PROCEDURE MANUAL

Prepared By :
LabduB Clinical Laboratory

labdub@gmail.com
Dumaguete City, Negros Oriental
https://labdub26.wixstudio.com/my-site

TABLE OF CONTENTS

TECHNICAL PROCEDURE:
ABO BLOOD TYPING

Procedure Title: ABO Blood Group Determination by Forward and Reverse Typing
Department: Blood Bank (Immunohematology)
Procedure Type: Manual Serologic Testing – Tube Method
Effective Date: August 28, 2026
Prepared by: Princess Mekaella S. Araneta
Reviewed by Clinical Pathologist: Seth Mikylla Loise S. Auza
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

ABO blood typing utilizes the principle of antigen-antibody agglutination reaction. Forward typing is performed by mixing red blood cells suspension with specific antisera against A and B antigens to detect the presence of corresponding antigens on the surface of the red blood cells. Reverse typing involves testing patients' serum or plasma with A_1 and B reagent red blood cells to detect corresponding ABO antibody. The forward and reverse grouping must be concordant before reporting the blood group.

SPECIMEN REQUIREMENTS

I. Collection

Collect whole blood from the patient through venipuncture, usually from a suitable vein such as the median cubital vein. The blood should be collected in an EDTA (lavender-top) tube to prevent clotting.

II. Specimen Type

The required specimen is **properly collected whole blood**. It must contain enough blood for the ABO blood typing procedure and should not be excessively hemolyzed, clotted, or otherwise compromised.

III. Labeling

The specimen must be **clearly and correctly labeled immediately after collection**. The label should include the patient's **full name and other required identifiers**, such as patient ID or date of birth, according to laboratory policy.

IV. Patient Identification

The patient's identity must be **verified before blood collection and testing** by checking the patient's identifiers against the request form or laboratory record. This helps prevent testing the wrong patient's specimen.

V. Specimen Handling/Transport

After collection, the blood specimen should be **properly mixed with the anticoagulant** by gently inverting the tube. It should be handled and transported according to the laboratory's established procedure to maintain specimen quality.

VI. Specimen Rejection

Specimens that are **unlabeled, mislabeled, clotted, insufficient, or significantly compromised** should not be tested and must be handled according to laboratory policy.

VII. Discrepant Results

If the ABO typing results are **inconsistent or questionable**, the result must be investigated. The patient's identification, specimen quality, and testing procedure should be checked, and repeat or additional testing may be performed according to laboratory policy.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

I. Reagents

- Anti-A reagent
- Anti-B reagent
- A_1 reagent red cells
- B reagent red cells
- Isotonic saline

II. Supplies

- Clean test tubes
- Test tube rack
- Transfer pipettes/dropper
- Applicator sticks or mixing sticks
- Appropriate labels
- Personal protective equipment

III. Equipment

- Serologic centrifuge
- Timer
- Other equipment specified by the laboratory's validated procedure

IV. Storage Requirements

- Store Anti-A reagent according to manufacturer's instructions
- Store Anti-B reagent according to manufacturer's instructions
- Store A_1 reagent red cells according to manufacturer's instructions
- Store B reagent red cells according to manufacturer's instructions
- Store isotonic saline according to manufacturer's instructions
- Keep reagents in properly labeled containers
- Observe recommended storage temperature
- Do not use expired reagents
- Protect reagents from contamination and deterioration

CALIBRATION

Not applicable to the ABO blood typing procedure.

QUALITY CONTROL

I. Control Materials

Use appropriate **positive and negative control materials** for ABO blood typing according to the reagent manufacturer's instructions and the laboratory's SOP.

II. Reagent Inspection

Check the **appearance, labeling, expiration date, and storage conditions** of Anti-A, Anti-B, A_1, and B reagents before testing.

III. Expected Control Results

Control reactions must produce the **expected positive and negative reactions** before patient specimens are tested.

IV. QC Failure

If control results are **unexpected or unacceptable**, do not report patient results. Investigate the possible cause and resolve the problem before repeating QC testing.

V. Documentation

Record all **QC results, corrective actions, and repeat QC results** according to the laboratory's quality control policy.

STEP-BY-STEP INSTRUCTIONS

I. Forward ABO Typing

1. Prepare the patient's red blood cell suspension according to the laboratory's validated procedure and reagent manufacturer's instructions.
2. Label clean test tubes as Anti-A and Anti-B.
3. Add the required amount of Anti-A reagent to the Anti-A tube.
4. Add the required amount of Anti-B reagent to the Anti-B tube.
5. Add the patient's red cell suspension to the corresponding tubes.
6. Mix the contents of each tube thoroughly.
7. Centrifuge according to the validated procedure or manufacturer's instructions.
8. Gently resuspend the cell button.
9. Examine each tube for agglutination.
10. Record the reaction strength and results.

II. Reverse ABO Typing

1. Label separate tubes as A_1 cells and B cells.
2. Add the patient's serum or plasma to the appropriately labeled tubes.
3. Add the corresponding A_1 reagent red cells to the A_1 tube.
4. Add the corresponding B reagent red cells to the B tube.
5. Mix the contents of each tube.
6. Centrifuge according to the validated procedure or manufacturer's instructions.
7. Gently resuspend the cell button.
8. Examine each tube for agglutination.
9. Record the reaction strength and results.

CALCULATIONS

Not applicable to the ABO blood typing procedure.

ABO blood typing is a qualitative test in which the presence or absence of agglutination is interpreted to determine the ABO blood group.

REPORTING RESULTS

Report the ABO blood group according to concordant forward and reverse typing results:

Forward Typing	Reverse Typing	ABO Group
Anti-A: Positive; Anti-B: Negative	A_1 cells: Negative; B cells: Positive	A
Anti-A: Negative; Anti-B: Positive	A_1 cells: Positive; B cells: Negative	B
Anti-A: Positive; Anti-B: Positive	A_1 cells: Negative; B cells: Negative	AB
Anti-A: Positive; Anti-B: Positive	A_1 cells: Positive; B cells: Positive	O

PROCEDURE NOTES

I. Patient Identification

Verify the patient's identity using at least the required identifiers before specimen collection and testing. Ensure that the information on the specimen label matches the patient's request form or laboratory record to prevent identification errors.

II. Reagent Instructions

Follow the manufacturer's instructions and the laboratory SOP when using Anti-A, Anti-B, A_1, B, and other reagents. Use the specified volumes, reaction conditions, and interpretation criteria for accurate ABO typing.

III. Reagent Condition

Do not use reagents that are expired, contaminated, improperly stored, or showing signs of deterioration. Check the reagent condition and storage requirements before beginning the test.

IV. Reaction Reading

Observe and interpret agglutination reactions carefully and within the required reading time. Record the reaction strength accurately according to the laboratory's validated procedure.

V. Contamination Prevention

Prevent contamination of patient specimens, reagents, test tubes, pipettes, and other materials during testing. Use clean supplies and avoid mixing reagent bottles, pipettes, or specimens with one another.

VI. Result Recording

Record all ABO typing results accurately, clearly, and completely. Ensure that the recorded results correspond to the correct patient specimen and include all required reaction observations.

VII. Unexpected Results

Unexpected, inconsistent, or discrepant ABO typing results must not be reported immediately. Repeat the testing and investigate possible causes, including specimen identification, reagent condition, and testing procedure, before reporting the final result.

VIII. Laboratory Safety

Wear appropriate PPE, including gloves and laboratory protective clothing, when handling blood specimens and reagents. Follow standard laboratory safety and infection-control precautions throughout the procedure.

LIMITATIONS OF METHODS

I. Weak or unusual ABO subgroups

Some people have uncommon ABO subgroups that may react only weakly, making the blood type harder to identify.

II. Recent transfusion, diseases, age, or other conditions

These can cause the forward and reverse typing results to disagree, creating an ABO discrepancy.

III. Problems with the specimen or testing process

Wrong patient/sample identification, expired or deteriorated reagents, contamination, or incorrect technique can produce inaccurate results.

IV. ABO typing is not enough for compatibility

ABO typing tells you the ABO group, but it does not determine Rh(D) status or establish complete transfusion compatibility.

V. Discrepancies must be investigated

If the forward and reverse typing do not match, the result should not be reported immediately. Further investigation is needed to resolve the discrepancy.

A TROUBLESHOOTING OR BACK-UP PLAN

I. Verify the patient's identification and specimen labeling.

Check the patient's identification and compare it with the information on the specimen label and laboratory request form. This ensures that the specimen belongs to the correct patient and prevents identification errors.

II. Check reagent expiration dates, storage conditions, and appearance.

Inspect the reagents to make sure they are not expired, have been stored at the required temperature and conditions, and show no signs of contamination, discoloration, or deterioration. Improper reagents may produce inaccurate results.

III. Verify that the correct reagents and cells were used.

Confirm that the appropriate anti-A, anti-B, and reagent red blood cells were used for the test. Using an incorrect reagent or cell suspension can lead to an incorrect ABO interpretation.

IV. Repeat the test using the validated laboratory procedure.

If the results are questionable or inconsistent, repeat the ABO typing following the laboratory's approved procedure. This helps determine whether the discrepancy was caused by a testing or procedural error.

V. Check the centrifuge and other equipment for proper operation.

Make sure that the centrifuge and other equipment used during testing are functioning correctly and are being operated according to laboratory procedures. Improper equipment operation can affect agglutination and test results.

VI. Review both forward and reverse typing results.

Compare the results from forward typing with those from reverse typing. Normally, the results should correspond with each other. Any disagreement between the two methods should be investigated before reporting the blood type.

VII. Refer to the specimen if the discrepancy persists.

If the ABO discrepancy remains after appropriate troubleshooting, refer the specimen to the appropriate senior laboratory personnel or reference laboratory according to the laboratory's established policy. Further testing may be necessary to determine the cause of the discrepancy.

VIII. Do not issue a final ABO result until the discrepancy is resolved.

A final ABO blood type should not be reported while there is an unresolved discrepancy. The result must be appropriately investigated and resolved to help prevent an incorrect blood type from being reported.

TECHNICAL PROCEDURE:
BLOOD GLUCOSE TEST

Procedure Title: Blood Glucose Test
Department: Clinical Chemistry
Procedure Type: Quantitative Blood Glucose Determination
Effective Date: September 19, 2026
Prepared by: Ayeszha Khirzt A. Balderas
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

The blood glucose test measures the amount of glucose in a patient's blood. Glucose is the body's main source of energy, and its concentration in the blood is carefully regulated by hormones such as insulin and glucagon.

In the laboratory, the patient's blood sample is tested using an appropriate glucose testing method. The amount of glucose present is measured and compared with the established reference interval. The result may help assess and monitor conditions such as diabetes mellitus, hypoglycemia, and other disorders affecting glucose metabolism.

The test should be performed carefully because proper specimen collection, handling, and testing conditions can affect the accuracy of the result.

