphology while preserving nucleic acids and proteins.
- **Dehydration and Clearing**: Tissue water is replaced with ethanol, and subsequently, xylene to make tissue compatible with embedding media.
- **Embedding**: Tissue is infiltrated with paraffin to create a stable block for sectioning. Proper orientation during this step ensures diagnostically relevant structures are sectioned.
- **Sectioning**: Thin sections (typically 3–5 µm) are cut to allow light to pass through, which is essential for detailed microscopic visualization.

Role of Staining:

Staining transforms otherwise translucent tissues into structures with distinct contrasts. **H&E** is the gold standard for general pathology:

- **Hematoxylin**: Highlights nuclei and chromatin.
- **Eosin**: Stains cytoplasmic components, collagen, and extracellular matrix

SPECIMEN REQUIREMENTS

Sample Collection:

- Tissue should be collected aseptically and handled with care to avoid crushing or tearing, which can introduce artifacts during processing .
- Place in **10% neutral buffered formalin** immediately, using a volume of at least 20 times the tissue size to ensure proper penetration and fixation .
- Tissue thickness: ≤ 5 mm to enable adequate fixative penetration and prevent autolysis .

Labeling:

- Ensure proper labeling with identifiers such as the patient's name, collection date, and type of specimen to avoid misdiagnosis.

Storage:

- Store fixed tissue at room temperature. Avoid freezing unless required for specific tests like immunohistochemistry, which benefits from fresh or frozen tissue.

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

Reagents

1. 10% Neutral Buffered Formalin
2. Xylene
3. Graded Ethanol Series (70%, 95%, and Absolute)
4. Paraffin Wax
5. Hematoxylin
6. Eosin
7. Mounting Media (e.g., Permount)

Supplies

1. Tissue Cassettes
2. Glass Slides and Coverslips
3. Filter Papers

Equipment

1. Tissue Processor
2. Microtome
3. Water Bath
4. Hotplate
5. Microscope

CALIBRATION

Manual Calculations

- **Stain Preparation**: Prepare correct dilutions for stains like hematoxylin or eosin. For example, a 1% eosin solution requires dissolving 1 g of dye in 100 mL of distilled water.
- **Dehydration Cycles**: Calculate the duration for each step in tissue dehydration using ethanol concentrations (e.g., 70%, 95%, absolute).

Machine Calibration

1. **Microtome Calibration**:
    - Verify section thickness adjustments, typically 3–5 µm for routine samples. Perform daily alignment checks to avoid chatter or uneven sections.
    - Confirm the quality of sections using reference slides.
2. **Tissue Processor Calibration**:
    - Check reagent levels and ensure accurate timing for fixation, dehydration, clearing, and paraffin infiltration. Replace reagents regularly to prevent contamination.
3. **Microscope Calibration**:
    - Validate magnification levels with a stage micrometer. Ensure consistent illumination for accurate observation across samples.
4. **Temperature-Controlled Equipment**:
    - Regularly check water baths and ovens for stable operation. Maintain water bath temperatures at 45–50°C for optimal section flattening.

QUALITY CONTROL

A. Pre-Analytical Quality Control

1. **Sample Integrity**:
    a. Ensure samples are promptly fixed in 10% neutral buffered formalin to prevent degradation. Tissue thickness should not exceed 5 mm for effective fixative penetration.

b. Verify labeling for accuracy, including patient identifiers and date.

2. **Reagent and Equipment Preparation**:
    a. Inspect reagents for contamination or degradation before use. Replace expired or compromised reagents.
    b. Perform equipment checks for tissue processors, embedding stations, and microtomes. Address malfunctions immediately to minimize disruptions.

B. Analytical Quality Control

1. **Control Slides**:
    - Incorporate positive and negative control slides with every batch of staining to validate reagent efficacy and protocol adherence.
    - Use known control tissues to confirm staining accuracy, especially for specialized stains like periodic acid-Schiff or trichrome.
2. **Slide Evaluation**:
    - Examine slides for artifacts, such as air bubbles, wrinkles, or uneven staining, and address procedural issues if found.
    - Implement dual-pathologist review for diagnostic consistency in complex cases.

C. Post-Analytical Quality Control

1. **Result Verification**:
    - Ensure results are reviewed and signed by qualified personnel. Investigate and document any inconsistencies or errors.
    - Employ pathologist oversight to confirm diagnostic findings before issuing reports.
2. **Documentation and Review**:
    - Maintain comprehensive logs of quality control measures, including calibration, reagent lot numbers, and control slide results.

- Conduct periodic audits of laboratory practices to ensure compliance with internal and external standards.

STEP-BY-STEP INSTRUCTIONS

1. **Specimen Collection and Fixation**

- Collect tissue samples aseptically and immediately immerse in 10% neutral buffered formalin to preserve morphology.
- Ensure no delays in transportation to the laboratory, as this can compromise tissue integrity.

2. **Grossing**

- Trim the specimen into thin slices (3–4 mm thick) to optimize fixative penetration.
- Ensure accurate labeling and avoid contamination by cleaning work surfaces between specimens.
- Use appropriate cassettes for sample type and avoid overloading to ensure proper reagent access during processing.

3. **Processing**

- **Dehydration**: Place tissues in ascending ethanol concentrations (e.g., 70%, 95%, absolute) to remove water.
- **Clearing**: Immerse in xylene to remove alcohol and prepare tissues for infiltration.
- **Embedding**: Use molten paraffin to encase tissues, ensuring correct orientation for diagnostic sections.

4. **Sectioning**

- Cut paraffin blocks into 3–5 µm thick sections using a calibrated microtome.
- Float sections in a warm water bath to flatten them before mounting on glass slides.

- Avoid wrinkles and folds, as these artifacts can obscure microscopic evaluation.

5. **Staining (Hematoxylin and Eosin)**

- **Deparaffinization**: Immerse slides in xylene to remove wax.
- **Hydration**: Pass slides through descending ethanol concentrations and rinse in water.
- **Hematoxylin Application**: Stain nuclei to a deep blue color.
- **Bluing**: Treat with a weak alkaline solution to stabilize the hematoxylin stain.
- **Eosin Application**: Counterstain cytoplasmic components pink.
- **Dehydration and Clearing**: Pass slides through ethanol and xylene again before coverslipping.

6. **Microscopic Examination**

Examine slides under a microscope to assess staining quality and identify morphological abnormalities. Ensure no air bubbles or mounting artifacts are present.

REPORTING RESULTS

Parameter	Reference Range	Notes
Staining		
Hematoxylin	Nuclei should stain a deep, clear blue, with no overstaining in cytoplasmic areas.	Ensures clarity and contrast in nuclear features for diagnostics.
Eosin	Cytoplasm and connective tissues should appear pink to orange-pink, with sufficient contrast from nuclei.	Provides a balanced counterstain for cellular components, aiding visualization of structural details.
Section Thickness		

Routine paraffin sections	3–5 µm for most tissue types.	Standard thickness ensures optimal visualization under light microscopy.
Special cases	≤1 µm for electron microscopy.	Ultra-thin sections allow for detailed subcellular imaging.
Fixation Time	Minimum of 6–24 hours in 10% neutral buffered formalin.	Prevents tissue autolysis and maintains cellular integrity.
	Longer fixation times (>48 hours) may cause hardening, impacting sectioning quality.	Overfixation can render tissues brittle and difficult to process.
Processing Parameters	Adequate dehydration, clearing, and infiltration are critical.	Proper processing prevents artifacts like brittleness or incomplete infiltration.
	Overprocessing (e.g., prolonged clearing in xylene) can cause brittleness, while underprocessing may result in poor infiltration and artifacts.	Ensures tissue sections maintain structural integrity and are free of preparation defects.
Artifact-Free Slides	Sections must be free of folds, wrinkles, chatter, or air bubbles.	These issues compromise diagnostic clarity by obscuring tissue structures.
Control Slide Evaluation	Positive control slides should demonstrate consistent staining quality and morphology.	Example: A liver control slide should exhibit well-defined nuclei and uniform eosin staining across hepatocytes.
Expected Morphological Features		
Normal Tissue	Preserved architecture with	Indicates optimal sample

	sharp nuclear and cytoplasmic contrast.	preparation and absence of pathological changes.
Abnormalities	Distorted tissue structures, uneven staining, or cell abnormalities.	These findings may suggest disease processes or preparation errors, requiring further investigation.

LIMITATION OF METHODS

Tissue Artifacts:

- Poor fixation or delayed processing can result in tissue degradation, autolysis, or drying artifacts. These compromise the clarity and integrity of tissue morphology.

Over-/Under-staining:

- Variations in staining protocols, reagent preparation, or exposure times can lead to uneven staining, reducing diagnostic accuracy.

Sampling Errors:

- A poorly collected or misrepresented tissue sample might not be representative of the lesion, leading to a potential misdiagnosis.

Equipment Limitations:

- Malfunctioning microtomes, improper sectioning techniques, or calibration issues can cause chatter or tears in tissue sections.

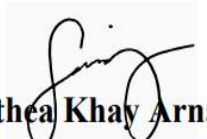
TROUBLESHOOTING

Problem	Cause	Solution
Uneven or poor	Stain degradation, incorrect pH,	Regularly replace reagents,

staining results	or insufficient reagent exposure time.	calibrate pH of hematoxylin solutions, and standardize staining durations.
Chatter marks on tissue sections	Dull or misaligned microtome blade, or over-hardened paraffin blocks.	Replace or realign microtome blade, and ensure blocks are properly chilled before sectioning.
Wrinkled or folded tissue sections	Improper water bath temperature or incorrect handling during mounting.	Maintain water bath at 45–50°C, and carefully transfer sections onto slides to avoid folds.
Air bubbles under the coverslip	Insufficient dehydration or clearing before coverslipping.	Ensure complete dehydration and clearing; avoid introducing bubbles during coverslip application.
Faded or uneven staining intensity	Contaminated or old staining reagents.	Replace staining reagents and ensure proper staining protocols are followed consistently.

EFFECTIVE DATE

October 6, 2024



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Laboratory Director

COMPLETE BLOOD COUNT (CBC)

For Hematology Section

PRINCIPLE OF COMPLETE BLOOD COUNT (CBC)

The CBC assesses the quantity and quality of red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), hematocrit (Hct), and platelets. It is performed using an automated hematology analyzer or manual microscopy for differential count. This test evaluates overall blood health and identifies conditions like anemia, infections, and hematological disorders.

SPECIMEN REQUIREMENTS

- **Type of Sample:** Venous Blood
- **Collection Tube:** EDTA (lavender-top) tube
- **Volume Requirements:**
    - **Adults:** 2 ml
    - **Children:** 0.5 ml
- **Handling and Storage:**
    - Mix gently by inversion (8-10 times)
    - Analyze within 6 hours; store at 2–8°c for up to 24 hours if necessary

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENTS

- **Reagents/Media:** Control materials for quality assurance, Wright's stain (if manual smear is needed).
- **Supplies:** Microscope slides, EDTA tubes, biohazard disposal containers.
- **Equipment:**
    - Automated hematology analyzer.
    - Microscope for manual smear examination.
    - Personal Protective Equipment (PPE).

CALIBRATION

Calculation (if Manual)

For manual hematocrit:

$$hematocrit\ (\%) = \left(\frac{height\ of\ packed\ red\ cells}{total\ height\ of\ blood\ column}\right) \times 100$$

Calibration

- Perform daily calibration using manufacturer-recommended calibration reagents

- Document calibration results in the maintenance log

QUALITY CONTROL

1. Run low, normal, and high control samples at the start of each shift
2. Confirm QC values fall within acceptable ranges
3. Investigate and resolve out-of-control results before processing patient samples

STEP-BY-STEP INSTRUCTIONS

Pre-Analytical Phase:

1. Verify patient identification and requisition details
2. Collect venous blood into an EDTA tube following aseptic techniques
3. Label and inspect the sample for clots or hemolysis

Analytical Phase:

1. Automated Analysis:

- Load the EDTA tube into the hematology analyzer
- Select the CBC test protocol
- Review flagged results for errors or abnormal findings

2. Manual Differential (if required):

- Prepare a thin blood smear on a glass slide
- Stain using Wright's stain
- Examine under a microscope for cell morphology and count

Post-Analytical Phase:

1. Validate the results against QC data and investigate discrepancies
2. Document results in the Laboratory Information System (LIS)
3. Notify the physician of critical values

REPORTING RESULTS

Reference Ranges:

- RBC: 4.2–6.1 x 10^6/µL
- WBC: 4,000–11,000/µL
- Hb: 12–16 g/dL (females), 14–18 g/dL (males)

- Hct: 37–47% (females), 42–52% (males)
- Platelets: 150,000–400,000/µL

LIMITATIONS OF METHODS

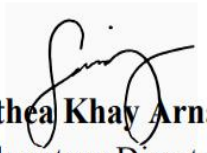
- Hemolysis or clotted samples may yield inaccurate results.
- Severe leukocytosis may falsely elevate hemoglobin or hematocrit readings.
- Improper calibration affects analyzer accuracy

TROUBLESHOOTING

- **Problem**: Analyzer flags inconsistent results.
  - **Solution:** Recalibrate and rerun the sample.
- **Problem**: Clotted blood sample.
  - **Solution:** Recollect the sample using proper mixing techniques.
- **Problem:** Hemoglobin interference due to lipemia.
  - **Solution**: Perform a plasma blank to correct interference.