SPECIMEN REQUIREMENTS

I. Collection

A venous blood specimen is collected from the patient using proper phlebotomy procedures. The patient may be required to fast before collection if a fasting blood glucose test is requested.

The primary collection container is a gray-top tube (Sodium Fluoride/Potassium Oxalate) to inhibit glycolysis. Alternatively, a green-top tube (Lithium Heparin) or SST (Serum Separator Tube) may be used if the specimen is centrifuged and separated immediately after collection.

II. Labeling

The specimen must be labeled immediately after collection. The label should contain the patient's full name or identification number, the date and time of collection, and any other information required by the laboratory. Proper identification is important to prevent patient and specimen mix-ups.

III. Rejection

A specimen may be rejected if it is:

- Unlabeled or incorrectly labeled
- Collected in an inappropriate container
- Insufficient in volume
- Grossly hemolyzed which may interfere with the method
- Clotted when plasma is required
- Improperly stored or transported
- Received beyond the acceptable stability period

If a specimen is rejected, the reason should be documented, and a new specimen should be requested when necessary.

IV. Storage and Transport

Specimens should be transported to the laboratory as soon as possible after collection. They should be stored under the temperature and time conditions recommended for the specific glucose testing method. Delays in separating serum or plasma from blood cells may cause glucose levels to decrease because the cells continue to use glucose.

V. Procedures for Submission to Central Laboratories

If glucose testing is not performed in the laboratory, the specimen should be properly identified, packaged, and transported in accordance with the receiving laboratory's requirements. The request form and specimen must be checked to ensure that the patient's information matches.

VI. Procedures for Microscopic Examination

Not applicable to the blood glucose test procedure because microscopic examination is not required for this.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

The following materials may be required depending on the glucose testing method used:

- Patient's blood specimen
- Appropriate blood collection tube
- Glucose reagent or testing kit
- Calibrators
- Quality control materials
- Micropipettes and appropriate tips
- Test tubes or reaction cuvettes
- Centrifuge, when applicable
- Chemistry analyzer or glucose meter
- Personal protective equipment (PPE)
- Gloves
- Laboratory coat
- Disinfectant
- Biohazard waste container

All reagents and equipment should be checked before use to make sure they are not expired, damaged, or contaminated.

CALIBRATION

The glucose analyzer or testing device should be calibrated according to the manufacturer's instructions. Calibration should be performed when required by the method, such as when a new reagent lot is introduced or when the instrument indicates that calibration is necessary.

Calibration results should be checked before patient specimens are tested. If calibration does not meet the required criteria, patient testing should not proceed until the problem has been corrected.

QUALITY CONTROL

I. Identify Control Materials to Use

Normal and abnormal glucose control materials should be used to check the performance of the testing system.

II. Preparation, Handling, and Storage

Control materials should be prepared, handled, and stored according to the manufacturer's instructions. Contamination should be avoided, and controls should not be used after their expiration date or stated stability period.

III. Frequency of Testing

Quality control should be performed according to the laboratory's quality control policy and the manufacturer's recommendations. Controls should also be tested when required after calibration, reagent changes, maintenance, or other situations that may affect test performance.

IV. Expected Results

Control results should fall within the acceptable ranges provided by the manufacturer or established by the laboratory.

V. Corrective Actions

If a control result is outside the acceptable range, patient results should not be reported until the problem is investigated. Possible causes such as expired reagents, incorrect preparation, instrument problems, or improper storage should be checked. Quality control should be repeated after the problem has been corrected.

VI. Recording and Storage of QC Data

All quality control results, including any corrective actions taken, should be properly recorded and stored in accordance with laboratory policy.

VII. Alternatives (If no QC materials are available)

Patient testing should ideally not be performed without appropriate quality control materials. If QC materials are temporarily unavailable, testing should follow the laboratory's established contingency procedure, and the situation should be reported to the appropriate laboratory personnel.

STEP-BY-STEP INSTRUCTIONS

I. Preparation

1. Wash or sanitize hands and put on the appropriate PPE.
2. Check the patient's identification and test request.
3. Check the specimen for proper labeling, volume, and condition.
4. Prepare the required reagents, supplies, and equipment.
5. Check the expiration dates and storage conditions of the reagents.
6. Make sure the instrument is functioning properly and that required QC has been completed.

II. Quantitative Testing

- **Method:** Glucose oxidase–peroxidase (GOD-POD/Trinder) colorimetric method (Shaker & Swift, 2023)

1. Bring the glucose reagent, calibrator, controls, and patient serum/plasma specimen to the required testing conditions, as specified by the reagent manufacturer's instructions.
2. Prepare labeled reaction tubes or cuvettes for the blank, standard/calibrator, control, and patient specimen.
3. Add the required volume of glucose reagent to each tube.
4. Add the required volume of calibrator, control, or patient specimen to the corresponding tube.
5. Mix thoroughly without causing contamination or excessive foaming.
6. Incubate the reaction mixture at the temperature and for the time specified by the reagent manufacturer.
7. Measure the absorbance of the reaction at 505 nm (or 500–520 nm, depending on reagent specifications) against the reagent blank using a calibrated chemistry analyzer or spectrophotometer.
8. Determine the glucose concentration using the calibration curve or the analyzer's programmed calculation.
9. Verify that the QC results are within the acceptable range before accepting patient results.
10. Review the patient's result for analytical flags, errors, or results outside the analytical measurement range before reporting.

III. Qualitative Testing

Not applicable to the blood glucose test procedure.

The blood glucose test described in this procedure is a quantitative test intended to determine the glucose concentration in the patient's blood.

IV. Interpretation

The patient's glucose result should be compared with the appropriate reference interval and interpreted together with the patient's clinical information.

A result that falls outside the expected range does not automatically establish a diagnosis. Further testing or clinical evaluation may be needed.

CALCULATIONS

For automated chemistry analyzers, the glucose concentration is calculated automatically using the programmed calibration curve.

For manual or semi-automated spectrophotometric determinations, calculate the glucose concentration using the following formula:

$$Glucose\ Concentration = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard}\ x\ Concentration\ of\ the\ Standard$$

REPORTING RESULTS

I. Reference Intervals

Reference intervals should be based on the laboratory's established values and the specific type of glucose test performed, such as fasting or random blood glucose. The laboratory's current reference interval should always be used when reporting patient results.

Values:

Fasting Blood Glucose (Normal): *70 - 99 mg/dl*
Impaired*: 100 - 125 mg/dl*
Diagnostic of Diabetes Mellitus*: 126 mg/dl and above*

II. Procedures for reporting abnormal results

Results that are significantly outside the laboratory's established limits should be reviewed before reporting. Critical results should be handled according to the laboratory's critical-value policy and communicated to the appropriate healthcare professional when required.

III. Reporting format

The result should be reported using the appropriate unit, such as **mg/dL** or **mmol/L**, together with the applicable reference interval.

Example:

Blood Glucose: ____ *mg/dL*
Reference Interval: ____ *mg/dL*

PROCEDURE NOTES

I. Special Precautions

- Always verify the patient's identity before testing.
- Follow proper infection-control and biosafety practices.
- Treat all blood specimens as potentially infectious.
- Wear appropriate PPE throughout the procedure.
- Do not use expired or improperly stored reagents.
- Avoid contamination of specimens and reagents.
- Follow the manufacturer's instructions for the specific analyzer or testing kit.

II. Possible Sources of Error

Possible errors may result from:

- Improper specimen collection
- Delayed separation of serum or plasma
- Incorrect specimen identification
- Improper reagent storage
- Expired reagents
- Incorrect pipetting
- Instrument malfunction
- Inadequate calibration
- Failure of quality control
- Incorrect timing or temperature during testing

III. Answers to Common Problems

Common Problem	Possible Cause / Corrective Action
Glucose result is unexpectedly high or low	Check specimen identification, QC results, calibration, and possible specimen-related factors. Repeat testing when appropriate.
QC result is outside the acceptable range	Check reagent condition, control preparation, calibration, instrument function, and storage conditions. Repeat QC after correction.
Instrument displays an error	Check the instrument message and follow the manufacturer's troubleshooting instructions.

Specimen is hemolyzed or unsuitable	Evaluate whether the specimen can be tested according to the method. Request a new specimen if necessary.
Insufficient specimen	Do not proceed if the volume is inadequate. Request an appropriate new specimen when needed.

LIMITATIONS OF METHODS

The accuracy of a blood glucose test can be affected by factors related to the specimen, reagents, equipment, and testing procedure. Glucose concentration may also be affected by the time between blood collection and processing. Certain medications, substances, or interfering compounds may affect some glucose testing methods. Results should therefore be interpreted together with the patient's clinical condition and other laboratory findings. The limitations and potential interfering substances listed by the manufacturer of the specific glucose reagent or analyzer should also be followed.

A TROUBLESHOOTING OR BACK-UP PLAN

If the primary analyzer or testing method cannot be used, testing should be performed using an approved backup method or, if available, another validated instrument. If the problem involves reagents, equipment, calibration, or quality control, patient testing should be temporarily stopped until the issue is resolved. All troubleshooting activities should be documented, and the laboratory supervisor or appropriate personnel should be informed when necessary.

TECHNICAL PROCEDURE:
BLOOD SMEAR EXAMINATION

Procedure Title: Blood Smear Examination
Department: Clinical Microscopy
Procedure Type: Standard Procedure Type
Effective Date: September 21, 2026
Prepared by: Mishka Isabel F. Salaveria
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

Blood smear examination is a laboratory procedure in which a thin layer of peripheral blood is spread onto a glass slide, stained, and examined microscopically. It is used to evaluate the morphology and distribution of blood cells and may provide information about hematologic abnormalities, infections, and other blood-related conditions.

The examination consists of:

1. **Blood smear preparation** – a small amount of peripheral blood is spread evenly across a clean glass slide to produce a thin smear with an appropriate examination area.
2. **Staining** – the prepared blood smear is stained using an appropriate Romanowsky-type stain, such as Wright or Wright-Giemsa stain, to differentiate blood cell structures.
3. **Microscopic examination** – the stained smear is examined using the microscope to evaluate red blood cells, white blood cells, and platelets, including their morphology and distribution.
4. **Reporting of findings** – observed blood cell characteristics and significant abnormalities are recorded according to the laboratory's established reporting format.

SPECIMEN REQUIREMENTS

I. Collection

A peripheral blood specimen should be collected using an appropriate anticoagulated blood specimen, commonly whole blood collected in an EDTA tube. The specimen should be properly mixed after collection to prevent clot formation. A blood smear should be prepared as soon as possible after collection to preserve normal blood cell morphology and minimize changes caused by storage.

II. Labeling

The blood specimen tube should be properly labeled with the patient's complete name, identification number, date and time of collection, and other required identifiers according to laboratory policy. The information on the specimen tube must correspond with the laboratory request form.

III. Rejection

A blood specimen may be rejected when it is unlabeled or improperly labeled, insufficient in quantity, collected in an inappropriate container, clotted when an anticoagulated specimen is required, hemolyzed when unsuitable for the requested examination, contaminated, excessively delayed, or otherwise unsuitable according to established laboratory policies. When a specimen is rejected, recollection may be requested when necessary.