EFFECTIVE DATE

October 6, 2024



Althea Khay Arnaiz
Laboratory Director

CROSSMATCHING

For Blood Banking Section

PRINCIPLE OF CROSSMATCHING

Crossmatching in blood banking is a critical procedure that ensures compatibility and safe transfusion between the donor's blood and the recipient. The main reason for this procedure is to prevent transfusion reactions from happening inside the recipient's system, which is usually caused by the body's immune system reacting against the donated blood.

A. Compatibility of Blood

- First, the blood group (ABO, Rh) of both the donor and recipient is confirmed. This ensures that the antibodies of the donor blood will not react against the recipient's red blood cells.

B. Screening for Antibodies

- The recipient's serum is tested for unexpected antibodies that may react with antigen's present on the donor's RBCs. Such antibodies may arise due to pregnancy, past transfusions, or exposure to foreign blood antigens.

C. Types of Crossmatching: Major and Minor Crossmatching

- *Major Crossmatch*: Involves testing the donor's RBCs with the recipient's plasma/serum to detect antibodies in the recipient's serum that could react with the antigen on the donor's RBCs.
- Types of Major Crossmatching:
    - *Immediate Spin Crossmatch (ISX):* Usually done for routine blood transfusions wherein there is a known ABO and Rh compatibility. This is a quick test involving the donor's RBC with the recipient's plasma or serum to see for agglutination or hemolysis. The pair is a match if no reaction is observed.
    - *Antiglobulin Crossmatch:* A test usually done for recipients with history of alloimmunization (e.g., pregnancies, or previous transfusions). This test is done to check for incompatible antibodies in the recipient's serum that could cause and adverse reaction of the donor's RBCs.
- *Minor Crossmatch:* Involves testing the recipient's RBCs with the donor's plasma/serum to detect antibodies in the donor's serum that could react with the antigens on the recipient's RBCs.

D. Compatibility Assessment

- If there is no agglutination or hemolysis observed in either of the tests, the blood is compatible, and the donor's blood products are safe for transfusion.
- If there is observed reaction in either of the tests, the transfusion is not compatible, which prompts the patient to be sought another unit of compatible blood.

E. Emergency Transfusions

- During emergency situations, where there is not enough time to perform a complete crossmatch, type-specific blood is given based on the recipient's known blood type. Alternatively, for safety reasons, a negative O (O-) blood may be used for patients of unknown blood type.

SPECIMEN REQUIREMENTS

A. Recipient's blood
   - Type: Serum or Plasma
   - Volume: 5 – 10 mL
   - Collection method: Red-top tube (serum); Light blue-top tube (plasma)

B. Donor's blood
   - Type: Erythrocytes (RBCs)
   - Volume: 1 unit of blood (300 – 450 mL)
   - Collection method: Collected in an anticoagulant-preserved bag, wherein RBCs are separated from the plasma

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

Reagents

Reagents needed for crossmatching are organized in the table below:

Reagent	Purpose
Anti-A, Anti-B, Anti-D sera	ABO and Rh compatibility
Coombs' Reagent (AHG)	Indirect antiglobulin test to detect antibodies in recipient serum
0.9% Sodium Chloride (Saline)	Washing RBCs, diluting patient plasma/serum

Donor RBCs	To test for compatibility with the recipient's serum
Screening Cells	To identify unexpected antibodies in recipient's plasma
Antibody Screening Reagents (LISS, PEG)	Enhancers for antibody screening and detection
Glycine-HCl Solution	Elution of antibodies from RBCs for identification

Supplies and Equipments

- Microscope
- Centrifuge
- Test Tubes and Racks
- Blood Bank Incubator
- Blood Bank Refrigerator
- Pipettes and Pipettors
- Timer or Stopwatch
- Test Panels
- Reagent Storage

CALIBRATION

Calibration

- Perform daily calibration using manufacturer-recommended calibration reagents
- Document calibration results in the maintenance log

Calculations

In blood banking, crossmatching typically involves testing the compatibility of the donor's RBCs and the recipient's serum which could lead to adverse reactions during transfusion. Although, most of the process is qualitive, which is looking for agglutination or hemolysis, there are still certain aspects of the process that requires calculations. Below are the different calculations needed for certain needs:

A. Dilution of Plasma/Serum for Antibody Screening

Final volume= Desired dilution × Initial volume of serum

B. Amount of Reagents (Coombs Reagent) for Antiglobulin Crossmatch

1drop × n patients= n drops of Coombs reagent

N drops × amount mL/drop= mL of Coombs reagent

QUALITY CONTROL

To ensure that quality is observed in every crossmatching test, the personnel involved should adhere to the following:

A. Pre-Analytical Quality Control

- Identify the patients involved properly
- Samples must be collected in the right tube and with the right anticoagulants
- Keep records of the blood products, patient information, and crossmatching results

B. Analytical Quality Control

- Ensure the reagents used are not expired
- All laboratory equipment involved in the tests, such as centrifuges, microscopes, refrigerator, and incubators should be regularly calibrated to maintain precision and accuracy for consistent results
- Control samples should be run alongside with the patients' sample to confirm the accuracy of the crossmatching

C. Post-analytical Quality Control

- Crossmatching results should be verified by qualified personnel to ensure the veracity of the results
- If there are abnormalities found, an investigation must be conducted
- Documentation of the crossmatch results alongside the details of the donor and recipient must be noted of, especially if there are antibodies found in the recipient's sample

STEP-BY-STEP INSTRUCTIONS

Major Crossmatching by Immediate Spin Crossmatch (ISX)

1. If using serum, prepare 3%-5% *EDTA-saline* suspension of test red cells, in each donor samples, A1 and B controls in appropriately labeled test tubes. If

using plasma, prepare 3%-5% *saline* suspension of test red cells, in each donor samples, A1 and B controls in appropriately labeled test tubes

2. In labeled 10 or 12 × 75-mm size test tubes, add 2-3 drops of serum or plasma and 1 drop of each of the prepared appropriate red cell suspensions.
3. Mix the contents of the test tubes then, centrifuge, dislodge the cell buttons gently, examine macroscopically for agglutination, grade and record the results.

Major Crossmatching by Antiglobulin Crossmatch

1. Prepare recipient's serum and donor's RBCs. And anti D serum, and AHG.
2. Label three test tubes: T (Test Serum), PC (Positive Control), and NC (Negative Control).
3. In test tube T, take 2 drops of test serum.
4. In test tube PC, take 1 drop of anti D serum.
5. Add one drop of 5% saline suspension of the pooled 'O' Rho positive cells in each tube.
6. At 37°C, incubate all test tubes in one hour.
7. Wash the cells three time in normal saline, removing excess serum with no free antibodies.
8. Add two drops of Coombs serum to each tube.
9. Let the tubes stand for 5 minutes and then centrifuge at 1,500 rpm for one whole minute.
10. Resuspend the cells and examine macroscopically and microscopically.

Minor Crossmatching

1. Prepare recipient's RBCs and donor's plasma. If using serum, prepare 3%-5% *EDTA-saline* suspension of test red cells, in each donor samples, A1 and B controls in appropriately labeled test tubes. If using plasma, prepare 3%-5% *saline* suspension of test red cells, in each donor samples, A1 and B controls in appropriately labeled test tubes
2. In labeled 10 or 12 × 75-mm size test tubes, add 2-3 drops of serum or plasma and 1 drop of each of the prepared appropriate red cell suspensions.
3. Mix and incubate the tubes at 37 degrees Celsius for about 60 minutes.
4. Decant the serum completely and wash the cells three times in saline.

5. Add two drops of Anti-human Globulin (AHG) and mix. Allow it to stand in room temperature for 5 minutes.
6. For 1 minute, centrifuge at 1500 rpm.
7. Observe macroscopically and microscopically for agglutination.

REPORTING RESULTS

Major Crossmatching by Immediate Spin Crossmatch (ISX)

Interpretation: If donor RBCs are nonreactive, and the controls are in accord with patient's ABO group, then **issue for blood unit for transfusion**. If reactive, and the controls are in accord with patient's ABO group, suspect cold-reactive auto- or alloantibodies, then **crossmatch by LISS Antiglobulin.** If nonreactive and, and the controls are not in accord with patient's ABO group, **tests are invalid and confirm red cell ABO group of donor and recipient. Blood may be released if there is ABO compatibility.** If reactive, and the controls are not in accord with patient's ABO group, **do not release blood unit. Instead, issue group O RBCs and investigate problem.**

Major Crossmatching by Antiglobulin Crossmatch

Interpretation: No agglutination, safe to transfuse. If agglutination is observed, do not transfuse blood unit.

Minor Crossmatching

Interpretation: Compatible donor and recipient blood should show no agglutination.

LIMITATIONS OF METHODS

Unwanted Positive Reactions:

- Auto- or alloantibodies.
- Contaminated samples.
- Polyagglutination.
- Polycarboxyl-dependent antibody.
- Passive antibody.

Unwanted Negative Reactions:

- Omission of red cells or test sample.

- Contaminated sample.
- Weakly reactive Anti-A and/or Anti-B.
- Newborn sample.
- Weakly expressed A and/or B antigens on donor red cells.

TROUBLESHOOTING

- Problem: False positive crossmatch.

○ **Solution:** Repeat the crossmatch.

- Problem: False negative crossmatch.

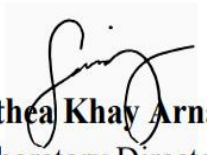
○ **Solution:** Repeat the crossmatch.

- Problem: Inconsistent or weak agglutination.

○ **Solution**: Recheck the test by running an additional test with a different donor to rule out variability in one of the components.

EFFECTIVE DATE

October 6, 2024



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DNA SEQUENCING

For Molecular Biology Section

PRINCIPLE OF DNA SEQUENCING

DNA sequencing depends on either chain termination or chemical degradation. According to Libretexts (2022), Sanger sequencing, or chain termination sequencing, is a DNA sequencing technique created by Frederick Sanger in 1977. This approach involves amplifying the DNA fragment sequenced using DNA polymerase and adding modified nucleotides, specifically dideoxynucleotides (ddNTPs). The target DNA sequence is a template for a unique Polymerase Chain Reaction (PCR) known as chain-termination PCR.Chain-termination PCR produces millions to billions of copies of the desired DNA sequence, each oligonucleotide ending at a random length (n) with 5'-ddNTPs (Merck, 2024).

Sequencing by chemical degradation required chemical alterations of the DNA and further cleavage and electrophoresis. The process entails employing polynucleotide kinase to radioactively label the 5′-P ends of double-stranded DNA (dsDNA) with 32P-dATP. The resultant ssDNA molecules are then separated by electrophoresis after the DNA is denatured with DiMethyl SulfOxide (DMSO) at 90°C. The adenosine (A), cytosine (C), guanosine (G), and thymidine (T) residues are modified by nitrogenous base-specific reactions, which permits the chemical cleavage of the ssDNA at the 5′-P side of such locations. The A and T reactions also produce some modest G and C cleavage, which will eventually manifest as a weaker signal. The ssDNA fragment can be separated based on size using polyacrylamide gel electrophoresis and autoradiography. The radiolabeled DNA band pattern on an X-ray film that encodes the DNA sequence can then be detected, allowing the sequence to be deduced.

SPECIMEN REQUIREMENTS

Sanger Sequencing Specimen Requirements

Sample Type	**DNA Mass (ng)**	**Volume of 10µM primer to add (µl)**	**Total Volume (µl)**
Plasmid			
Single-stranded DNA	200	3	12

Sample Type	DNA Mass (ng)	Volume of 10µM primer to add (µl)	Total Volume (µl)
Double-stranded DNA (up to 10 kb)	1000	3	12
Purified PCR Product			
<100 - 200 bp	2 - 6	3	12
200 - 500 bp	6 - 15	3	12
500 - 1000 bp	10 - 40	3	12
1000 - 2000 bp	20 - 80	3	12
>2000 bp	80 - 200	3	12

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

Reagents

Sanger sequencing requires the following:

- The DNA that needs to be sequenced is called template DNA.
- Short single-stranded DNA molecules called primers complement the target DNA sequence.
- DNA building blocks are called deoxynucleotide triphosphates, or dNTPs. These include dATP, dCTP, dGTP, and dTTP.
- Chain terminators marked with fluorescent dyes are dideoxynucleotide triphosphates or ddNTPs (ddATP, ddCTP, ddGTP, and ddTTP).
- The enzyme DNA polymerase is responsible for the synthesis of DNA.
- The DNA polymerase reaction can be enhanced by a sequencing buffer.

Supplies

The following supplies or equipment are needed for Sanger sequencing.

- Microcentrifuge tubes are used to store and process samples.

- Precise measurement and transfer of liquids are achieved via micropipettes.
- Disposable tips for micropipettes are also needed.
- PCR tubes or plates are essential to amplify DNA fragments for PCR.
- Agarose gel separates DNA fragments according to size.
- UV transilluminator visualizes DNA bands in agarose gels,
- Gel loading dye is for DNA sample visualization in the same media.
- Staining DNA samples in gels is conducted via ethidium bromide.
- Gel electrophoresis machine is required to separate DNA based on size and charge.

Equipment

The following equipment is necessary for Sanger sequencing.

- Thermocycler
- Sequencing machine
- Water bath
- Centrifuge
- Computer. For data analysis

QUALITY CONTROL

According to Your Genome (n.d.), DNA should undergo quality control after the sequencing to determine if the processes have adequately worked, if the instruments have operated precisely, and if the DNA sample is not contaminated with other samples. The following steps are required to check if DNA is of suitable quality.

- Check how much DNA, in clusters, is in each mm2 of every lane in the sequencing machine. Each lane must have hundreds of thousands to millions of DNA clusters.
- The number of clusters outside the recommended range indicates an error during the sequencing. The sample should be rejected from further testing.
- Measure the strength of the signal from the DNA bases in the sequence, which has to be as bright as possible, especially in the first base. A dull signal reveals an error.

The next step is to check whether the DNA sample's purity is contaminated with the DNA from other samples. Know the following.

- Align the DNA sequence against the reference genome. Verify if it matches with the species it should be. Expect a 98-99% match with the reference genome. A 100% match is unlikely due to genetic variations between individuals of the same species.
- Tags are added to the DNA sample before the sequencing, which acts as barcodes in identifying DNA fragments. These should not appear after the sequencing because they result from contamination or human error.
- Primary analysis of the data sequence is completed after three to four days. After that period, it will be either passed or failed. Failed samples will be discarded, and Sanger sequencing will be redone. Samples that have passed will be stored in a data repository, along with information on which species it is from and which study the genome was sequenced for (Your Genome, n.d.).

STEP-BY-STEP INSTRUCTIONS

Adhere to the instructions of Sanger Sequencing cited from Cornell University (2020).

1. **Choose between tubes or plates**.
   - Tubes may be used for samples with fewer than sixteen samples.
   - Plates may be used for samples with more than sixteen samples.
2. **Order the suitable tubes, plates, and cover**
   a. **Tubes.** Please use one of the following 500ul standalone screw-top vials:
      - USA Scientific: item number 1405-9799.
      - VWR: 16466-052 (Tubes with caps).
      - VWR: 16466-036 (Tubes only); 16466-084 (Caps only).
      - Fisher Scientific: catalog number 50-476-676.

   b. **Plates.** Submit samples in 96-well plates if you have large batches. This enables quicker results to be obtained and the samples processed more effectively. Any kind of PCR-type plate can be used.

   c. **Covers.** Use strip caps instead of adhesive seal if plates will be shipped by mail. Strip caps are not required to deliver samples personally but cover the plates with an adhesive seal.
3. **Prepare the DNA.**
   a. **Purify your DNA.** The following are commonly used.

- **Purification Column Kit (Qiagen or Promega)**. Adhere to the instructions precisely. The volume of liquid added to the column and the quantity of DNA determine the purification result. Do not overfill your columns. Follow the suggested volumes and amounts.
- **ExoSAP-IT purification kit (Thermo Fisher)**. If you choose this approach, quantify DNA using a Qubit. When estimating the sample concentration following an ExoSAP-IT purification, do not use a NanoDrop, as this will result in inaccurate results.
- **Gel cutting.** Gel purification is required when the intended PCR product is contaminated with additional amplification products. Run your sample out on a gel to cut out and purify the band of interest.
- **AMPure XP beads (Beckman Coulter).** Although this approach is not the most affordable, it produces excellent outcomes.

b. **Evaluate DNA purity.** The sample's absorbance ratio (A260/A280) needs to be at least 1.8. Results of poor quality or even unsuccessful sequencing are produced by values below 1.7.

c. **Evaluate DNA integrity.** Use an agarose gel to assess the integrity of your DNA. Sequencing is more likely to fail if your DNA seems severely damaged.

4. **Identify DNA concentration.** Measure the optical density (OD) at 260. 50 ug/mL of double-stranded DNA is approximately equivalent to an OD_{260} of 1.0.
5. **Calculate the amounts of primers and DNA templates**.
   - Mix DNA templates and primers with the same primers used for the PCR.
   - Request to add universal primers to your DNA templates.
6. **Fill up plates or tubes with the calculated volumes.**
   a. For plates:
      - Fill up the columns with samples.
      - Empty H12 and G12 for controls.
      - Add wells that were missed so that samples are not shifted.
   b. For tubes:
      - Label caps of sample tubes with appropriate numbers in the same order when entering the samples.
      - Write the sample name on the tube’s side.
7. **Analyze and interpret the results.** Note the following comments.

- **Ran Well**. This indicates that the sample was read out appropriately, and the sequencing succeeded.
- **Low signal strength.** This may indicate multiple issues, including (1) little to no DNA was necessary, (2) primer was miscalculated, and (3) high background noise.
- **Failed.** This means priming did not occur, the plate's well was empty, and no results were generated. This could be due to mismatching between template and primer, issues with the primer, poor quality samples, incorrect primer or template concentrations, or contaminated templates.
- **Homopolymer.** A homopolymer is an extended sequence of one or more repeating bases. The polymerase commonly slips when homopolymers are present, which has a significant impact on the downstream sequences. Using an alternative primer or sequencing in the other direction is the simplest way to solve the problem. Another option is to attempt an alternative sequencing chemical, like the dGTP kit (for C or G repeats only), which we may supply upon request.
- **Noisy or Deteriorated.** Low peaks beneath big peaks on the electropherogram indicate substantial background noise in a sequence. Because of the noise, the software miscalls or fails to call bases, interfering with the sequence. The most common cause of noisy sequencing is poor DNA quality, while other possible causes exist. Furthermore, proteins, salts, ethanol, isopropanol, PEG, and other contaminants that are left over after DNA preparation can disrupt the process.
- **Drop Off.** Drop-off sequences typically occur because the template is a PCR product, though a secondary structure can also result in a drop-off. The end of your product is the drop-off point if your template is a PCR product.\
- **Overlap.** When two distinct sequencing reactions occur in the same tube, overlapping sequences arise. Both sequences are indistinguishable from each other. Two priming sites in the template, two distinct prims in the sequencing reaction, including two plasmids in a single sequencing reaction, or insufficient PCR cleanup can all result in overlaps.

REPORTING RESULTS

Report results of Sanger sequencing as stated by Ellard et al. (n.d.).

1. **Variant or mutation found.** Mutations found for the first time in a particular lineage should be verified by a second test using a new template if there is no reliable tube transfer checking method (such as barcode scanning or witnessed transfers) to ensure sample identity at every stage.
2. **No mutation was found.** Reporting negative results is appropriate for the nucleotide range for which high-quality sequencing data was analyzed. Within the report, the scope of the analysis should be specified. The following could lead to erroneous negative results:
    a. Allelic dropout-causing polymorphisms in the primer binding sites of PCR or sequencing
    b. Tissue mosaicism
    c. Augmentation of the more minor allele in a more significant insertion
    d. Deletion of a whole gene or exon
    e. The potential effect of high GC sequence content
        - The primer binding sequences must be examined for SNPs in predictive tests if the family mutation is not detected and the outcome depends on amplification using a single primer pair.
3. **Reports.** This shows the sequencing data wherever available with the given clinical information. When a reliable number is available for negative results, the "clinical sensitivity"—the percentage of patients with the relevant phenotype in which a mutation is found by the testing approach used—should be provided. If this information is not accessible to users elsewhere, it may also be used to indicate the technical sensitivity of the testing procedure.

LIMITATION OF METHODS

- Sanger sequencing is limited to sequencing brief DNA fragments. Its throughput is constrained.
- It is slow and costly for large genomes since it can only sequence one fragment at a time.
- It may require more handling and preparation time.
- Its low sensitivity makes studying complicated mixes and detecting uncommon mutations challenging.

- Because contaminated or damaged DNA might produce inaccurate results, Sanger sequencing requires comparatively pure and high-quality DNA samples.

TROUBLESHOOTING

Dye Blobs

After the cycle sequencing processes are purified, unincorporated dye terminators still in solution result in dye blobs. Typically, sequencing kits co-migrate with the approximately 85–90 bp designated pieces. In more extreme cases, these blobs can also be found in regions between 125 and 140 bp and about 60 to 65 bp. Although they can also appear as "G" blobs, dye blobs are usually observed as broad "C" or "T" peaks. Figure 1 is an example of a dye blob.

Figure 1

Severe dye blobs in the 60–65bp and 125–140bp regions

Table 2

Dye Blob Causes and Recommended Solutions

Causes	**Recommended Solutions**
The sample bypassed the purification material when employing spin columns/spin plates for sequencing cleanup.	Make sure the sample is moved to the middle of the purification substance. The purification material may be circumvented if the sample is administered along the clean-up column walls. When dispensing quickly, use a single-channel pipette and/or place the tip just above the spin column or plate.

During ethanol precipitation, the concentration of ethanol is very high. As a result, the sequencing product precipitates with unincorporated dye terminators and salts.	Reperform the procedure with the correct ethanol concentration.
Incorrect ratio of reagents.	Vortex the solution for ten seconds at maximum speed before dispensing. Ensure the sample is mixed properly before dispensing pre-mixed solutions to maintain the appropriate reagent ratio.
Insufficient mixing during the vortexing step	Follow the vortexing instructions and ensure the plate is securely fastened to the vortexer.

G/C compression

Subtle G or C peak shoulders or unresolvable sections of GC bases are frequently the consequence of G/C compression caused by either an excess sequencing template or an inadequate denaturation of GC-rich regions of the sequencing template. See Figure 2 for an example.

Figure 2

G/C Compression Due to an Excess of Sequencing Template

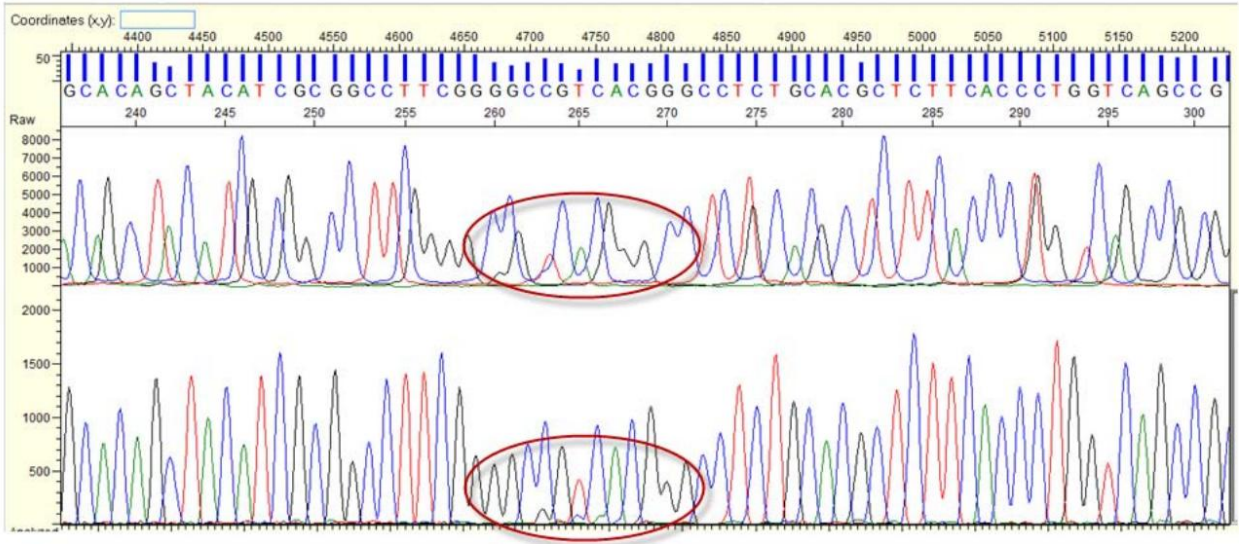


Table 3

G/C Compression Causes and Recommended Solutions

Causes	**Recommended Solutions**
An excessive amount of gDNA input during PCR results in excessive sequencing templates when using the sequencing kit.	Make sure to use less ≤20 ng of input DNA when using the sequencing kit.
	Reinject the sample. Compressions are usually removed following the initial injection.
Insufficient denaturation of the artificially produced DNA from GC-rich areas, mainly when using dGTP sequencing chemical for sequencing.	As of right moment, no remedial action is known. G/C compression may be resolved by using a new sequencing primer that is situated closer to the GC-rich area.