IV. Storage and Transport

Blood specimens should be transported to the laboratory as soon as possible after collection. Specimens should be stored according to the laboratory's established procedures. Excessive delay or improper storage may cause changes in blood cell morphology and may affect the accuracy of microscopic examination.

V. Procedures for Submission to Central Laboratories

The specimen label should be verified against the laboratory request form before submission. The blood specimen tube should be properly closed and placed in the designated transport system. Required documentation should be completed, and the specimen should be transported according to established laboratory biosafety and specimen-transport procedures.

VI. Procedures for Microscopic Examination

For microscopic examination, a properly prepared and stained blood smear is examined using a light microscope. The stained smear is first examined under low-power magnification to assess the quality of the smear and locate the appropriate area for examination. The smear is then examined under high-power magnification to evaluate the morphology and distribution of red blood cells, white blood cells, and platelets. The oil-immersion objective may be used for detailed examination of blood cell morphology and differential leukocyte identification. Red blood cells are evaluated for their size, shape, and color, while white blood cells are identified according to their morphology and cellular characteristics. Platelets are assessed for their distribution, size, and estimated quantity. Any abnormal or unusual cellular findings are recorded according to the laboratory's established reporting format.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

The **reagents** required for blood smear examination include an appropriate blood smear stain, such as Wright stain or Wright-Giemsa stain, and other staining solutions or buffers required by the selected staining procedure.

Supplies include clean glass microscope slides, spreader slides, anticoagulated blood specimens, staining containers or racks, gloves, absorbent materials, and appropriate biohazard waste containers.

Equipment includes a light microscope with appropriate objectives, including an oil-immersion objective, a slide staining rack, and other equipment required for blood smear preparation and staining.

CALIBRATION

All equipment used for blood smear examination should be checked regularly to ensure that it is clean and functioning properly. The microscope should be properly maintained, including checking the illumination, optical system, and objectives. Equipment maintenance and calibration or verification should be performed according to the manufacturer's instructions and the laboratory's established procedures. Maintenance and verification results should be properly documented.

QUALITY CONTROL

I. Identify Control Materials to Use

Use appropriate quality-control materials, reference slides, or known blood smear preparations when required by the laboratory's quality-control program.

II. Preparation, Handling, and Storage

Prepare, handle, and store quality-control materials according to the manufacturer's instructions and laboratory procedures.

III. Frequency of Testing

Quality control should be performed according to the laboratory's QC policy, manufacturer instructions, and applicable laboratory requirements.

IV. Expected Results

Quality-control slides or preparations should demonstrate acceptable staining quality and appropriate cellular morphology.

V. Corrective Actions

If QC results are unacceptable:

- Check the staining procedure and staining reagents.
- Verify stain preparation, storage, and expiration.
- Check microscope operation and cleanliness.
- Prepare and examine another control smear.
- Replace reagents or materials when necessary.
- Notify the appropriate supervisor when the problem persists.

VI. Recording and Storage of QC Data

Record QC results, corrective actions, and relevant observations according to laboratory policy.

STEP-BY-STEP INSTRUCTIONS

I. Preparation of Blood Smear

1. Mix the anticoagulated blood specimen gently and thoroughly.
2. Place a small drop of blood near one end of a clean glass slide.
3. Place a clean spreader slide at an appropriate angle in front of the blood drop.
4. Allow the blood to spread along the edge of the spreader slide.
5. Push the spreader smoothly and steadily across the slide to produce a thin blood smear.
6. Allow the smear to air-dry completely.

II. Staining

1. Place the dried blood smear on a staining rack.
2. Apply the appropriate blood smear stain according to the laboratory's validated staining procedure.
3. Allow the stain to react for the required time.
4. Rinse the smear appropriately according to the staining procedure.
5. Allow the stained smear to dry.

III. Microscopic Examination

1. Examine the stained smear under low-power magnification to assess smear quality and locate the appropriate examination area.
2. Examine the smear under high-power magnification.
3. Use the oil-immersion objective when detailed cellular examination is required.
4. Evaluate RBC morphology, WBC morphology, and platelet appearance and distribution.
5. Perform differential leukocyte identification when required.
6. Record significant findings according to the laboratory's reporting format.

IV. Quantitative Testing

Quantitative or semi-quantitative assessment during blood smear examination may include estimation of platelet numbers and differential leukocyte distribution. When required, the findings should be reported according to the laboratory's validated procedure and reporting system.

V. Qualitative Testing

Qualitative examination of a blood smear involves the identification and description of blood cell morphology and other abnormal findings through microscopic examination. A properly prepared and stained blood smear is examined under the microscope, using the appropriate magnification to evaluate red blood cells, white blood cells, and platelets. The cells are assessed for their size, shape, color, distribution, and other morphological characteristics. Abnormal findings such as anisocytosis, poikilocytosis, abnormal white blood cell morphology, platelet abnormalities, blood parasites, or abnormal cells are recorded according to the laboratory's established reporting format.

Examples include:

Parameter	**Possible reporting**
RBC morphology	Normal, anisocytosis, poikilocytosis, hypochromia, other abnormalities
WBC morphology	Normal, reactive changes, abnormal morphology
Platelet morphology	Adequate, decreased, increased, clumping, abnormal size
Blood parasites	Not seen / seen
Abnormal cells	Not seen / present; describe morphology

Blood smear examination is particularly useful for evaluating blood cell morphology and detecting abnormalities that may require further laboratory investigation.

VI. Interpretation

Results should be interpreted according to the laboratory's established reference criteria and the patient's clinical information.

Examples:

- **RBC morphology**: Changes in RBC size, shape, or color may be associated with different hematologic conditions and should be interpreted together with other laboratory findings.
- **WBC morphology**: Abnormal WBC morphology or unusual leukocyte populations may provide information about infection, inflammation, or hematologic disorders.
- **Platelets**: Decreased or increased platelet estimates and abnormal platelet morphology may be clinically significant and should be correlated with the platelet count and other laboratory findings.
- **Blood parasites**: Identification of blood parasites may provide evidence of a parasitic infection and should be reported according to laboratory procedures.
- **Abnormal cells**: Unusual or atypical cells should be documented and, when necessary, referred for further evaluation.

Blood smear findings should not be interpreted in isolation and should be correlated with the patient's clinical information and other laboratory results.

CALCULATIONS

Blood smear examination generally does not require mathematical calculations when morphological findings are evaluated directly under the microscope. When a differential leukocyte count or platelet estimate is performed manually, results should be calculated and reported according to the laboratory's validated procedure.

For example, a differential count may be reported as percentages of:

- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils

REPORTING RESULTS

I. Reference Intervals

Common expected peripheral blood smear findings include:

Parameter	Typical expected finding
RBC morphology	Predominantly normal size and shape
RBC color	Predominantly normochromic
WBC morphology	Normal mature leukocyte morphology
Platelets	Adequate and normally distributed
RBC inclusions	None normally observed
Abnormal cells	None observed
Blood parasites	None observed

II. Procedures for reporting abnormal results

Unexpected or abnormal blood smear findings should be evaluated and verified when necessary. The specimen condition, smear preparation, staining quality, microscope performance, and quality-control results should be reviewed. Significant microscopic abnormalities should be correlated with the patient's CBC results and other laboratory findings. Repeat examination or additional testing may be performed when indicated. Verified abnormal findings should be reported according to laboratory policy, with critical findings communicated to the appropriate personnel when required.

III. Reporting format

Results should be reported using the laboratory’s approved format and units.

Microscopic Examination:

- **RBC morphology**: Normocytic, normochromic
- **WBC morphology**: No significant abnormality observed
- **Platelets**: Adequate
- **RBC inclusions**: None seen
- **Abnormal cells**: None seen
- **Blood parasites**: None seen

Differential leukocyte count, when performed:

- **Neutrophils**: ___ %
- **Lymphocytes**: ___ %
- **Monocytes**: ___ %
- **Eosinophils**: ___ %
- **Basophils**: ___ %

PROCEDURE NOTES

I. Special precautions

All blood specimens should be treated as potentially infectious. Appropriate personal protective equipment should be worn during specimen handling and testing. Proper hand hygiene and laboratory safety procedures should be observed. Blood smears should be prepared carefully to avoid exposure to blood. Slides should be handled properly to prevent breakage and contamination. Staining reagents should be handled according to laboratory safety procedures, and appropriate waste disposal should be followed.

II. Possible sources of error

Possible Error	Effect
Old blood specimen	Altered or deteriorated blood cell morphology
Clotted specimen	Uneven distribution or loss of cellular components
Poorly mixed specimen	Uneven cell distribution
Blood drop too large	Smear may be too thick
Improper spreader angle	Smear may be too thick or too thin
Uneven spreading technique	Irregular smear distribution
Improper staining time	Poor cellular differentiation
Incorrect stain preparation	Abnormal or poor staining
Dirty microscope slide	Artifacts or interference
Incorrect microscope focusing	Difficulty identifying cells

Contaminated specimen	Misleading microscopic findings
Inadequate examination area	Important abnormalities may be missed

High vitamin C can interfere with some urine dipstick reactions, including glucose and nitrite testing.

III. Answers to common problems

Common Problem	**Possible Cause / Corrective Action**
Smear is too thick	Use a smaller blood drop or adjust the spreading technique
Smear is too thin	Use an appropriate blood drop and spreading angle
Poor staining	Check stain quality, staining time, and staining procedure
Cells are difficult to identify	Check microscope focusing and illumination
Excessive staining background	Check staining and washing procedures
Unexpected cellular findings	Verify smear quality and repeat examination when indicated
Platelets appear clumped	Review specimen collection and preparation and correlate with the platelet count
Results appear inconsistent	Review specimen condition, smear quality, staining, and microscopic examination

LIMITATIONS OF METHODS

Blood smear examination has several limitations. The quality of microscopic findings depends on specimen condition, smear preparation, staining quality, microscope performance, and examiner experience. Delayed examination or improper specimen storage may cause changes in blood cell morphology. A blood smear provides important morphological information but does not replace other hematologic tests such as a complete blood count. Some abnormalities may require additional laboratory testing or expert review for confirmation. Blood smear findings should therefore be interpreted together with the patient's clinical information and other laboratory results.

A TROUBLESHOOTING OR BACK-UP PLAN

When an unexpected result or problem with blood smear examination occurs, the specimen and testing process should first be evaluated. Patient identification, specimen condition, anticoagulant, smear preparation, staining procedure, reagent quality, and microscope performance should be checked. The smear may be prepared or examined again when appropriate. If staining reagents or equipment are unavailable or malfunctioning, an approved alternative procedure may be used when available. The problem and corrective action should be documented, and unresolved problems should be referred to the laboratory supervisor or other authorized personnel. Questionable results should not be released until the issue has been appropriately resolved.