Primer Impurity

In this situation, the primer is contaminated.

Figure 3

Example of Mixed Sequences Due to a 10% Primer Impurity

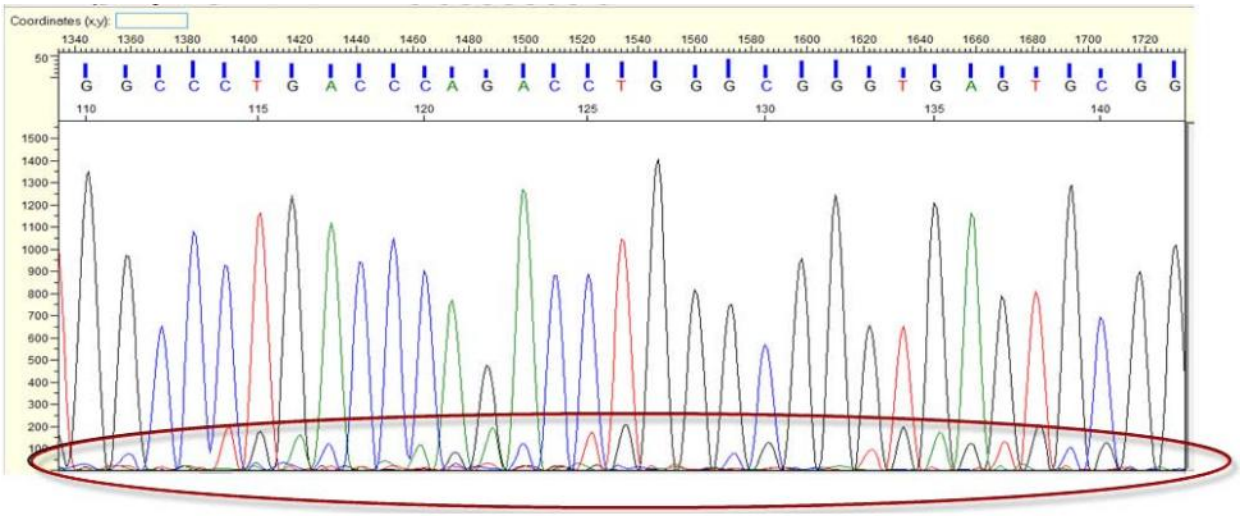


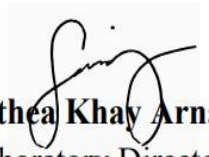
Table 4

Primer Impurity Causes and Possible Solutions

Causes	Recommended Solutions
Inadequate purification of PCR primer	Redo through HPLC- purified primers
Inadequate sequencing of PCR primer	

EFFECTIVE DATE

October 6, 2024



Althea Khay Arnaiz
Laboratory Director

Enzyme-Linked Immunosorbent Assay (ELISA)

For Immunoserology Section

PRINCIPLE OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Enzyme-linked immunosorbent Assay is a technique used to quantify substances like antibodies, proteins, and hormones in a sample (liquid). It involves antigen-antibody interactions and enzyme detection systems. Antibodies bind to specific antigens to catch particular antibodies needed for a procedure in a sample. Then the antibody is immobilized and a second antibody is linked to an enzyme and binds to a specific antigen complex. A color change will then occur after adding a particular substrate and becomes more vibrant according to the concentration of the molecule, this is then measured by machines such as a spectrometer which uses a standard curve to know the concentration of the analyte in the sample.

ELISA serves as a way for a non-invasive diagnosis to be done on a patient because it can pinpoint different biomarkers through blood, urine, saliva, etc. ELISA can also be automated through different systems inside the laboratory, this allows for high-output testing with the principle of quality and time management in mind, considering that ALISA deals with a wide dynamic range accommodating both high and low analyte concentrations. ELISA has different applications such as in detecting diseases, screening drugs, detecting pathogens in food, and monitoring pollutants in the surroundings thus making it a versatile test not only in the clinic but also in different areas of experience

SPECIMEN REQUIREMENTS

ELISA requires appropriate specimen collection and handling in order to ensure the best results possible during processing. Among the different specimen types serum, plasma, urine, saliva, tissue lysates together with other bodily fluids are the most common depending on what is being tested. Specific samples like blood should be processed through sterile methods to obtain specific requirements for certain procedures. These must follow a specific set of conditions to prevent any analytical error. Specimens should also be stored properly to prevent specimen deterioration before analysis and should be disposed of properly after analysis.

Sample Collection Procedure

Sample collection techniques for ELISA can differ in accordance to the specimen that is to be examined, a sterile techniques is a must in collecting these samples. For blood, it has to be extracted from the patient and properly kept, this ensures that the sample's integrity is secured, anticoagulants must be applied to avoid materials. Urine collection must also follow the mid stream collection method and must be placed in sterile containers before analysis, this is also true with saliva and tissue samples. Each specimen should be labeled and stored at an appropriate temperature of 2-8 degrees celsius or frozen for long term use, this may vary depending on the assay's requirements.

Handling and Storage Requirements/Transport

It is important to ensure that the requirements for the samples are met to ensure the integrity of the results. Samples from the blood like serum and plasma should be store at 2-8 degrees celcius and must be analyzed within a 48 hour time period while frozen at -20 degrees celsius for longer use. Depending on the stability of the analyte, urine, saliva, and other bodily fluids are stored similarly with short or ong term freezing. To maintain tissue and cell lysatates's protein integrity they should be stored at -80 degrees celsius. In storing, constants thawing and refrigeration should be avoided to avoid the degradation of samples, and should be stored in aliquots to minimize handling. In transportations, all these conditions must be met with the addition of different transport guidelines and leakproof containers. Proper storage/transport should always be observed, so that the quality of the samples are maintained before and during testing.

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENTS

Reagents/Media

- Coating Buffer
- Blocking Buffer
- Primary Antibody
- Secondary Antibody
- Substrate Solution
- Sample Dilution Buffer

Supplies and Equipment

1. Disposable Gloves
2. Sterile Collection Containers
3. Specimen Labels
4. Microscope Slides and Coverslips
5. Plastic Pipettes
6. Microplates
7. Plate Reader/Absorbance Reader
8. Microplate Washer
9. Reagent Bottles and Tubes
10. Incubator
11. Tweezers
12. Seals Plates
13. Timer
14. Centrifuge
15. Microscope

CALIBRATION

Calculation

Dilution Factor - If samples have been pre-diluted before testing, the concentration value from the standard curve should be multiplied by the dilution factor to give the actual concentration in the original sample.

Corrected Concentration=Measured Concentration×Dilution Factor

Preparation of Standard Curve - In quantitative assays, a standard curve is created by plotting known concentrations of the target analyte. ODs are plotted against the known concentrations to create the curve.

Y= A+ (B-A)/(1+(X/C)^D)

Where;

Y = Absorbance (OD)

X = Concentration of the analyte

A, B, C, D = Parameters derived from curve fitting

QUALITY CONTROL

Pre-Assay

- Preparation of reagent
- Equipment calibration
- Validation of the standard curve
- Inspection of plate

Internal Controls

- Positive Control
- Negative Control
- Blank Control
- Monitoring the Performance of the Assay
- Replicate
- Washing consistently
- Consistent Incubation

Post-Assay

- Evaluation of Standard Curve
- Validation of Control
- Inter Plate Consistency

STEP-BY-STEP INSTRUCTIONS

1. **Sample Collection -** typically collected through venipuncture and stored inside serum/plasma tubes in accordance witht the requirements of the assay. For serum samples, the blood should be allowed to clot for 30 mins to 1 hour at room temperature. While for plasma, the blood should be put inside a centrifuge immediately. And should be stored in a 2–8°C container if testing within 48 hours or frozen for longterm use.

2. **Preparation of Plate** - using the diluted capture antigen/antibody, coat the wells of 96-well microplate, incubate it in 4 degrees celsius overnight or 1-2 hours at room temperature. The plate should be washed 3-5 times with wash buffer.

3. **Add Blocking Buffer** - a blocking buffer should be added to each well in order to block nonspecific binding sites and incubate in room temperature for the next 1-2 hours, then was the plate like before.

4. **Addition of Sample** - in accordance with the assay, dilute the serum or plasma in the sample dilution buffer and add about 100 microliters to each well, standards and controls should also be added into the plate to increase accuracy. Then incubate the plate in room temperature for 1-2 hours then wash for any components that did not bind.

5. **Detecting of Antibody -** prepare the detection antibody that is conjugated with an enzyme and add 100 microliters to each well and incubate for 1-2 hours in room temperature. The plate should be washed 3-5 times to remove the antibodies that did not bind.

6. **Add Substrate -** add 100 microliters of the substrate solution to each well.

7. **Stop the reaction** - add about 50-100 microliters of the stop solution like sulfuric acid to each well to stabalized the development of color (normally yellow).

8. **Read Absorbance -** prepare a microplate reader at 450 nm for HRP-based assays and measure the optical density.

9. **Analyze the Data -** Finally, using the optical density (OD) values gathered during the procedure, make a standard curve and interpolate the values of the unknown sample to the curve. Compare it to the reference range of the assay kit and if there are any dilution factors involved, adjustments should be made. Calculations are provided above.

REPORTING RESULTS

Component	Reference Range
Vitamin D (25-OH)	Deficient: <20 ng/mL. Insufficient: 20–29 ng/mL. Sufficient: 30–100 ng/mL.
Thyroid-Stimulating Hormone (TSH)	Reference range: 0.5–5.0 µIU/mL
Cytokines	Normal range: <5 pg/mL
Hepatitis B Surface Antigen	Negative: Below the assay cutoff. Positive: Above the assay cutoff.
Allergen-Specific IgE	Class 0: <0.35 kU/L (negative). Class 1–6: Increasing levels of sensitization
HIV Antibody Test	Negative: Optical Density (OD) below the cutoff value. Positive: OD above the cutoff value

LIMITATIONS OF METHODS

1. Issues of the quality of the sample- hemolysis , lipemia, or improper storage may cause the blood sample to be of poor quality which can cause problems in the data.

2. Sensitivity Limitation - low analyte concentrations may be overlooked by the detention threshold of ELISA making it less suitable for detecting these small amounts compared to PCR.

3. False results that are Negative or Positive - similar antigen reactions can cause false positives while a low level of the analyte may cause false negatives.

4. Time and Labor Intensive - the feasibility of the resource limiting setting may be compromised to to the requirement of multiple incubation periods, washing, and specific equipment requirements that are done and used by skilled personnel.

5. Current and previous Infections - an antibody based test of ELISA is unable to recognize if the infections are active or from a previous expose because antibodies persist after the first infection.

6. Focus on a single analyte - ELISA can only measure on analyte per well which makes it limited compared to other technologies with almost the same purpose that are designed to have multiple targets.

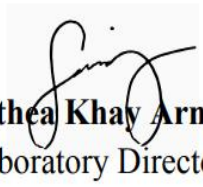
TROUBLESHOOTING

- **Problem:** No Signal or Low Signal
  - **Solution:** Make sure the reagents are stored properly and the expiration dates are checked regularly. When using a more sensitive method, increase the concentration of the sample.
- **Problem:** Well Variability
  - **Solution:** Make sure the plates are curated evenly during the incubation period, and the measurement tools are regularly calibrated. Proper washing techniques should also be observed in order to prevent any data gathering problems.
- **Problem:** Extensive Backgroud Signal
  - **Solution:** High background signal should be managed by washing properly to remove all of the unbound reagents, use blocking buffers that reduce nonspecific binding, and careful optimization of incubation time for the

substrate, which ensures the reaction stops promptly overdeveloping the substrate.