TECHNICAL PROCEDURE:
COMPLETE BLOOD COUNT (CBC) TEST

Procedure Title: Complete Blood Count (CBC) Test
Department: Hematology
Procedure Type: Laboratory Diagnostic Procedure
Effective Date: August 21, 2026
Prepared by: Ma. Chloe Jane Argoncillo
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

The Complete Blood Count (CBC) is a routine hematology test that measures the number and characteristics of major blood cell components. These major blood components include red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), hematocrit (Hct), and platelets. CBC is generally performed with an automated hematology analyzer, which measures and counts blood cells using methods such as electrical impedance, optical analysis, and photometric measurement, or manual microscopy for a differential count. The test provides essential information about a patient's hematological status and may help detect abnormalities associated with anemia, infection, inflammation, and other disorders.

SPECIMEN REQUIREMENTS

I. Specimen Type
Whole blood obtained through venipuncture.

II. Collection Tube
EDTA anticoagulant tube (lavender-top).

III. Volume Requirements
- **Adults:** 2mL
- **Pediatric:** 0.5mL

IV. Specimen Handling and Storage
- Mix the EDTA tube carefully by inverting it approximately (8-10 times) after collection.
- Whenever possible, perform analysis within 6 hours.
- If immediate testing is not possible, maintain the specimen under 2-8°C for up to 24 hours if necessary.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

I. Reagents/Media (use the reagents appropriate for specific analyzer)

- Diluent
- Lysing reagent
- Cleaning solution
- Materials for quality control
- Calibration materials (if necessary)
- Wright's stain (if manual smear is necessary)

II. Supplies

- Microscope slides
- EDTA blood collection tubes
- Biohazard disposal containers
- Tourniquet
- Sterile needles

III. Equipment

- Automated hematology analyzer
- Microscope (for manual smear examination)
- Microscope slides
- Personal Protective Equipment (PPE)

CALIBRATION

- Carry out calibration based on the manufacturer-recommended procedures.
- Report all calibration results in an appropriate calibration log.
- Do necessary additional calibrations after instrument maintenance, repairs, or inconsistent quality control results.

QUALITY CONTROL

1. Read and analyze the quality control materials at the start of each testing.
2. Use appropriate control levels and confirm if QC values fall within the established acceptable ranges.
3. Investigate causes of unacceptable results before processing patient samples.
4. Record and document all necessary actions.

STEP-BY-STEP INSTRUCTIONS

I. Pre-Analytical Phase

1. Confirm patient's identity and verify laboratory request.
2. Collect venous blood into an EDTA tube following the appropriate collection technique.
3. Invert the tube carefully approximately (8-10) times after collection to ensure adequate mixing of blood and anticoagulant.
4. Check specimen for visible clots or hemolysis.
5. Proper labeling of specimen.

II. Analytical Phase

1. Automated Analysis:
   - Load EDTA tube into hematology analyzer
   - Select CBC analysis program
   - Review the test after the analyzer completes the procedure for any

2. Manual Differential (if necessary):
   - Prepare the blood smear on a glass slide
   - Apply Wright's stain
   - Examine the sample correctly for cell morphology and count

III. Post-Analytical Phase

1. Review once the procedure is completed for accuracy
2. Validate the results against QC data and investigate for errors
3. Document complete and correct results in the Laboratory Information System (LIS)
4. Alert attending physician for critical values following the laboratory critical value policy.
5. Retain or dispose of the specimens according to the laboratory guidelines.

CALCULATIONS

I. Manual Hematocrit

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

REPORTING RESULTS

The following complete blood count results are standard results for adults. Blood most often is measured in cells per liter (cells/L) or grams per microliter.

I. **White Blood Cells** (WBC)
   - **Women:** 4,500 to 10,000 cells/mcL
   - **Men:** 4,500 to 10,000 cells/mcL

II. **Red Blood Cells** (RBC)
   - **Women:** 4.2 to 5.4 million cells/mcL
   - **Men:** 4.7 to 6.1 million cells/mcL

III. **Hemoglobin** (Hgb)
   - **Women:** 12.1 to 15.1 gm/dL
   - **Men:** 13.8 to 17.2 gm/dL

IV. **Hematocrit** (Hct)
   - **Women:** 36.1% to 44.3%
   - **Men:** 40.7% to 50.3%

V. **Platelets** (Plt)
   - **Women:** 150,000 to 450,000/dL
   - **Men:** 150,000 to 450,000/dL

PROCEDURE NOTES

I. **Special Precautions**
   - Confirm the patient's identity before specimen collection and testing.
   - Treat all blood specimens as potentially infectious.
   - Follow appropriate biosafety and infection-control practices.
   - Wear suitable PPE while handling blood specimens.
   - Use the correct EDTA tube for CBC collection.
   - Mix EDTA specimens gently and adequately after collection.
   - Do not use visibly clotted specimens for routine CBC analysis.
   - Follow the operating instructions of the specific hematology analyzer.
   - Dispose of needles, tubes, and contaminated materials in their appropriate waste containers.

II. Possible Sources of Error

Possible errors may result from:

- Incorrect patient identification
- Improper venipuncture
- Wrong collection tube
- Incorrect blood volume
- Failure to mix the EDTA specimen properly
- Clotted specimen
- Hemolysis
- Delayed specimen processing
- Improper specimen storage
- Expired or contaminated reagents
- Instrument malfunction
- Calibration problems
- QC failure
- Incorrect analyzer settings
- Failure to investigate analyzer flags

III. Answers to Common Problems

Common Problems	**Possible Cause / Corrective Action**
Specimen is clotted	Insufficient mixing or delayed mixing. Collect a new EDTA specimen.
Insufficient blood volume	Tube was not filled adequately. Recollect the specimen if required.
CBC result has an analyzer flag	Possible presence of abnormal cells or technical issues. Review the flag and perform a blood smear.
QC result is outside the acceptable range	Check reagents, controls, calibration, and condition, then repeat testing when required.
Specimen is hemolyzed	Difficulty in collection or improper handling. Evaluate specimen acceptability and recollect when necessary.
Analyzer fails to produce a result	Check the instrument, reagent used, and sample position. Troubleshoot if necessary according to the manufacturer's procedure.

LIMITATIONS OF METHODS

- Incorrect specimen collection may alter the CBC results.
- Improper calibration affects the analyzer accuracy.
- Clotted specimens yield inaccurate results.
- A delay in analysis affects the cell integrity and morphology.

A TROUBLESHOOTING OR BACK-UP PLAN

- **Problem**: The specimen has a visible clot.
- **Solution**: Reject and dispose of the specimen according to proper laboratory procedures.

- **Problem**: The analyzer gives questionable results.
- **Solution**: Repeat sample analysis after correcting the issue.

- **Problem**: Quality control results are out of the expected range.
- **Solution**: Recalibrate the analyzer and do not proceed with patient testing.

TECHNICAL PROCEDURE:
GRAM STAINING

Procedure Title: Gram Staining of Bacterial Smears
Department: Microbiology
Procedure Type: Qualitative Differential Stain
Effective Date: August 21, 2026
Prepared by: Wendy Summer Faye P. Austero
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

Gram staining is a **differential staining procedure** used to classify bacteria according to differences in their cell-wall properties.

The procedure uses four main reagents:

1. **Crystal violet** – primary stain
2. **Gram's iodine** – mordant
3. **Decolorizer** – removes the crystal violet-iodine complex from cells that do not retain it
4. **Safranin** – counterstain

Gram-positive bacteria generally retain the crystal violet-iodine complex and appear **purple to blue**, while Gram-negative bacteria are decolorized and subsequently take up the counterstain, appearing **pink to red**.

I. Clinical Reason for Performing the Test

Gram staining is often performed as an initial diagnostic test for suspected bacterial infections because it can quickly identify the presence and type of bacteria. Differentiating bacteria into Gram-positive and Gram-negative categories provides information that can help guide the initial selection of appropriate antibiotics. This rapid preliminary result is particularly useful in infections such as pneumonia, urinary tract infections, and meningitis.

SPECIMEN REQUIREMENTS

I. Collection

Specimens must be collected using aseptic techniques to minimize contamination.

- **Sputum:** Collect from a deep cough into a sterile container.
- **Blood:** Collect from two separate venipuncture sites and inoculate each into a culture bottle.
- **Cerebrospinal fluid (CSF):** Collect through lumbar puncture.
- **Ascitic, pleural, and synovial fluids:** Collect using sterile methods; these specimens may require centrifugation before staining.
- **Urine:** Midstream collection in a sterile container is preferred. For catheterized specimens, collect directly from the catheter port rather than the collection bag.
- **Swabs:** Specimens from the throat, nose, wounds, rectum, or cervix should be collected from the infected area while avoiding contamination of adjacent tissues.

All specimens must be clearly labeled and transported promptly to preserve bacterial integrity.

II. Labeling

Specimens must be clearly labeled to ensure positive patient identification and maintain specimen integrity. In accordance with CLIA requirements, the laboratory must have procedures for specimen labeling that include the patient name or a unique patient identifier and, when appropriate, the specimen source. The CDC further recommends using at least two patient identifiers and including relevant specimen information such as the collection site, collection date, and specimen type.

III. Rejection

Gram-stain specimens shall be rejected when they are unlabeled or improperly labeled, collected in an inappropriate container, insufficient in quantity, excessively delayed in transport, or visibly compromised in a manner that may affect the reliability of the examination.

IV. Storage and Transport

Specimens for Gram staining shall be collected in appropriate sterile containers and transported to the laboratory as soon as possible after collection. Specimens shall be protected from contamination and leakage during transport.

If immediate processing is not possible, specimens shall be handled according to the requirements appropriate for the specific specimen type to preserve specimen quality.

V. Procedures for Submission to Central Laboratories

Specimens requiring testing at a central or reference laboratory shall be referred according to the laboratory's established written procedures. The receiving laboratory must be CLIA-certified or meet equivalent requirements. The submitting laboratory shall follow the receiving laboratory's current specimen requirements, including those for specimen preparation, handling, transportation, and submission. Specimens shall be maintained under appropriate conditions and handled in a manner that preserves their integrity until transfer to the receiving laboratory. The date and time of specimen receipt shall be documented.

VI. Procedures for Microscopic Examination

After the staining procedure is completed and the slide has dried, examine the stained smear using a bright-field microscope. First, locate the stained cells using the 40× objective. Apply a drop of immersion oil to the smear and examine the preparation using the 100× oil-immersion objective. Examine multiple microscopic fields to evaluate the stained microorganisms.

During microscopic examination, observe and record the Gram reaction of the organisms, their color, shape, arrangement, and location on the smear. Gram-positive organisms appear purple, while Gram-negative organisms appear pink. Common bacterial shapes include cocci and bacilli.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

I. Reagents

- Crystal violet
- Gram's iodine
- Decolorizer, such as 95% ethanol
- Safranin
- Distilled or tap water for rinsing

II. Supplies

- Clean glass microscope slides
- Slide rack
- Timer
- Absorbent or bibulous paper
- Pencil or laboratory marker
- Personal protective equipment (PPE)

III. Equipment

- Bright-field microscope with a 100× objective
- Immersion oil

IV. Preparation of Reagents

If reagents are prepared in the laboratory, preparation shall follow the laboratory's validated formulation.