EFFECTIVE DATE

October 6, 2024



Althea Khay Arnaiz
Laboratory Director

Liver Function Tests (LFTs)

For Clinical Chemistry Section

PRINCIPLE OF LIVER FUNCTION TESTS (LFTs)

Liver function tests are blood tests used to help find the cause of your symptoms and monitor liver disease or damage. Some of these tests measure how well the liver is performing its regular functions of producing protein and clearing bilirubin, a blood waste product. Other liver function tests measure enzymes that liver cells release in response to damage or disease.

Some common liver function tests include:

- **Alanine transaminase (ALT).** When the liver is damaged, ALT is released into the bloodstream and levels increase. This test is sometimes referred to as SGPT. An elevation of the enzyme activity in serum is a strong indicator of parenchymal liver disease.
- **Aspartate transaminase (AST)**. An increase in AST levels may mean liver damage, liver disease or muscle damage. This test is sometimes referred to as SGOT.
- **Alkaline phosphatase (ALP).** Higher-than-usual levels of ALP may mean liver damage or disease, such as a blocked bile duct, or certain bone diseases, as this enzyme is also present in bones. A rise in serum alkaline phosphatase occurs in all forms of cholestasis, particularly in obstructive jaundice. It is also elevated in diseases of the bone (Paget's disease, hyperparathyroidism, rickets, osteomalacia), fractures and malignant tumors. Children and juveniles can also exhibit significant increases in serum alkaline phosphatase levels as a result of increased osteoblast activity following
  accelerated bone growth.

SPECIMEN REQUIREMENTS

The specimen that needs to be collected during the sample collection is blood, specifically either serum or plasma.

Alanine transaminase (ALT)

- **Specimen Type and Requirements:** Serum, and EDTA- or heparin-anticoagulated plasma are acceptable for this procedure. Other anticoagulants are not acceptable. The NHANES Biochem study uses refrigerated serum. This test is analyzed from NHANES Vial 018.
- **Specimen Volume**: Optimum/Minimum volume: 100 pL in a sample cup or 2 mL microtube (9 pL for test; remainder for dead volume).

- **Specimen Stability and Storage**: Separated serum or plasma should be removed from the cells within 1 hour of collection. Separated serum or plasma is stable for seven days at 4°C, six months at -20°C and longer at -70°C.
- **Interferences or limitations:** Icteric index <60: no interference. Hemolytic index <200: no interference. Lipemic index <150: no interference. Drugs: No interference was found at therapeutic concentrations using common drug panels. Exception: Calcium dobesilate and Isoniazid can cause artificially low and Furosemide artificially high ALT results at therapeutic concentrations. Cyanokit (Hydroxocobalamin) may cause interference with results. In very fare cases, gammopathy, in particular IgM (Waldenstrom's macroglobulinemia), may cause unreliable results.
- **Specimen Handling and Transport:** Mix specimens well, allow clot to fully form (if serum), and centrifuge 10 minutes at 2000 x g before use. Aliquot a minimum of 0.1 mL. Store sample in refrigerator until shipment. Ship at refrigerated temperature. Specimens must be at room temperature prior to assay.

Aspartate transaminase (AST)

- **Interferences:**

  1) Interference with >30.0 mg/dL bilirubin or >3+ lipemia.

  2) Do not use hemolyzed specimens.

  3) Specimens with >3+ lipemia should be treated with Lipoclear clarifying agent prior to analysis (see Lipoclear procedure).
- Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
- Fasting is not required.
- A minimum of 0.6 mL serum is needed for the Multi-Analyte Panel.
- Sample volume for individual test is 25 µl added to 250 µl reagent.

- Sample is run singly as part of Multi-analyte Biochemistry Panel.

Alkaline phosphatase (ALP)

- **Specimen Type and Requirements:** Use serum or plasma (lithium heparin, ammonium heparin, and sodium heparin are acceptable anticoagulants) for the procedure. Other anticoagulants are not acceptable. The NHANES Biochem study uses refrigerated serum. This test is analyzed from NHANES Vial 018.
- **Specimen Volume: Optimum/Minimum volume:** 100 µL in a sample cup or 2 mL microtube (2.8 µL for test; remainder for dead volume).
- **Specimen Stability and Storage:** Separated serum or plasma should be removed from the cells within 1 hour of the collection. Separated serum or plasma is stable for seven days at 4ºC, two months at -20°C and longer at –70ºC .
- **Interferences or limitations:** Icteric index <60 Hemolytic index <200 Lipemic index <2000 Drugs (therapeutic concentrations of commonly used drug panels): no interference. In very rare cases, gammopathy, in particular IgM (Waldenstrom's macroglobulinemia), may cause unreliable results.
- **Specimen Handling and Transport:** Mix specimens well, allow clot to fully form (if serum), and centrifuge 10 minutes at 2000 x g before use. Aliquot a minimum of 0.1 mL. Store sample in refrigerator until shipment. Ship at refrigerated temperature. Specimens must be at room temperature prior to assay.

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENTS

Alanine transaminase (ALT)

a. ***Reagents and Supplies***

- Roche Cat. No. 20764957322, ALT (ALTL) reagent kit (500 tests):

b. ***Reagent Preparation***

- R1 reagent: TRIS buffer: 224 mmol/L, pH 7.3 (37 °C); L-alanine: 1120 mmol/L; albumin (bovine): 0.25%; LDH (microorganisms): ≥45 ukat/L; stabilizers; preservative. No preparation necessary.
- R2 reagent: 2-Oxoglutarate: 94 mmol/L; NADH: ≥ 1.7 mmol/L; additives; preservative. No preparation necessary.

Storage and stability. Keep reagents stored at 2-8°C until use. The reagents are stable for 12 weeks refrigerated on the analyzer.

c. ***Equipment/Instrumentation***

- Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN 46250)
- The Millipore Elix Gulfstream Clinical 35 System is designed to meet CLSI Clinical Laboratory Reagent Water (CLRW) standards. Water purification is achieved by reverse osmosis, electrodeionization, bactericidal 254 nm UV lamp and 0.22 pm filtration.

d. ***Specimens are run in singleton***

Aspartate transaminase (AST)

a. ***Instrumentation:*** **Beckman Coulter UniCel® DxC800 Synchron Clinical System**

b. ***Materials***

1. Beckman Micro Tube (*Part #448774*)
2. S/P Plastic Transfer Pipet (*Cat. #P5214-10*)
3. S/P Brand Accutube Flange Caps (*Cat. #T1226-37*)

c. ***Reagent Preparation:*** Beckman Synchron System AST Reagent (*Part #442665, 200 tests/cartridge or #476831, 400 tests/cartridge*)

1) 200 test cartridge: Prior to use transfer the entire contents of smallest reagent compartment (C) to largest reagent compartment (A) using a disposable transfer pipette. Gently invert cartridge several times to mix.

2) 400 test cartridge: Transfer entire contents of one bottle AST (A-reagent) into the largest compartment (A). Replace cartridge caps and mix gently.

3) Unopened reagent is stable until expiration date when stored at 2-8°C.

4) Premixed reagent is stable for 30 days when installed on the instrument or stored at 2- 8°C, unless the expiration date is exceeded.

5) Do not freeze.

6) Contains sodium azide. Avoid skin contact with reagent. Use water to wash skin.

d. Standards Preparation: None required.

e. Control Material

1) Bio-Rad Liquid Unassayed Multiqual level 1 and 3 (Part#697 and #699).

- Thaw bottle of control and mix very well.
- Thawed control is stable 7 days. Mix well prior to each use.

  After August 19, 2009 Bio-Rad Multiqual has been used in our lab as the regular control for this analyte.

Alkaline phosphatase (ALP)

a. ***Reagents and Supplies***

- Roche product #03333752190, ALP2S reagent kit (200 tests):

b. ***Reagent Preparation***

- R1 reagent. 2-amino-2-methyl-1-propanol: 1.724 mol/L, pH 10.44 (30 °C); magnesium acetate: 3.83 mmol/L; zinc sulfate: 0.766 mmol/L; N-(2-hydroxyethyl)- ethylenediamine triacetic acid: 3.83 mmol/L. No preparation required.
- R2 reagent. p-nitrophenyl phosphate: 132.8 mmol/L, pH 8.44 (30 °C); preservatives. No preparation required.
- Storage and stability. Keep reagents stored at room temperature until use. The reagents are stable for 8 weeks refrigerated on the analyzer.

c. ***Equipment/Instrumentation***

- Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN 46250)
- The Millipore Elix Gulfstream Clinical 35 System is designed to meet CLSI Clinical Laboratory Reagent Water (CLRW) standards. Water purification is achieved by reverse osmosis, electrodeionization, bactericidal 254 nm UV lamp and 0.22 μm filtration.

d. ***Specimens are run in singleton***

CALIBRATION

Alanine transaminase (ALT)

Roche Calibrator for Automated Systems (C.F.A.S.), catalog #10759350190. The calibrator is stable until the expiration date on the bottle when stored at 4ºC. The lyophilized calibrator is prepared with 3.0 mL of deionized water. Pipette the water, and then dissolve by gentle swirling within 30 minutes. Avoid formation of foam while mixing. The prepared calibrator is stable for eight hours at room temperature, two days at 4ºC, and one month at –20ºC (frozen once).

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substratespecific absorptivity. Calibration frequency: A two-point calibration (H2O + C.F.A.S.) must be performed when there is a reagent lot change. The Cobas 6000 will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.

Manual calibration should be performed if:

- A reagent lot change has not occurred in the past 6 months
- After major service or repairs
- As needed for troubleshooting

If calibration fails perform the following corrective action steps in sequence:

- Check reagent and calibrator for appropriate lot numbers, expiration dates, preparation and storage conditions.
- Repeat calibration with new calibrator.
- Repeat calibration with new reagent and new calibrator
- If successful calibration is not achieved, discontinue testing and notify the supervisor.

Aspartate transaminase (AST)

a. Calibrators: None required.

b. Calibration: Calibration is based on physical principles of dilution ratio extinction coefficient and time. Known samples verify calibration.

Alkaline phosphatase (ALP)

Roche Calibrator for Automated Systems (C.F.A.S.), catalog #10759350190. The calibrator is stable until the expiration date on the bottle when stored at 4ºC. The lyophilized calibrator is prepared with 3.0 mL of deionized water. Pipette the water into the bottle, and then dissolve by gentle swirling within 30 minutes. Avoid formation of foam while mixing. The prepared calibrator is stable for eight hours at room temperature, two days at 4ºC, and one month at –20ºC (frozen once).

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing

absolute values and the substrate-specific absorptivity. Calibration frequency: A two-point calibration (H2O + C.F.A.S.) must be performed when there is a reagent lot change. The Cobas 6000 will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.

Manual calibration should be performed if:

- A reagent lot change has not occurred in the past 6 months
- After major service or repairs
- As needed for troubleshooting

If calibration fails perform the following corrective action steps in sequence:

- Check reagent and calibrator for appropriate lot numbers, expiration dates, preparation and storage conditions.
- Repeat calibration with new calibrator.
- Repeat calibration with new reagent and new calibrator
- If successful calibration is not achieved, discontinue testing and notify the supervisor.

QUALITY CONTROL

Alanine transaminase (ALT)

- Normal pooled serum control (CQ). Stable at -80°C for up to 4 years, at refrigerated temperature for up to 1 day and at room temperature for up to 4 hours.
- Roche Precipath U Plus Control (catalog #12149443160). Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457). Stable until expiration date on package when unopened and stored at 2-8°C. To prepare, open bottle 1 and pipette in exactly 3.0 mL of diluent. Dissolve by gentle swirling for 30 minutes. Prepared control is stable for 12 hours at room temperature, 5 days at 2-8°C, and one month at -20°C (when frozen once).
- Both levels of quality control are analyzed at the start of the day and results are verified for acceptability prior to testing specimens. Quality control is also analyzed at the end of the shift, with change in reagent, after major maintenance, or as needed for troubleshooting.

- The analytical measurement range (AMR) must be validated every 6 months or after major maintenance or service procedures. The laboratory enrolls in the College of American Pathologist (CAP) linearity program. Alanine aminotransferase is included in the LN2 kit that is shipped twice per year. Follow kit instructions for preparation. Analyze samples in duplicate. Results are due within two to four weeks of receipt of kit. Results are submitted online to the CAP website by the lead or supervisor. The linearity report is available online at the CAP website shortly after the due date. Confirm reported values are within acceptability limits. Place instrument printouts, worksheets and CAP results in the CAP three ring binder.
- New Lot Verification: Each new reagent lot must be verified for acceptability before being placed into use. Calibration, quality control, and comparison of at least 5 patient samples on the old and new lots must be performed and found to be within acceptable limits before a new lot can be placed into use.
- Quarterly Technical Progress Report:

Progress reports for each quarter of the calendar year are submitted to NHANES. The report includes :

1) A summary of the status of all specimens including date of arrival, deviations from protocol, handling, storage, and manipulation of vials and all laboratory procedures used.

2) A description of any major problems such as missing data, out of range values or inconsistent data and what was done to overcome them, internal and external quality control for runs containing NHANES specimens, instrument calibration and repairs, reagent lots and dates of use.

Aspartate transaminase (AST)

a. Blind QC Specimens are included in the samples received from NHANES.
b. Controls are assayed in early A.M. and if a new reagent pack is loaded, controls are assayed again. One level is assayed in middle of the day and both control levels are assayed after running NHANES samples.

c. BioRad Liquid Unassayed Multiqual Controls Levels 1 and 3 are assayed for CDCNHANES runs to allow long term control use. Multiqual controls are analyzed at beginning and end of runs with CDC-NHANES samples. Before August 19, 2009 Beckman Triad Custom Unassayed Chemistry Controls Levels 2 and 3 were analyzed in early AM and at times when CDC-NHANES were not being analyzed. As of August 19, 2009 we have discontinued use of Beckman Triad controls.

d. Acceptable Answers:

   1) Controls must be within ±2 S.D.

   2) Refer to Quality Control Flow Chart for action decisions guidelines

Alkaline phosphatase (ALP)

- Normal pooled serum control (CQ). Stable at -80°C for up to 4 years, at refrigerated temperature for up to 1 day and at room temperature for up to 4 hours.
- Roche Precipath U Plus Control (catalog #12149443160). Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457). Stable until expiration date on package when unopened and stored at 2-8°C. To prepare, open bottle 1 and pipette in exactly 3.0 mL of diluent (bottle 2). Dissolve by gentle swirling for 30 minutes. Prepared control is stable for 12 hours at room temperature, 5 days at 2-8°C, and one month at -20°C (when frozen once).
- Both levels of quality control are analyzed at the start of the day and results are verified for acceptability prior to testing specimens. Quality control is also analyzed at the end of the shift, with change in reagent, after major maintenance, or as needed for troubleshooting.
- The analytical measurement range (AMR) must be validated every 6 months or after major maintenance or service procedures. The laboratory enrolls in the College of American Pathologist (CAP) linearity program. Alkaline phosphatase is included in the LN2 kit that is shipped twice per year. Follow kit instructions for preparation. Analyze samples in duplicate. Results are due within two to four weeks of receipt of kit. Results are submitted online to the CAP website by the lead or supervisor. The linearity report is available online at the CAP website shortly after the due date. Confirm reported values are within acceptability limits. Place instrument printouts, worksheets and CAP results in the CAP three ring binder.

- New Lot Verification: Each new reagent lot must be verified for acceptability before being placed into use. Calibration, quality control, and comparison of at least 5 patient samples on the old and new lots must be performed and found to be within acceptable limits before a new lot can be placed into use.
- Quarterly Technical Progress Report:

  Progress reports for each quarter of the calendar year are submitted to NHANES. The report includes:

  1) A summary of the status of all specimens including date of arrival, deviations from protocol, handling, storage, and manipulation of vials and all laboratory procedures used.
  2) A description of any major problems such as missing data, out of range values or inconsistent data and what was done to overcome them, internal and external quality control for runs containing NHANES specimens, instrument calibration and repairs, reagent lots and dates of use.

STEP-BY-STEP INSTRUCTIONS

Alanine transaminase (ALT)

a. ***Instrument Operation*:** The Roche/Hitachi Cobas 6000 analyzer series is a fully automated, random-access, software controlled system for immunoassay and photometric analyses intended for qualitative and quantitative in vitro determinations using a wide variety of tests. The Cobas 6000 analyzer series is optimized for workloads using a combination of photometric and ionselective electrode (ISE) determinations (c501 module), and electrochemiluminescence (ECL) signal in the immunoassay analysis module (e601 module). The ISE system is used in the quantitation of sodium, potassium and chloride. The photometric system can measure colorimetric or immunoturbidimetric reactions utilizing end point or kinetic (rate) absorbance measurements. Test ordering end execution on the Cobas 6000 and data entry in the STARLIMS host computer system may be done manually or these tasks may be executed via a barcode-based bi-directional interface. The Cobas 6000 can utilize both'of these two systems simultaneously.

b. ***Professional Judgement:*** Check results for error flags and take appropriate corrective action. Investigate alert values and delta checks.

c. ***Result Entry***

STARLIMS test code: ALT

Manual entry.

• Results are reported to the whole number, as in x, U/L.

• Report low results as 3 U/L.

• Check results for error flags and take appropriate corrective action.

• Investigate alert values and delta checks.

Aspartate transaminase (AST)

a. Preliminaries

- Enter test in L.I.S. as a part of a panel according to procedure listed in this document (See Attachment A).

b. Sample Preparation

- Procedure for labeling Micro tube (CX tube) and transferring serum (See Attachment B).

c. Operation

- Refer to Operation Procedures for programming controls/patients and loading sectors/racks in the Beckman Coulter Synchron UniCel DxC 600/800 System Instructions For Use (IFU) manual or DxC800 and DxC600 Operating Procedure

d. Recording of Data

1. Operator will review and verify results in the L.I.S.
2. The L.I.S. reorders tests to verify any critical results. These results are stored in the L.I.S. along with the original results. Original values are used when repeat results match the original within 3 CV.
3. Project supervisor will export data from the L.I.S. into an Excel file. The data is copied in into another Excel file for further evaluation.
4. An Excel spreadsheet printout of the results for each container ID is made and comments noted.
5. Project supervisor reviews the results. If problems noted with results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.
6. Daily log sheets are completed and any problems or issues noted.

e. Replacement and Periodic Maintenance of Key Components

f. Calculations - Synchron Systems perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

Alkaline phosphatase (ALP)

1. ***Instrument Operation:*** The Roche/Hitachi Cobas 6000 analyzer series is a fully automated, random-access, software controlled system for immunoassay and photometric analyses intended for qualitative and quantitative in vitro determination using a wide variety of tests. The Cobas 6000 analyzer series is optimized for workloads using a combination of photometric and ion-selective electrode (ISE) determinations (c501 module), and electrochemiluminescence (ECL) signal in the immunoassay analysis module (e601 module). The ISE system is used in the quantitation of sodium, potassium and chloride. The photometric system can measure colorimetric or immunoturbidimetric reactions utilizing end point or kinetic (rate) absorbance measurements. Test ordering end execution on the Cobas 6000 and data entry in the STARLIMS host computer system may be done manually or these tasks may be executed via a barcode-based bi-directional interface. The Cobas 6000 can utilize both of these two systems simultaneously.
2. ***Professional Judgement:*** Check results for error flags and take appropriate corrective action. Investigate alert values and delta checks.
3. ***Result Entry***

   STARLIMS test code: ALKP

   Manual entry:

   - Results are reported in whole numbers in U/L.
   - Report low results as <2 U/L.
   - Check results for error flags and take appropriate corrective action.
   - Investigate alert values and delta check.

REPORTING RESULTS

Alanine transaminase (ALT)

Out of Range results: Certain tests have pre-programmed limits that trigger an automatic re-analysis by the COBAS. These limits may be low-end values or high-

end values (but within technical range). If the duplicate value is in agreement with the initial value, then the initial value is reported.

Results are reported to the whole number, as in x, U/L. Report low results as 3 U/L.
a. Reportable Range of Test Results: Reportable Range 3-7000 U/L
Intra-assay %CV (10 within-day replicates at a concentration of 45.4 U/L) 1.1%
Intra-assay %CV (10 within-day replicates at a concentration of 111.5 U/L) 0.5%
Inter-assay %CV (between day replicates at a concentration of 21 U/L) 2.4%
Inter-assay %CV (between day replicates at a concentration of 133 U/L) 2.0%

Dilutions: Linear range of the method: 3 - 700 U/L (serum). Specimens exceeding the high limit are automatically diluted (1:10) by the instrument; results from samples diluted using the rerun function are automatically multiplied by a factor of 10. If a manual dilution is required, dilute the specimen in normal saline, and muitiply the result by the dilution factor. For example, to perform a 1:5 dilution, pipette 50 pL of the patient sample into 200 pL of normal saline. Mix thoroughly, perform the assay, and multiply the result by a factor of 5. The maximum allowable dilution is 1:10.
b. Reference Range: Serum, female: 0-31 U/L Serum, Male: 0-40 U/L
c. Critical Results: None
d. Analytical Measurement Range: 3-700 U/L
Reportable Range 3-7000 U/L
Limit of Detection (standard 1 + 3 SD): 3 U/L

Aspartate transaminase (AST)

a. Analytical Range:

1. 5-400 IU/L; Up to 2600 IU/L using ORDAC on the DXC800.
2. Samples which are out of ORDAC (Overrange Detection and Correction) range high should be reanalyzed after doing a manual dilution of the sample. The dilution factor must be entered into the sample information. If the dilution factor is not entered into the system, the printout must be multiplied by the dilution factor to obtain the final answer.
3. Limits of detection (LOD) are established by Beckman Coulter and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.

4. Samples with high enzyme activity may consume all of the NADH Substrate before the first absorbance measurement is taken after sample addition. These samples can either report very low enzyme activity or suppress the result at “OIR LO”.
5. Samples with results suppressed “OIR LO” should be diluted 1:20 with saline and reanalyzed.
6. Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the AST determination is 5 IU/L.
7. 0 is not a reportable value.

Serum or Plasma Age Group	*Reference Range IU/L*
0-6 Y	18-63
6-10 Y	21-44
10-20 Y	13-38
>20 Y	13-33

Alkaline phosphatase (ALP)

Out of Range results: Certain tests have pre-programmed limits that trigger an automatic re-analysis by the COBAS. These limits may be low-end values or high-end values (but within technical range). If the duplicate value is in agreement with the initial value, then the initial value is reported.

Results are reported to the whole number, as in x, U/L. Report low results as <2 U/L.

a. ***Reportable Range of Test Results:*** Reportable Range 2-6000 U/L

Intra-assay %CV (10 within-day replicates at a concentration of 91.2 U/L) 0.7% Intra-assay %CV (10 within-day replicates at a concentration of 223.7 U/L) 0.6%Inter-assay %CV (between day replicates at a concentration of 64 U/L) 1.7% Inter-assay %CV (between day replicates at a concentration of 215 U/L) 3.3%

Dilutions: Linear range of the method: 2 - 1200 U/L (serum). Specimens exceeding the high limit are automatically diluted (1:5) by the instrument; results from samples diluted using the rerun function are automatically multiplied by a factor of 5. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor. For example, to perform a 1:5 dilution, pipette 50 µL of

the patient sample into 200 µL of normal saline. Mix thoroughly, perform the assay, and multiply the result by a factor of 5. The maximum allowable dilution is 1:5.

b. ***Reference Range:*** Plasma, female: 35-104 U/L Plasma, Male: 40-129 U/L

c. ***Critical Results:*** None

d. ***Analytical Measurement Range:*** 2-1200 U/L

e. ***Reportable Range of Test Results:*** Reportable Range 2-6000 U/L Limit of Detection (standard 1 + 3 SD): 2 U/L

LIMITATIONS OF METHODS

Alanine transaminase (ALT)

a. Limit of Detection (standard 1 + 3 SD): 3 U/L
   Analytical Measurement Range: 3-700 U/L

b. Icteric index <60: no interference. Hemolytic index <200: no interference. Lipemic index <150: no interference. Drugs: No interference was found at therapeutic concentrations using common drug panels. Exception: Calcium dobesilate and Isoniazid can cause artificially low and Furosemide artificially high ALT results at therapeutic concentrations. Cyanokit (Hydroxocobalamin) may cause interference with results.In very rare cases, gammopathy, in particular IgM (Waldenstrom's macroglobulinemia), may cause unreliable results.

Aspartate transaminase (AST)

a. Hemoglobin causes falsely elevated AST results. Do not do test if sample is hemolyzed.
b. Bilirubin <30 mg/dL has no significant interference.
c. Lipemia >3+ demonstrates positive interference.
d. Pyruvate demonstrates positive interference.
e. Refer to References for other interferences caused by drugs, disease and preanalytical variables.