For Hucker's crystal violet, FDA describes preparation using crystal violet and ethanol combined with an ammonium oxalate solution. The mixture is allowed to stand for 24 hours and then filtered before use.

Gram's iodine is prepared using iodine, potassium iodide, and distilled water.

For Hucker's counterstain, a safranin stock solution is prepared using safranin O and 95% ethanol. A working solution is then prepared by diluting the stock solution with distilled water.

The decolorizer may consist of 95% ethanol. Commercially prepared Gram stain reagents may also be used in accordance with the manufacturer's instructions.

V. Storage Requirements

Prepared reagents shall be stored in appropriately labeled containers under the conditions specified by the validated laboratory procedure or the reagent manufacturer.

Gram's iodine should be protected from light. When prepared in-house, it should be stored in a dark container to reduce degradation.

All reagents should be checked before use for proper labeling, expiration or preparation date, and signs of contamination or deterioration. Commercial reagents should be stored according to the manufacturer's instructions.

CALIBRATION

Not applicable to the Gram staining procedure.

Gram staining is a manual microscopic procedure and does not require calibration of the staining procedure itself. However, laboratory equipment used for the examination should be maintained and checked in accordance with the laboratory's established equipment maintenance procedures and applicable manufacturer requirements.

QUALITY CONTROL

I. Identify Control Materials to Use

Known Gram-positive and Gram-negative control organisms should be used to verify the performance of the Gram-staining reagents and staining procedure.

- **Gram-positive control**: *Staphylococcus aureus*
- **Gram-negative contro**l: *Escherichia coli*

These organisms are used as reference controls because their expected Gram reactions are known. Gram-stain QC slides containing S. aureus and E. coli are also commercially available for evaluating staining reagents and staining technique.

II. Preparation, Handling, and Storage

Known control organisms or prepared control slides shall be used to verify the performance of the Gram-staining procedure. Control materials shall be handled and stored according to the manufacturer's instructions or the laboratory's validated procedure, when applicable.

Control materials shall be protected from contamination and damage and shall not be used beyond their expiration or acceptable storage period.

III. Frequency of Testing

Gram-stain reagents and staining performance should be checked using positive and negative reference organisms with each new batch of stains and at least weekly during patient testing. More frequent QC testing should be performed when required by the laboratory's validated procedure, reagent manufacturer's instructions, or applicable regulatory requirements.

IV. Expected Results

Control organism	**Expected result**
Staphylococcus aureus	Gram-positive; purple/violet cocci
Escherichia coli	Gram-negative; pink/red bacilli

V. Corrective Actions

When QC results are unacceptable, patient results shall not be reported until the cause of the QC failure has been identified and acceptable QC results have been obtained. The staining reagents, control material, staining procedure, and equipment shall be checked to identify the source of the problem. Corrective actions shall be documented.

VI. Recording and Storage of QC Data

QC results shall be recorded for each quality-control run. Records shall include the date of testing, control material used, observed Gram reaction, staining results, reagent information, and corrective actions taken when applicable. QC records shall be maintained for the period specified by the laboratory's record-retention requirements.

VII. Alternatives (If no QC materials are available)

If prepared control organisms or commercial QC slides are unavailable, patient Gram-stain results shall not be released until an acceptable QC material or validated alternative QC method is available.

STEP-BY-STEP INSTRUCTIONS

I. Quantitative Testing

Not applicable to the Gram staining procedure.

Results are interpreted based on the observed Gram reaction and the characteristics of the microorganisms, rather than on a numerical measurement.

II. Qualitative Testing

1. Position the slide rack over the sink and place the fixed slide on it.
2. Flood the slide with **crystal violet**. Allow it to remain on the slide for at least 15 seconds.
3. Gently rinse the slide with water, then tilt the slide to remove excess water.
4. Flood the slide with **Gram's iodine**. Allow it to remain for at least 15 seconds.
5. Gently rinse the slide with water, then tilt the slide to remove excess water.
6. Apply the **decolorizer** to the slide and rinse immediately with water to help prevent over-decolorization.
7. Tilt the slide to remove excess water.
8. Flood the slide with **safranin**. Allow it to remain for at least 15 seconds.
9. Gently rinse the slide with water, then tilt the slide to remove any excess water.
10. Gently blot the slide with bibulous paper, taking care not to wipe the smear from the slide.
11. Allow the stained slide to air dry.
12. Once the slide is dry, examine it using a bright-field microscope.
13. First locate the cells using the 40× objective.
14. Place a drop of immersion oil in the center of the smear and examine the slide using the 100× oil-immersion objective.
15. Record the observed Gram reaction. Gram-positive cells appear purple, while Gram-negative cells appear pink.

III. Interpretation

Interpret the stained smear according to the color, shape, and arrangement of the observed microorganisms.

- **Gram-positive organisms:** Appear purple or violet because they retain the primary crystal violet stain.
- **Gram-negative organisms:** Appear pink to red after losing the primary stain during decolorization and taking up the counterstain.
- **Cocci:** Round or spherical bacterial cells.
- **Bacilli:** Rod-shaped bacterial cells.

The Gram reaction and bacterial characteristics should be recorded according to the laboratory's approved reporting format. Gram staining provides preliminary information about the organisms present and does not by itself establish identification to the species level.

CALCULATIONS

Not applicable to the Gram staining procedure.

Gram staining is primarily a qualitative microscopic examination rather than a quantitative test.

REPORTING RESULTS

I. Reference Intervals

Not applicable to the Gram staining procedure.

Gram stain results are qualitative and are reported based on the observed Gram reaction, cell morphology, and arrangement of microorganisms.

II. Procedures for reporting abnormal results

Gram stain findings that indicate the presence of microorganisms shall be documented in the patient's laboratory report. Findings requiring urgent communication shall be immediately relayed to the requesting physician or appropriate healthcare personnel according to the laboratory's reporting protocol.

III. Reporting format

Gram stain results shall be reported based on the observed Gram reaction, cell morphology, and arrangement of microorganisms.

Gram Reaction	Cell Morphology	Arrangement	Reported Result
Gram-positive	Cocci	Clusters	Gram-positive cocci in clusters
Gram-positive	Cocci	Chains	Gram-positive cocci in chains
Gram-positive	Cocci	Pairs	Gram-positive cocci in pairs
Gram-positive	Bacilli	Single/Chains	Gram-positive bacilli
Gram-negative	Cocci	Clusters	Gram-negative cocci in clusters
Gram-negative	Cocci	Chains	Gram-negative cocci in chains
Gram-negative	Cocci	Pairs	Gram-negative cocci in pairs
Gram-negative	Bacilli	Single/Chains	Gram-negative bacilli

PROCEDURE NOTES

I. Special Precautions

Handle all specimens and stained slides using appropriate laboratory safety practices. Use aseptic techniques when handling specimens to minimize contamination. During the decolorization step, rinse the slide promptly after applying the decolorizer to prevent over-decolorization. When blotting the stained slide, handle it carefully to avoid removing the smear. Use immersion oil only with the appropriate oil-immersion objective.

II. Possible Sources of Error

Several factors may affect the accuracy and interpretation of Gram-stained smears. Thick or clumped smears may make microscopic examination difficult and may interfere with proper decolorization. The decolorization step should be carefully controlled, as insufficient or excessive decolorization can result in inaccurate Gram reactions. Thicker smears may require additional decolorization time. Fresh cultures should be used whenever possible, since older cultures may lose cell wall integrity, causing Gram-positive organisms to appear Gram-negative or Gram-variable.

III. Answers to Common Problems

Common Problem	Possible Cause / Corrective Action
Smear does not adhere to the slide	The slide may require additional cleaning before preparing the smear.
Smear is too thick	Spread the specimen more thinly across the slide to allow proper staining and examination.
Cells appear distorted or fragmented	Avoid overheating the slide during fixation.
Smear washes off during staining	Ensure that the smear is properly fixed before staining.
Gram-positive organisms appear Gram-negative	Over-decolorization may have occurred. Review the decolorization step and ensure that the slide is not excessively decolorized.
Gram-negative organisms appear Gram-positive	Under-decolorization may have occurred. Review the decolorization step.
Staining results are unreliable	Check the staining reagents for expiration, deterioration, or contamination and verify their performance using appropriate QC materials.
Uneven staining occurs	Ensure that the staining rack is level so that the staining solution covers the slide evenly, and use gentle washing during the rinse steps.

LIMITATIONS OF METHODS

Gram staining provides rapid preliminary information about the presence and Gram reaction of bacteria but has limitations in identifying microorganisms precisely. Compared with molecular techniques and genetic sequencing, Gram staining is less specific and cannot provide the same level of information regarding bacterial species and antimicrobial resistance patterns. Therefore, Gram staining serves primarily as a preliminary diagnostic method and may require additional testing for more precise identification and characterization of microorganisms.

A TROUBLESHOOTING OR BACK-UP PLAN

If the Gram-staining procedure cannot be performed or the staining reagents become unsuitable for use, testing shall be temporarily discontinued until the cause of the problem has been identified and corrected.

If acceptable quality-control results cannot be obtained, patient specimens shall not be reported until the staining procedure has been verified to produce reliable results. The problem and corrective actions taken shall be documented.

If the Gram-staining system remains unsuitable for use after corrective action, the specimen shall be referred to an appropriate backup laboratory or testing facility for Gram-stain examination, when necessary.

TECHNICAL PROCEDURE:
HELICOBACTER PYLORI STOOL ANTIGEN TEST

Procedure Title: Detection of *Helicobacter pylori* Antigen in Stool Samples
Department: Clinical Microbiology
Procedure Type: Immunochromatographic Assay (Rapid Test)
Effective Date: September 20, 2026
Prepared by: Seth Mikylla Loise S. Auza
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

The *Helicobacter pylori* (*H. pylori*) stool antigen test is an immunochromatographic method used to detect *H. pylori* antigens in a patient's stool specimen. The test works through an antigen-antibody reaction. When *H. pylori* antigen is present, it reacts with specific antibodies in the test device and produces a visible test line. A control line must also appear to confirm that the test has functioned properly. The presence of both control and test lines indicates a positive result, while the presence of only the control line indicates a negative result.

SPECIMEN REQUIREMENTS

The required specimen for this procedure is a fresh stool specimen collected in a clean, dry, and leak-proof container. The specimen should contain enough material for testing and should not be contaminated with urine, water, disinfectants, or other substances. Fresh specimens are preferred because improper storage or prolonged delays may affect the accuracy of the test.

Examples of acceptable specimens:

- Freshly collected stool
- Adequate quantity of stool
- Stool collected in a clean, dry container
- Properly sealed and identified specimen

I. Collection

The patient should be instructed to collect the stool specimen in a clean, dry container without contaminating it with urine or toilet water. After collection, the container should be securely closed and immediately labeled with the required patient information. The specimen should then be submitted to the laboratory as soon as possible for processing.

Important collection points:

- Avoid contamination with urine or water.
- Use the laboratory-approved specimen container.
- Collect an adequate amount of stool.
- Close the container securely after collection.

II. Labeling

The specimen must be labeled correctly to ensure proper patient identification and prevent reporting errors. The information on the specimen container must match the laboratory request form.

The label should include:

- Patient's complete name
- Patient identification number
- Date and time of collection
- Specimen type
- Other identifiers required by the laboratory

III. Rejection

A specimen should be rejected when it does not meet the laboratory's acceptance criteria. This includes specimens that are improperly labeled, leaking, contaminated, insufficient, improperly stored, or transported outside the acceptable conditions. The reason for rejection should be documented, and recollection should be requested when necessary.

Examples of reasons for rejection:

- Unlabeled or incorrectly labeled specimen
- Mismatch between specimen and request form
- Leaking or damaged container
- Stool contaminated with urine or other substances
- Insufficient specimen
- Improper storage or transportation
- Expired specimen stability period

IV. Storage and Transport

The stool specimen should preferably be tested as soon as possible after collection. If testing cannot be performed immediately, the specimen should be stored according to the manufacturer's instructions for the specific test kit. During transportation, the specimen should be placed in an appropriate biohazard specimen bag and protected from leakage and contamination. The required temperature and maximum transport time must be followed.

Transport requirements may include:

- Properly sealed specimen container
- Biohazard specimen bag
- Completed laboratory request form
- Appropriate temperature during transport
- Prompt delivery to the testing laboratory

V. Procedures for Submission to Central Laboratories

When *H. pylori* testing is referred to a central or reference laboratory, the submitting laboratory should first verify the patient's identification, test request, specimen type, and specimen acceptability. The required request form should be completed, and the specimen should be packaged according to applicable biological specimen transport requirements. The date and time of dispatch should be documented, and the result received from the central laboratory should be reviewed and entered into the patient's laboratory record.

VI. Procedures for Microscopic Examination

Microscopic examination is not normally used as the primary method for detecting *H. pylori* in stool. Stool antigen testing is generally used for noninvasive detection. Other diagnostic methods include urea breath testing, gastric biopsy examination, rapid urease testing, histologic examination, and molecular methods. When gastric biopsy specimens are examined, appropriate staining methods may be used to demonstrate *H. pylori* organisms.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

I. Reagents

The reagents required depend on the specific test kit used by the laboratory. For a rapid stool antigen test, the kit commonly contains the test device, extraction buffer, and specimen collection applicator. Positive and negative controls may also be provided. All reagents should be stored according to the manufacturer's instructions and should not be used beyond their expiration date.

Common materials include:

- *H. pylori* antigen test cassette/device
- Extraction buffer
- Specimen collection applicator
- Positive control, when provided
- Negative control, when provided

II. Supplies

The laboratory should prepare all necessary supplies before beginning the procedure. These include personal protective equipment and materials needed for specimen handling, testing, cleaning, and waste disposal.

Examples:

- Disposable gloves
- Laboratory gown or coat
- Stool collection container
- Disposable pipette/dropper
- Extraction tubes
- Timer
- Biohazard waste container
- Laboratory disinfectant

III. Equipment

The equipment required depends on the testing method. A rapid stool antigen test generally requires minimal equipment, such as a timer and appropriate storage facilities. If additional laboratory methods are performed, equipment such as microscopes, centrifuges, pipettes, or specialized incubation systems may be required.

CALIBRATION

For a qualitative rapid immunochromatographic test, routine instrument calibration is generally not required because the test device produces a visual result. However, any equipment used in the testing process must be properly maintained and calibrated according to laboratory policy. Measuring devices such as micropipettes and temperature-monitoring equipment should undergo regular verification or calibration.

Calibration and maintenance examples:

- Micropipette calibration
- Refrigerator temperature monitoring
- Timer verification
- Equipment preventive maintenance
- Documentation of calibration activities

QUALITY CONTROL

Quality control ensures that the testing system is functioning properly and that patient results are reliable. The control line included in the test device serves as an internal procedural control. If the control line does not appear, the test is considered invalid. External positive and negative controls may also be tested according to the manufacturer's instructions and laboratory quality management system.

External QC may be performed during:

- New reagent or kit lot verification.
- New shipment.
- Troubleshooting.
- Changes in testing conditions.
- Routine QC according to laboratory policy.

STEP-BY-STEP INSTRUCTIONS

Before testing, verify the patient's identity, test request, specimen condition, and expiration date of the test kit. Prepare the work area and wear appropriate personal protective equipment. Allow the test components to reach the temperature specified by the manufacturer.

For specimen processing, the required amount of stool is collected using the provided applicator and placed into the extraction buffer. The specimen is mixed thoroughly according to the manufacturer's instructions. The extracted specimen is then added to the designated sample well of the test device.

After adding the specimen, the timer is started and the test is allowed to develop for the time specified by the manufacturer. The result must be read within the manufacturer's recommended reading period. After interpretation, the result is recorded and all used materials are disposed of as biohazardous waste.

Basic sequence:

- Verify patient and specimen.
- Check kit expiration and condition.
- Prepare specimen.
- Add specimen to extraction buffer.
- Mix according to instructions.
- Apply extracted specimen to test device.
- Wait for the specified reaction time.
- Check the control line.
- Interpret and record the result.
- Dispose of materials properly.

I. Quantitative Testing

Weak or unclear results may be associated with insufficient specimen, improper extraction, or incorrect testing procedures. If quality-control results are unacceptable, patient testing should be stopped until the problem has been investigated and resolved.

Common problems and actions:

- **No control line:** Repeat using a new test device.
- **Weak/unclear result:** Review specimen preparation and repeat if appropriate.
- **Unexpected QC result:** Stop testing and investigate the QC problem.
- **Insufficient specimen:** Request a new specimen.
- **Repeated invalid results:** Notify the laboratory supervisor and use an alternative validated method or refer the specimen to an appropriate reference laboratory. The standard rapid immunochromatographic stool antigen test does not normally provide a quantitative measurement. Therefore, no concentration calculation or standard curve is generally required. If a quantitative or semi-quantitative laboratory assay is used instead, the laboratory must follow the specific manufacturer's calibration and calculation procedures.

II. Qualitative Testing

The rapid stool antigen test is primarily a **qualitative test**, meaning that it determines whether *H. pylori* antigen is detected or not detected rather than measuring its exact concentration.

Interpretation:

- **Positive:** Control line and test line are visible.
- **Negative:** Only the control line is visible.
- **Invalid:** No control line appears.

III. Interpretation

A **positive result** indicates that *H. pylori* antigen was detected in the stool specimen. A **negative result** indicates that *H. pylori* antigen was not detected by the assay. An **invalid result** means that the test did not produce the required control reaction and therefore cannot be interpreted. An invalid test should be repeated using a new test device.

CALCULATIONS

No mathematical calculation is normally required for a qualitative rapid stool antigen test. The result is determined by observing the test and control lines. If a quantitative method is used, the patient's result is calculated using the calibration curve or calculation method specified by the manufacturer.

For qualitative testing:

- No numerical calculation is required.
- Result is reported as Positive, Negative, or Invalid.

REPORTING RESULTS

The laboratory result should clearly state the test performed and the corresponding interpretation. Results should be reviewed and authorized according to the laboratory's reporting procedures.

Examples:

- **H. pylori stool antigen:** POSITIVE
- **H. pylori stool antigen:** NEGATIVE
- **H. pylori stool antigen:** INVALID – Repeat testing recommended.

The report should also contain the appropriate patient identification, specimen type, date and time of testing, and other required laboratory information.

PROCEDURE NOTES

The manufacturer's instructions should always be followed because specimen requirements, extraction procedures, reagent volumes, reaction times, and interpretation criteria may differ among test kits. Expired or damaged test devices should not be used. Results should only be interpreted within the manufacturer's specified reading period.

Important notes:

- Do not use expired kits.
- Do not use damaged test devices.
- Avoid specimen contamination.
- Follow the specified reaction time.
- Maintain appropriate QC documentation.
- Follow laboratory biosafety procedures.

LIMITATIONS OF METHODS

The stool antigen test detects *H. pylori* antigen but does not determine antibiotic susceptibility or provide a bacterial concentration. False-negative results may occur when the amount of antigen is below the detection limit or when factors affecting test sensitivity are present. Improper specimen collection, storage, transport, or testing can also affect the result. A negative result should therefore be interpreted together with the patient's clinical information and other relevant diagnostic findings.

Other limitations include:

- Manufacturer's package insert for the specific *H. pylori* stool antigen test kit used by the laboratory.
- Different test kits have different sensitivity and specificity.
- Medication use may affect test performance.

A TROUBLESHOOTING OR BACK-UP PLAN

If no control line appears, the test is invalid and should be repeated using a new test device. Weak or unclear results may be associated with insufficient specimen, improper extraction, or incorrect testing procedures. If quality-control results are unacceptable, patient testing should be stopped until the problem has been investigated and resolved.

Common problems and actions:

- **No control line:** Repeat using a new test device.
- **Weak/unclear result:** Review specimen preparation and repeat if appropriate.
- **Unexpected QC result:** Stop testing and investigate the QC problem.
- **Insufficient specimen:** Request a new specimen.
- **Repeated invalid results:** Notify the laboratory supervisor and use an alternative validated method or refer the specimen to an appropriate reference laboratory.

TECHNICAL PROCEDURE:
PREGNANCY TEST

Procedure Title: Human Chorionic Gonadotropin (hCG) Pregnancy Test
Department: Clinical Chemistry/Immunology
Procedure Type: Immunoassay
Effective Date: September 20, 2026
Prepared by: Janine Bernadette A. Barron
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

Human chorionic gonadotropin (hCG) is a hormone produced during pregnancy. Following implantation, hCG concentrations increase and can be detected in both blood and urine. Pregnancy tests detect hCG to determine pregnancy status. Blood testing can detect and quantify hCG, while urine testing is commonly used for qualitative detection.

I. Quantitative Serum β-hCG Test

Measures the concentration of β-hCG in a patient's serum using an immunoassay. The antigen-antibody reaction produces a measurable signal that is related to the concentration of hCG in the specimen. The result is reported as a numerical concentration, commonly in mIU/mL or IU/L, according to the assay used.

II. Qualitative Urine hCG Test

Detects hCG in urine using an immunochromatographic test device. When hCG is present at or above the detection limit, a visible test line appears. Qualitative point-of-care tests may have limited sensitivity at low hCG concentrations, particularly during very early pregnancy.