Alkaline phosphatase (ALP)

a. Limit of Detection (standard 1 + 3 SD): 2 U/L
   Analytical Measurement Range: 2-1200 U/L

b. Icteric index <60 Hemolytic index <200 Lipemic index <2000 Drugs (therapeutic concentrations of commonly used drug panels): no interference. In very rare cases, gammopathy, in particular IgM (Waldenstrom's macroglobulinemia), may cause unreliable results.

TROUBLESHOOTING

Alanine transaminase (ALT)

Should the testing system become inoperable, discontinue testing and notify the supervisor. While instrument trouble-shooting or repair occurs; keep specimens at refrigerated or frozen temperature depending on study specific requirements.

Aspartate transaminase (AST)

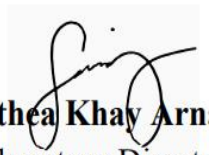
Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

Alkaline phosphatase (ALP)

Should the testing system become inoperable, discontinue testing and notify the supervisor. While instrument trouble-shooting or repair occurs; keep specimens at refrigerated or frozen temperature depending on study specific requirements.

EFFECTIVE DATE

October 6, 2024



Althea Khay Arnaiz
Laboratory Director

Papanicolaou (Pap) Smear Examination

For Cytology Section

PRINCIPLE OF PAPANICOLAOU (PAP) SMEAR EXAMINATION

The Papanicolaou (Pap) smear is a cytological examination to diagnose cervical cancer and any other form of precancerous changes or abnormal cell formation in the cervix. Cells are taken from the cervix via spatula or brush and stained to determine them by microscopic examination for abnormal cellular morphology and malignancy.

SPECIMEN REQUIREMENTS

Patient Preparation

A patient should avoid practices that may interfere with the test results, such as douching, sexual intercourse, and vaginal medication use, for at least 48 hours before undergoing the test. The middle of the menstrual cycle is ideal, as this is when the cervix is least inflamed.

Specimen Collection

Specimen collection for the Pap smear involves scraping or brushing of the cervical cells obtained with a spatula or cytobrush. The cells are then smeared on to a glass slide or collected in liquid preservative.

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

Reagents or Media:

- Papanicolaou stain (hematoxylin, eosin, and OG-6 or equivalent components)
- Fixative (such as an alcohol-based fixative for slides)
- Liquid Preservatives for liquid-based cytology (such as ThinPrep, SurePath)

Supplies or Equipment:

- Cervical Spatula or cytobrush
- Glass slides (for conventional smears)
- Cytology brush or broom (for liquid-based systems)
- Microscope
- Light microscope slide warmer (for drying)
- Immersion oil (if using a high-powered microscope lens)
- Fixative spray (if doing conventional smears)

CALIBRATION

Manual: There is no direct calculation in a conventional Pap smear procedure. However, interpretation or cellular pattern, frequency, and distribution must be recorded.

Calibration (if a machine is involved): If liquid-based cytology systems are used, calibration of an automated machine like ThinPrep or SurePath, is needed. These machines require periodic maintenance and calibration to ensure proper preparation, labeling, and analysis of the sample.

QUALITY CONTROL

- Fix the slides or specimens to prevent deterioration of the cells.
- Regular maintenance and calibration of the microscope and any automated equipment used for analysis.
- Proper staining techniques along with control slides should be done to obtain staining consistency.
- Laboratory workers should be trained to recognize the normal and abnormal cytological features.
- Regular interlaboratory comparisons and participating in proficiency testing programs, like CAP or CLIA, must be performed to ensure accuracy.

STEP-BY-STEP INSTRUCTIONS

1. **Pre-Test Preparation of Patient:** As an essential preparation for the patient, it should be told not to have any vaginal douche, coitus, or application of any vaginal cream at least 48 hours before the test.
2. **Specimen Collection:** Put the patient in a lithotomy position. Put her on back, with the legs opened and flexed at the knees. Insert the speculum into the vagina and see the cervix. Use cervical spatula and cytobrush to collect the specimens of cells from ectocervix and endocervix. If using traditional smears, air-dry the cells onto a glass slide and spray on a fixative. If using liquid-based cytology, immerse the brush into the transport medium.
3. **Smear Function:** For traditional smears, the preparation needs to be fixed instantly by an alcohol-based fixative. This helps in retaining the cellular

structure. Liquid-based cytology systems fix the sample automatically within the transport liquid.

4. **Staining:** The slide prepared should be stained with Papanicolaou stain. This includes a series of stains by hematoxylin, orange G-6, and eosin which would help in staining the cell structures.
5. **Examination under the Microscope:** Study the slide at low and high power using a microscope to identify abnormal cells or presence of malignancy.
6. **Reporting:** Document your findings and issue a report that may include normal results, abnormal results, such as ASCUS, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and carcinoma.

REPORTING RESULTS

Normal: No abnormal cytologic features, the smear showed normal cervical cells.

Abnormal: This would have results like ASCUS (Atypical Squamous Cells of Undetermined Significance), LSIL, HSIL, or even shows presence of malignancy, for example, squamous cell carcinoma.

Inconclusive/Unsatisfactory: The specimen is too small to be analyzed (e.g., not enough cells or obscured by blood or inflammation).

LIMITATION OF METHODS

- **False negatives/positives:** The Pap smear fails to identify all cervical cancers or pre-cancerous changes, especially in the early stages.
- **Inadequate specimen:** A false negative or missed diagnosis may occur if there are too few cells are obscured by blood or inflammation.
- **Patient Factors:** Hormonal changes, for example pregnancy or infections, or recent menstruation, may affect the clarity and accuracy of the smear.

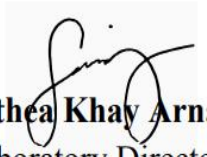
TROUBLESHOOTING

- **Problem**: Insufficient cell collection
  - **Solution:** Ensure proper technique is used when collecting cells from both the ectocervix and endocervix. Use appropriate brushes or spatulas.

- **Problem**: Slide not adequately fixed
  - **Solution:** Immediately fix the sample after collection to avoid cell degradation. Apply an appropriate fixative or immerse in liquid preservative.
- **Problem**: Poor Staining or inconsistent coloration
  - **Solution:** Ensure proper preparation and application of the Papanicolaou stain. Follow manufacturer protocols for staining times and dilution of reagents.

EFFECTIVE DATE

October 6, 2024



Althea Khay Arnaiz
Laboratory Director

Polymerase Chain Reaction (PCR) for Viral Detection

For Virology Section

PRINCIPLE OF POLYMERASE CHAIN REACTION (PCR) FOR VIRAL DETECTION

In Polymerase Chain Reaction (PCR), a single, brief section of a DNA molecule can be replicated several times, in vitro. With a basic set of reagents and a denaturing and annealing cycle that involves heating and cooling, a researcher can produce thousands of identical copies of a DNA template from a single copy. Taq polymerase, an isolate from Thermus aquaticus, is the DNA polymerase I enzyme used in PCR.

SPECIMEN REQUIREMENTS

Acceptable specimens for PCR viral detection include:

- nasopharyngeal swabs,
- throat swabs,
- blood,
- urine,
- stool, and
- saliva,

depending on the specific viral target. Collection should be performed using sterile, RNAse/DNAse-free equipment to prevent contamination and ensure sample integrity. Transport samples in a viral transport medium maintained at 2–8°C and process them within 24 hours. For longer storage, specimens shall be freezed at -80°C to preserve nucleic acid stability. Samples with improper labeling, insufficient quantity, or visible contamination will be rejected to maintain the accuracy and reliability of test results.

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

Reagents

- 2 µL Template DNA (10 ng-500 ng)
- 5 µl 10X Taq buffer with MgCl2
- 1 µl dNTP mix (10 mM each nt)
- 2.5 µL Forward Primer (10 µM stock)
- 2.5 µL Reverse Primer (10 µM stock)
- 36.8 µL Sterile dH2O
- 0.2 µL Taq DNA Polymerase (5 units/µL)
- Agarose Gel

Supplies and Equipment

- Thin-walled PCR tube
- Ice Bucket
- PCR Machine

CALIBRATION

Thermocycler calibration should be performed annually to ensure accuracy in temperature control and ramp speed, which are critical for reliable PCR amplification. On the other hand, micropipettes must be validated every three months using gravimetric or photometric methods to confirm precise volume delivery and prevent pipetting errors. The fluorescence detection system of the PCR machine should also be regularly checked and maintained according to the manufacturer's recommendations to ensure consistent and accurate signal detection during amplification.

QUALITY CONTROL

Quality control measures are essential to ensure the reliability of PCR results. Positive controls containing the target nucleic acid must be included to confirm the assay is performing correctly and detecting the intended sequences. Negative controls, which lack target nucleic acid, will also be used to identify potential contamination during the testing process. Internal controls, such as housekeeping genes or spiked nucleic acids, shall be incorporated to verify the efficiency and integrity of the amplification process. All control results are to be thoroughly reviewed before releasing patient results to confirm the test accuracy and reliability.

STEP-BY-STEP INSTRUCTIONS

1. Primer Design

- Refer to the protocol for designing primers.
- Use **Primer3** (clickable link) to help select the appropriate primers.

2. Reaction Setup

- Keep reagents on ice throughout the process.
- For each reaction (50 µL total), add the following into thin-walled PCR tubes:

- **2 µL Template DNA** (10 ng–500 ng)
- **5 µL 10X Taq buffer with MgCl2**
- **1 µL dNTP mix** (10 mM each nucleotide)
- **2.5 µL Forward Primer** (10 µM stock)
- **2.5 µL Reverse Primer** (10 µM stock)
- **0.2 µL Taq DNA Polymerase** (5 units/µL)
- **36.8 µL Sterile dH2O** (adjust to 50 µL total volume)

Tip: If performing multiple PCR reactions, prepare a **master mix** to save time. Combine all reagents (except primers and template) in a larger volume, and distribute into PCR tubes.

3. Master Mix Preparation

For a specific number of reactions, multiply each reagent by the number of reactions needed and add 10% extra for pipetting errors. In this example, we have:

- **10X Taq buffer with MgCl2**: 38.5 µL
- **dNTP mix (10 mM each nucleotide)**: 7.7 µL
- **Template DNA**: 15.4 µL
- **Sterile dH2O**: 283.3 µL
- **Taq DNA Polymerase**: 1.54 µL

Combine these in a 1.5 mL tube and gently mix by pipetting. Keep the tube on ice.

4. PCR Tube Preparation

- **Add primers**. Pipet the **forward primer** onto one side and the **reverse primer** onto the other side of each PCR tube.
- **Add master mix**. Dispense 45 µL of master mix (calculated from the total mix minus the primers) into each PCR tube.
- Gently tap the tubes to ensure all liquid is at the bottom.

5. PCR Machine Setup

- Place the prepared PCR tubes into the PCR machine.
- Set the following conditions:

- **Initial Denaturation**: 94°C for 2 minutes
- **Denaturation**: 94°C for 30 seconds
- **Annealing**: 55°C for 30 seconds (5°C below primer Tm)
- **Extension**: 72°C for 2 minutes (per 1 kb product length)
- Repeat steps for **25–30 cycles**
- **Final Extension**: 72°C for 5 minutes

6. Check PCR Product

- After PCR is complete, run **2 µL of the PCR reaction** on an agarose gel to check the size and concentration of the PCR product.

Tip: Use a gel loading dye to track the progress and visualize the PCR product on the gel.

REPORTING RESULTS

In PCR, amplification of DNA is observed through fluorescent dyes or sequence-specific probes. The quantification cycle (Cq) represents the number of cycles required for fluorescence to reach a threshold, and it is used to determine whether the sample is positive or negative. PCR efficiency, which impacts Cq values, reflects the amplification rate per cycle and is ideally close to 100% (a fold value of 2). PCR efficiency can be assessed through standard and amplification curves, though standard curves are prone to dilution errors. A low PCR efficiency requires additional cycles to reach the quantification threshold, resulting in a higher Cq.

qPCR measures DNA or RNA levels and assumes 100% assay efficiency, with results typically reported using Cq, delta-Cq, or delta-delta-Cq values. Efficiency correction is essential for accurate interpretation of clinical samples. Cq values, combined with clinical data, can be used to assess disease stage, monitor progression, and predict recovery. They can also assist contact tracers by identifying patients with a higher viral load, indicating greater potential for disease transmission.