SPECIMEN REQUIREMENTS

I. Collection

- **Serum**:

1. Identify the patient using the laboratory's approved identification procedure.
2. Collect venous blood using venipuncture.
3. Collect the specimen in the appropriate serum tube specified by the assay manufacturer.
4. Allow the specimen to clot when required.
5. Centrifuge the specimen according to laboratory protocol.
6. Separate the serum from cellular components when required.

Blood testing can provide a quantitative measurement of the amount of hCG present and may detect lower concentrations than urine testing.

- **Urine:**

1. Provide the patient with clean, dry urine container.
2. Collect fresh urine specimen.
3. First-morning urine may be preferred because hCG is generally more concentrated.
4. Avoid excessive fluid intake immediately before collection because diluted urine may decrease the concentration of hCG.

II. Labeling

Label the specimen with:

- Patient's name or identification number
- Date and time of collection
- Specimen type
- Collector's name or identification when required

III. Rejection

Reject or request recollection of specimens that are:

- Unlabeled or improperly labeled
- Collected in an inappropriate container
- Insufficient amount
- Leaking or contaminated
- Improperly stored or transported
- Unsuitable according to laboratory policy or the assay manufacturer's instructions.

IV. Storage and Transport

1. Transport specimens to the laboratory as soon as possible.
2. Transport specimens to the laboratory as soon as possible.
3. Store specimens according to the manufacturer's instructions.
4. Protect specimens from conditions that may affect analyte stability.
5. Avoid unnecessary repeated freeze-thaw cycles.

V. Procedures for Submission to Central Laboratories

If the test is referred:

1. Verify patient and specimen identification.
2. Complete the required laboratory request form.
3. Prepare and package the specimen according to the receiving laboratory's requirements
4. Maintain the required storage and transport conditions.

VI. Procedures for Microscopic Examination

Not applicable to the pregnancy test procedure.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

I. Quantitative Serum β-hCG

- Serum specimen
- Quantitative β-hCG immunoassay reagents
- Calibrators
- Quality-control materials
- Appropriate serum collection tube
- Centrifuge
- Micropipette or automated sampling system
- Immunoassay analyzer
- Disposable tips/cuvettes, as applicable
- Personal protective equipment

II. Qualitative Urine hCG

- Urine pregnancy test cassette or strip
- Clean urine container
- Disposable dropper, if provided
- Timer
- Gloves

CALIBRATION

1. Perform calibration according to the specific manufacturer's instructions.
2. Use the manufacturer's recommended calibrators.
3. Perform calibration when required by the assay, such as when indicated by the reagent lot, analyzer, or laboratory QC procedure.
4. Verify that calibration meets the manufacturer's acceptance criteria.
5. Do not report patient results when calibration is invalid.

Because quantitative hCG results may be reported and interpreted differently between laboratories and assays, the laboratory should use the calibration system and reporting conventions validated for its specific method.

QUALITY CONTROL

I. Identify Control Materials to Use

Use appropriate β-hCG control materials covering relevant concentration levels, as specified by the manufacturer.

For qualitative urine testing, use positive and negative controls when required or recommended by the test manufacturer.

II. Preparation, Handling, and Storage

Prepare, handle, and store control materials according to the manufacturer's instructions.

III. Frequency of Testing

Perform QC according to the laboratory's established QC program and whenever required by the assay manufacturer, including after relevant calibration or reagent changes.

IV. Expected Results

QC results must fall within the laboratory's established acceptable range before patient results are reported.

V. Corrective Actions

If QC results are unacceptable:

1. Do not report patient results.
2. Check reagents, controls, calibration, and analyzer performance.
3. Repeat QC.
4. Recalibrate or troubleshoot when necessary.
5. Document the corrective action.

VI. Recording and Storage of QC Data

Record QC results, expected ranges, dates, reagent/control lot numbers, operator identification, and corrective actions according to laboratory policy.

VII. Alternatives (If no QC materials are available)

Follow the laboratory's approved alternative QC procedure. For qualitative devices, built-in procedural controls may provide an internal indication that the test has functioned correctly.

STEP-BY-STEP INSTRUCTIONS

I. Quantitative Testing

- **Serum β-hCG**:

1. Verify patient’s identity and request.
2. Check the serum specimen for correct identification, adequate volume, and suitability.
3. Prepare the β-hCG analyzer, reagents, calibrators, and QC materials.
4. Perform calibration according to the manufacturer's instructions.
5. Run the required QC materials.
6. Verify that QC results are acceptable.
7. Load the patient's serum specimen into the analyzer or prepare it according to the assay's instructions.
8. Perform the quantitative β-hCG assay.
9. The assay measures the signal produced by the reaction between β-hCG and the assay's specific antibodies.
10. The analyzer determines the β-hCG concentration using its validated calibration system.
11. Review the result for analytical flags and QC acceptability.
12. If the result exceeds the assay's analytical measurement range, perform dilution and repeat testing according to the manufacturer's instructions.
13. Record the final β-hCG concentration with the appropriate unit.
14. Interpret the result using the reference interval or decision limits validated for the specific assay.

Quantitative hCG results should be reported carefully because different laboratories may use different reference intervals, reporting conventions, and interpretive approaches.

II. Qualitative Testing

- **Urine hCG**:

1. Verify the patient's identity and urine specimen.
2. Check the test device for expiration, damage, and proper storage.
3. Follow the manufacturer's instructions for specimen preparation.
4. Add the required amount of urine to the test device or use the test strip as directed.
5. Start the timer.
6. Allow the test to develop for the specified period.
7. Read the result within the manufacturer's specified reading time.

Interpretation:

- **Positive:** Control line and test line are visible.
- **Negative:** Only the control line is visible.
- **Invalid:** The control line does not appear; repeat the test using a new device.

Qualitative urine tests can produce false-negative results when hCG concentrations are below the test's detection capability. Studies have demonstrated limitations of some point-of-care hCG devices in detecting low hCG concentrations associated with very early pregnancy.

III. Interpretation

- **Quantitative Serum β-hCG:**

Report the numerical β-hCG concentration in the unit specified by the assay.

Interpret the result according to the validated reference interval or decision limit of the laboratory's specific assay rather than applying a universal cutoff.

A quantitative result may be useful for assessing pregnancy status and, when clinically indicated, monitoring changes in hCG concentration over time. Blood testing can measure the amount of hCG rather than simply reporting whether it is detected.

- **Qualitative Urine hCG:**

Report the result as: **Positive**, **Negative**, or **Invalid**.

A negative result does not necessarily exclude very early pregnancy because hCG may not yet have reached the test's detection limit.

CALCULATIONS

I. Quantitative ß-hCG Result

For an automated quantitative ß-hCG immunoassay, the analyzer calculates the patient's ß-hCG concentration based on the assay's calibration curve. The final result is reported as a numerical concentration, commonly in mIU/mL.

$$Patient\ ß - hCG\ concentration = Analyzer - generated\ result$$

No manual calculation is normally required when using an automated quantitative immunoassay.

II. Qualitative Urine hCG

No mathematical calculation is required.

REPORTING RESULTS

I. Reference Intervals

Use the reference interval and interpretive limits established and validated for the specific quantitative β-hCG assay used by the laboratory.

Quantitative hCG reporting practices and reference intervals vary among laboratories; therefore, a single universal cutoff should not be substituted for the laboratory's validated method-specific values.

II. Procedures for reporting abnormal results

1. Verify the result and QC status.
2. Review specimen integrity and analyzer flags.
3. Repeat or confirm the test when required by laboratory policy.
4. Report results according to the laboratory's established procedures.
5. Follow the laboratory's policy for results requiring immediate notification.

III. Reporting format

- **Quantitative:**
  - **β-hCG**: _____ mIU/mL

- **Qualitative:**
  - **Urine hCG**: Positive / Negative / Invalid

PROCEDURE NOTES

I. Special precautions

- Treat blood and urine specimens as potentially infectious.
- Wear appropriate PPE.
- Follow standard precautions.
- Dispose of needles immediately in an approved sharps container.
- Check reagent and test-kit expiration dates.
- Follow the manufacturer's instructions for specimen volume and testing conditions.
- Do not interpret qualitative results outside the manufacturer's specified reading period.
- Avoid excessive fluid intake before urine collection because dilution may reduce detectable hCG concentration.

II. Possible sources of error

- Incorrect patient identification
- Improper specimen collection
- Insufficient specimen volume
- Diluted urine
- Improper storage or transport
- Expired or improperly stored reagents
- Incorrect reagent or specimen volume
- Calibration error
- Analytical interference
- Testing too early in pregnancy
- Reading a qualitative test outside the specified time
- QC failure

III. Answers to common problems

Common Problem	Possible Cause / Corrective Action
No control line in urine	Invalid test; repeat using a new test device
Negative urine result but pregnancy is suspected	Testing may have been performed too early or urine may be diluted; repeat testing or perform quantitative serum β-hCG as clinically indicated.
QC result is unacceptable	Check controls, reagents, calibration, and analyzer; resolve QC problem before reporting patient results.

β-hCG result exceeds measurement range	Perform the appropriate dilution and repeat according to the assay manufacturer's instructions.
Unexpected quantitative result	Review specimen quality, QC, calibration, analytical flags, and method-specific limitations.

LIMITATIONS OF METHODS

1. A pregnancy test detects hCG but does not independently establish the location or viability of a pregnancy.
2. Quantitative hCG results may differ between analytical methods because assays may use different calibration systems and reporting practices.
3. A qualitative urine test may fail to detect very early pregnancy when hCG concentration is below the test's detection limit.
4. A negative result should therefore be interpreted in relation to the timing of testing and clinical circumstances.
5. Urine concentration can affect qualitative test performance.
6. Some substances, medications, or medical conditions may interfere with hCG testing and should be considered according to the assay manufacturer's information.
7. Quantitative urine hCG testing has also been discussed in the literature as potentially useful in situations where urine hCG concentration provides clinically relevant information; however, its use depends on the specific laboratory method and clinical application.

A TROUBLESHOOTING OR BACK-UP PLAN

1. Stop testing if an instrument or QC problem could affect result accuracy.
2. Check the analyzer, reagents, calibration, controls, and specimen.
3. Repeat testing when appropriate.
4. Perform troubleshooting according to the manufacturer's instructions.
5. If the quantitative analyzer remains unavailable, refer the specimen to an approved laboratory with a validated β-hCG method.
6. If a qualitative urine test is invalid, repeat the procedure using a new test device.
7. If a urine result is negative but early pregnancy remains clinically suspected, repeat testing or perform quantitative serum β-hCG as appropriate.
8. Document all troubleshooting and corrective actions.

TECHNICAL PROCEDURE:
ROUTINE URINALYSIS EXAMINATION

Procedure Title: Routine Urinalysis Examination
Department: Clinical Microscopy
Procedure Type: Standard Procedure Type
Effective Date: September 20, 2026
Prepared by: Dominique Ashley R. Zamora
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

Routine urinalysis is a laboratory examination of urine that evaluates its physical, chemical, and microscopic characteristics. It is used to detect abnormalities associated with the urinary system and may provide information about renal, metabolic, and systemic conditions.