LIMITATIONS OF METHODS

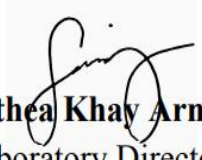
- **Sample Quality**. Poor or degraded samples can lead to either false negatives. Or false positives
- **Contamination**. Improper handling may cause false positives or false negatives.
- **Specificity**. Cross-reactivity with non-target sequences may occur if primers or probes are not highly specific.

TROUBLESHOOTING

1. **No amplification in positive control.**
   Degraded reagents, incorrect thermocycler settings, or pipetting errors can cause the lack of amplification in the positive control. To resolve this, verify the quality of reagents, check the thermocycler programming, and ensure accurate pipetting. Re-setup the reaction if necessary.
2. **High background signal or noise of the machine.**
   Contamination of reagents or the work area often leads to high background signals or noise. To fix this, decontaminate the workspace, replace reagents, and use fresh controls to rule out contamination before rerunning the PCR.
3. **Weak or inconsistent internal control signal.**
   Weak or inconsistent internal control signals are usually caused by poor nucleic acid extraction or reaction inhibition. To address this, assess extraction efficiency, dilute the template if necessary, and remove inhibitors before rerunning the PCR.

EFFECTIVE DATE

October 6, 2024



Althea Khay Arnaiz
Laboratory Director

Urine Drug Screening

For Drug Test and Toxicology Section

PRINCIPE OF URINE DRUG SCREENING

The validity of urine drug screen results is dependent on specimen integrity. While direct-observation collections provide specimens of the greatest credibility, non-witnessed collections can be effective if safeguards are in place to ensure the donor does not have access to substances that may affect test results (water, chemicals, substitute urine, etc.).

SPECIMEN REQUIREMENTS

Drug testing

Testing for specimen substitution and/or adulteration is performed by most laboratories upon request and may include one or both of the following: temperature strips affixed to urine collection containers to monitor the temperature of the collected specimen at the point of collection, chemical tests for adulteration and dilution (pH, specific gravity, creatinine, oxidants, etc.).

Toxicology

In the setting of acute illness, serum, and urine samples are acquired for laboratory testing without specific concern for drug screening. Urine specimen requirements for screening do exist, mostly in the context of mandated or routine testing. Typically, a collection of the specimen should occur within 4 minutes of providing a sample and with a volume of at least 30 mL. The urine temperature should be between 32.2 C (90 F) and 37.7 C (100 F), with a pH of 4.5 to 8.5. These requirements are essentially an attempt to ensure the sample is from the individual providing it and without added diluents or substances that might interfere with testing.

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

Reagents or Media:

- **Immunoassay:** Enzyme Immunoassays (EIAs) and antibodies
- **Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Mass Spectrometry (LC-MS):** GC derivatization reagents, calibration standards, and solvents

Supplies & Equipment:

- Collection Kit consisting of a specimen cup with temperature strip
- The requisition form provided by the laboratory

- Bluing (coloring) agent to add to the toilet bowl/water tank to prevent a patient from diluting the specimen.
- Single-use disposable gloves are recommended for collectors to use while handling specimens.
- Tamper-evident tape for securing faucets, toilet tank tops, and other appropriate areas, and signs, when necessary, that can be posted to prevent entry into collection areas.
- Automated chemistry analyzers
- Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

CALIBRATION

Immunoassays

Drug testing immunoassays are typically calibrated for qualitative analysis using a single standard measurement and at a calibrated cutoff concentration. Some immunoassays support the use of multiple calibration points for semi-quantitative application and reporting of results. Regardless of qualitative or semi-quantitative assay configuration, the combination of antibody cross-reactivity (binding affinity), reagent formulation, and limits of the analytical instrumentation measurement range define the immunoassay's relative sensitivity, linearity, and cutoff precision. By measuring whether the cumulative antigen-antibody response of a donor's specimen is equal to or greater than the calibrated cutoff, the immunoassay test provides results that are used to differentiate "negative" from "presumptive positive" specimens. Immunoassay test results that are equal to or greater than the cutoff are always considered "presumptive" positive for the following reasons:

• Immunoassays do not provide a definitive identification of the measurand.

• False positive, non-drug class cross-reactivity or interference cannot be distinguished from true positive results.

Gas Chromatography-Mass Spectrometry (GC-MS)

Internal Standards

If injection volumes vary or if the concentration may change easily because the solvent is volatile, we can still create a calibration using an internal standard as a

reference. The internal standard is a compound added to every solution that is used as a reference to compare to the analyte's signal. The species, which we call an internal standard, must be different than the analyte.

Because the analyte and the internal standard receive the same treatment, the ratio of their signals is unaffected by any lack of reproducibility in the injection procedure or changes in concentration due to evaporation. If a solution contains an analyte of concentration C_A and an internal standard of concentration C_{IS}, then the signals due to the analyte, S_A, and the internal standard, S_{IS}, are

$$S_A = k_A C_A$$

$$S_{IS} = k_{SI} C_{IS}$$

$$\frac{S_A}{S_{IS}} = \frac{k_A C_A}{k_{IS} C_{IS}} = K \times \frac{C_A}{C_{IS}}$$

K is the relative sensitivity (relative response factor) for the analyte compared to the internal standard. Because K is a ratio of the analyte's sensitivity and the internal standard's sensitivity, it is not necessary to determine independently values for either k_A or k_{IS}

QUALITY CONTROL

False positives can occur due to cross-reactivity between other substances not being tested for and the immunoassay being used.

A major consideration when using drug testing is regarding the significance of a negative result. Clinicians should bear in mind that a negative result simply means that the particular substance being tested for was not detected. This may mean its level was not sufficient enough to be detected or that the use of that substance did not occur during the detection window. A negative result does not rule out the use of a substance or the presence of a substance use disorder. False negatives are not uncommon, particularly if the clinician is not aware of what is being tested for in a given immunoassay. A common example is in the testing of benzodiazepines, where

the immunoassay is directed at the detection of oxazepam and is not intended to detect benzodiazepines such as clonazepam or alprazolam.

Clinicians must understand the testing methodology of various drug testing modalities as well as their associated sensitivity, specificity, and significance of false-negative and false-positive results.

STEP-BY-STEP INSTRUCTIONS

Equipment Operation. Operators of testing equipment must be certified by the manufacturer. At no time may a non-certified person operate testing equipment except under the direct supervision of a certified operator for training purposes.

All protocols and procedures recommended by the manufacturer for the storage preparation, calibration, operation, and maintenance of testing equipment and use of reagents will be observed at all times.

Positive Test Results. All specimens testing positive for any drug must be subjected to a second test for that drug using the same technology. A fresh aliquot must be used whenever positives are retested. If a second test result is negative, the results of testing will be reported to the court as negative. If the second test is positive, the results of testing will be reported to the court as presumptive positive. In the case of a positive test, the sample must be stored in a secure freezer for up to 30 days unless the defendant signs a written statement admitting the positive test result. If ordered by the court, the sample should be sent to the contract laboratory for confirmation using GC/MS.

Hand-held Testing Devices. Only hand-held testing devices approved by the Administrative Office of U.S. Courts will be utilized in this project. Districts utilizing hand-held drug testing devices must have written guidelines and procedures for the use of these kits. The following are the minimum guidelines:

1. District staff are required to receive training on the use of the device prior to implementation.

2. The court is to be notified that hand-held devices are used in the initial screening of urine samples. Such tests are not to be used in violation hearings.

3. If an offender admits to a presumptive positive, document such admission in the chronological record and obtain a signed statement of admission from the defendant. Confirmation by the national laboratory is not necessary unless the results will be used in a violation proceeding. Confirmation from the national laboratory is required for all presumptive positive results contested by the defendant.

3. Cut-off levels of the hand-held devices must be the same as those used by the national laboratory. Do not request the national laboratory to test results from hand-held devices at any other cut-off levels. The current and legally defensible cut-offs are:

Marijuana metabolites: 50 ng/ml
Cocaine metabolites: 300 ng/ml
Opiate metabolites: 300 ng/ml
Phencyclidine: 25 ng/ml
Amphetamines: 1,000 ng/ml

5. Districts are required to maintain quality control by sending 1 of every 40 negative hand-held tests to the laboratory for testing. If the total amount of your purchase of these kits for the fiscal year is less than $2,500, you may use a purchase order.

If the total cost exceeds $2,500, then you must obtain price quotes from at least three vendors and maintain a written justification for the purchase in your records.

The testing area must be locked and secured by the certified operator at all times when the lab is not in use. Only certified personnel and persons being trained by certified personnel may be permitted in the testing area.

Supplies and equipment must be stored in a secure area, accessible only to authorized pretrial services personnel. At no time may defendants or the general public enter a

secure area. Access by cleaning or building maintenance personnel should be limited to working hours when pretrial services personnel are present.

Chain of Custody. Chain of custody documentation is critical to the integrity of the testing program. Possession of the specimen must be accounted for at all times. Changes in possession must be recorded. All personnel assigned to drug testing must be thoroughly familiar with all forms and must complete them at the required times. All such personnel must have a copy of this manual for reference at all times.

Recording Test Results (PS 21 Section B). The individual receiving the specimen from the collector must sign the first line in Section B and note the date and time. Likewise, the tester must sign the second line and note the date and time.

The tester must check the appropriate box reflecting the results of each test. A positive result should not be entered until a rerun has been completed. The person completing the rerun must sign line 3 and note the date and time.

REPORTING RESULTS

Test results, as well as details regarding the date and time of the urine drop and test, should be incorporated in the "health" section of the pretrial services report as additional information for consideration by the court. Other information concerning drug usage by the defendant should also be included here. The source(s) of information must be clearly cited.

Recommendations. A positive test result, in itself, should never result in a recommendation of pretrial detention. The pretrial services officer should treat positive test results as information to be considered when attempting to tailor appropriate conditions of release and should not trigger "automatic" recommendations. The officer should consider the overall circumstances of the case (see 18 U.S.C. 3142(g)) before making a recommendation. In the case of a positive test result, consideration may be given to ongoing drug monitoring by pretrial services, drug

treatment by a contract program in the community, or by an appropriate combination of both.

Recording Court Information (PS 21. Section C). This section must be completed after the defendant has appeared before a judicial officer and a determination has been made with respect to release or detention.

LIMITATION OF METHODS

- Urine drug tests do not provide information regarding the length of time since the last ingestion, the overall duration of use, or the state of intoxication.

- Sometimes, urine can be difficult to obtain due to dehydration, urinary retention (the person is unable to empty their bladder), or other reasons.

- Drug testing can be a useful tool, but it should not be the only tool for making decisions. Drug testing results should be considered alongside a patient's self-reports, treatment history, psychosocial assessment, physical examination, and a practitioner's clinical judgment.

- Drug testing can also produce false positives and false negatives.

TROUBLESHOOTING

1. False positive

A drug test shows the presence of a substance that isn't there. This can happen during the initial urine drug screening, which uses the immunoassay method (antibodies to detect drugs at the molecular level). Immunoassays are the most commonly available method of testing for drugs in urine. Immunoassays rely on a chemical reaction between an antibody and a drug the test is designed to identify. Sometimes, the antibodies can react to other chemicals that are similar to the drug—called cross-reactivity. Cross-reactivity can occur with some over-the-counter medicines,

prescription medicines, and certain foods, like poppy seeds. For example, some cough and cold medicines, antidepressants, and antibiotics can cause false positive results.

2. False negative

A drug test does not show the presence of a substance that is there. This can happen during the initial urine drug screening. A false negative result can happen when the cutoff level used is set too high, so small amounts of the drug or drug metabolites are missed. False negatives can also happen when contaminants are deliberately ingested or added to urine to interfere with a test's ability to detect a drug's presence.

Troubleshooting. A confirmatory test can be performed to confirm the initial screening test results. A medical review officer can also interview the patient and review the lab results to help resolve any discrepancies

3. Sample Tampering

One common method is dilution, either by dilution by adding an adulterant or by increasing fluid intake prior to the test. Common adulterants include household items, such as bleach, laundry detergent, and table salt. Commercial products directed at bypassing UDT also exist, such as UrinAid (glutaraldehyde), Stealth (containing peroxidase and peroxide), Urine Luck (pyridinium chlorochromate), and Klear (potassium nitrite). These products are easily obtained through various internet sources. Synthetic urine is another common adulterant. These adulterants can be used to bypass both presumptive and confirmatory tests.

Troubleshooting. Several on-site adulteration detection strips and devices are commercially available. Certain urinalysis tests contain individual strips that can detect pH, creatinine, glutaraldehyde, nitrites, PCC, and other oxidants.

EFFECTIVE DATE

October 6, 2024

Althea Khay Arnaiz
Laboratory Director

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