The examination consists of:

1. **Physical examination** – color, appearance/clarity, and other observable characteristics.
2. **Chemical examination** – testing of urine pH, specific gravity, protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite, and leukocyte esterase using reagent strips.
3. **Microscopic examinatio**n – identification and estimation of formed elements such as RBCs, WBCs, epithelial cells, casts, crystals, and microorganisms.

SPECIMEN REQUIREMENTS

I. Collection

A fresh urine specimen should be collected in a clean, dry, properly labeled urine container. A random urine specimen may be used for routine urinalysis, although a first-morning urine specimen may be preferred because it is generally more concentrated. When contamination is a concern, a clean-catch midstream specimen may be requested. The specimen should be delivered to the laboratory as soon as possible after collection.

II. Labeling

The urine specimen container should be properly labeled with the patient's complete name, identification number, date and time of collection, and other required identifiers according to laboratory policy. The information on the specimen container must correspond with the laboratory request form.

III. Rejection

A urine specimen may be rejected when it is unlabeled or improperly labeled, insufficient in quantity, collected in an inappropriate container, leaking, contaminated externally, excessively delayed without proper preservation, or otherwise unsuitable according to established laboratory policies. When a specimen is rejected, recollection may be requested when necessary.

IV. Storage and Transport

Urine specimens should be examined as soon as possible after collection. If immediate examination is not possible, the specimen should be stored according to the laboratory's established procedure. Unpreserved urine may require refrigeration when testing is delayed. Before examination, a refrigerated specimen should be handled according to the laboratory's validated procedure and mixed thoroughly. Delayed examination may cause changes in urine pH, bacterial growth, and deterioration of cells and casts.

V. Procedures for Submission to Central Laboratories

The specimen label should be verified against the laboratory request form before submission. The specimen container should be properly closed and placed in the designated transport system. Required documentation should be completed, and the specimen should be transported according to established laboratory biosafety and specimen-transport procedures.

VI. Procedures for Microscopic Examination

For microscopic examination, the urine specimen should first be mixed thoroughly. An appropriate volume of urine is transferred into a centrifuge tube when sediment examination by centrifugation is performed. The specimen is centrifuged according to the laboratory's validated procedure, after which the sediment is prepared for examination. A portion of the sediment is placed on a clean glass slide and covered with a coverslip. The preparation is examined using the microscope, with low-power magnification used to scan the specimen and high-power magnification used to examine formed elements such as cells, crystals, and microorganisms. Findings are recorded according to the laboratory's established reporting format.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

The **reagents** required for routine urinalysis include urinalysis reagent strips and appropriate quality-control materials. **Supplies** include clean urine specimen containers, centrifuge tubes, transfer pipettes, microscope slides, coverslips, gloves, absorbent materials, and appropriate biohazard waste containers. **Equipment** may include a clinical laboratory centrifuge, light microscope, urine analyzer when available, timer, and refractometer when specific gravity is measured instrumentally.

CALIBRATION

All instruments used for routine urinalysis should be checked to ensure that they are clean and functioning properly. Calibration should be performed according to the manufacturer's instructions and the laboratory's established procedures. Calibration results should be properly documented, and instruments that fail calibration should not be used for patient testing until the appropriate corrective action has been completed.

QUALITY CONTROL

I. Identify Control Materials to Use

Use appropriate urinalysis control materials for reagent-strip or automated urine testing.

II. Preparation, Handling, and Storage

- Prepare and store controls according to the manufacturer's instructions.
- Check expiration dates and storage conditions.
- Do not use deteriorated or expired control materials.

III. Frequency of Testing

Quality control should be performed according to the laboratory's QC policy, manufacturer instructions, and applicable regulatory requirements.

IV. Expected Results

Control results must fall within the established acceptable ranges before patient specimens are reported.

V. Corrective Actions

If QC results are unacceptable:

- Do not report patient results affected by the QC failure.
- Check reagent-strip expiration and storage conditions.
- Verify instrument operation.
- Repeat the control.
- Replace reagents or controls if necessary.
- Notify the appropriate supervisor when the problem persists.

VI. Recording and Storage of QC Data

Record QC results, corrective actions, and relevant observations according to laboratory policy.

VII. Alternatives (If no QC materials are available)

Testing should follow the laboratory's established contingency procedure. Patient results should not be reported when required QC cannot be verified.

STEP-BY-STEP INSTRUCTIONS

I. Physical Examination

1. Mix the specimen gently.
2. Observe and record the color.
3. Observe and record the appearance/clarity.
4. Examine other physical characteristics required by the laboratory.

Routine urinalysis includes visual examination of urine color and appearance.

II. Chemical Testing

1. Mix the specimen thoroughly.
2. Remove one reagent strip without touching the reagent areas.
3. Immediately close the reagent-strip container.
4. Completely immerse the reagent areas in the urine.
5. Remove the strip promptly.
6. Remove excess urine according to the manufacturer's instructions.
7. Read each reagent area at its specified time.
8. Compare the colors with the manufacturer's chart or allow the automated analyzer to interpret the strip.
9. Record the results.

Common reagent-strip parameters include:

- pH
- Specific gravity
- Protein
- Glucose
- Ketones
- Blood/hemoglobin
- Bilirubin
- Urobilinogen
- Nitrite
- Leukocyte esterase

III. Quantitative Testing

When quantitative testing is required, appropriate measurements may be performed using validated laboratory methods. Specific gravity may be measured using a refractometer, while quantitative urine protein, glucose, or other measurements may be performed when specifically required. All quantitative testing should follow the laboratory's validated procedure and the manufacturer's instructions for the equipment or method used.

IV. Qualitative Testing

Chemical examination may be performed using urine reagent strips. The specimen should be mixed thoroughly before testing. A reagent strip is removed from its container without touching the reagent areas, and the container should immediately be closed to protect the remaining strips. The reagent areas are completely immersed in the urine and the strip is removed promptly. Excess urine is removed according to the manufacturer's instructions. Each reagent area is read at its specified reaction time and compared with the manufacturer's color chart or interpreted by an automated analyzer when applicable. Results are then recorded appropriately.

Qualitative urine testing determines whether a substance is present or absent or provides a semi-quantitative result such as trace, 1+, 2+, 3+, or 4+.

Examples include:

Parameter	**Possible reporting**
Protein	Negative, trace, 1+ to 4+
Glucose	Negative/positive or graded
Ketones	Negative/trace/positive
Blood	Negative/trace/positive
Nitrite	Negative/positive
Leukocyte esterase	Negative/trace/positive
Bilirubin	Negative/positive

Dipstick testing is particularly useful for detecting substances such as protein, glucose, ketones, blood, nitrite, and leukocyte esterase.

V. Interpretation

Results should be interpreted according to the laboratory's established reference intervals and the patient's clinical information.

Examples:

- **Protein**: May indicate proteinuria and may be associated with renal disease, although temporary proteinuria can occur in some situations.
- **Glucose**: May occur when urinary glucose exceeds the renal threshold or in certain renal conditions.
- **Ketones**: May occur when the body is using fat as an energy source.
- **Blood**: May indicate hematuria or the presence of hemoglobin/myoglobin and should be correlated with microscopic findings.
- **Nitrite**: A positive result may indicate the presence of nitrate-reducing bacteria.
- **Leukocyte esterase**: Indicates the presence of an enzyme associated with WBCs and may support inflammation or UTI when correlated with other findings.
- **Specific gravity**: Provides an estimate of urine concentration.

A urinalysis result should not be interpreted in isolation.

CALCULATIONS

For routine reagent-strip urinalysis, calculations are generally not required when results are obtained directly from the reagent-strip color chart or automated analyzer.

If manual microscopic quantitation or other quantitative procedures are performed, results may be reported according to the laboratory's validated procedure, such as:

- RBCs/HPF
- WBCs/HPF
- Casts/LPF

Any required calculation must follow the laboratory's established formula and validated method.

REPORTING RESULTS

I. Reference Intervals

Reference intervals must be established or verified by the laboratory and should be interpreted according to the method used. Reference ranges can vary depending on the laboratory, population, and methodology.

Common routine findings include:

Parameter	Typical expected finding
Color	Yellow to amber
Appearance	Clear
Protein	Negative
Glucose	Negative
Ketones	Negative
Blood	Negative
Bilirubin	Negative
Nitrite	Negative
Leukocyte esterase	Negative

II. Procedures for reporting abnormal results

Unexpected or abnormal results should be evaluated and verified when necessary. The specimen condition, quality-control results, reagent status, and testing procedure should be reviewed. Chemical and microscopic findings should be correlated, and repeat testing may be performed when indicated. Verified abnormal findings should be reported according to laboratory policy, with critical findings communicated to the appropriate personnel when required.

III. Reporting format

Results should be reported using the laboratory's approved format and units.

Example:

Physical Examination

- **Color**: Yellow
- **Appearance**: Clear

Chemical Examination

- **pH**: 6.0
- **Specific Gravity**: 1.020
- **Protein**: Negative
- **Glucose**: Negative
- **Ketones**: Negative
- **Blood**: Negative
- **Nitrite**: Negative
- **Leukocyte Esterase**: Negative

Microscopic Examination

- **RBC**: ___ /HPF
- **WBC**: ___ /HPF
- **Epithelial cells**: ___ /HPF
- **Casts**: ___ /LPF
- **Crystals**: ___
- **Bacteria**: ___

PROCEDURE NOTES

I. Special precautions

All urine specimens should be treated as potentially infectious. Appropriate personal protective equipment should be worn during specimen handling and testing. Proper hand hygiene and laboratory safety procedures should be observed. Contamination of the specimen should be avoided. Reagent strips should be protected from moisture and excessive exposure to air, and the appropriate reaction times should be observed carefully. Urine specimens should be mixed properly before examination.

II. Possible sources of error

Possible Error	Effect
Delayed testing	Changes in pH, bacterial growth, deterioration of cells/casts
Improper storage	Altered chemical and microscopic results
Contaminated specimen	False or misleading findings
Expired reagent strips	Incorrect reagent reactions

Improper reagent-strip timing	Incorrect results
Excess urine left on strip	Color contamination between reagent areas
Improper QC	Unreliable patient results
High vitamin C intake	May cause false-negative results for some dipstick reactions
Improper labeling	Specimen identification error

High vitamin C can interfere with some urine dipstick reactions, including glucose and nitrite testing.

III. Answers to common problems

Common Problem	**Possible Cause / Corrective Action**
Unexpected reagent-strip result	Repeat test and check strip expiration/storage
QC is outside range	Check controls, reagents, instrument, and repeat QC
Specimen is very old	Follow laboratory rejection/recollection policy
Insufficient specimen	Request recollection when necessary
Unexpected microscopic findings	Verify specimen preparation and repeat examination if indicated
Reagent strip does not change color	Check reagent-strip expiration, storage, and procedure
Results appear inconsistent	Check specimen integrity and correlate physical, chemical, and microscopic findings

LIMITATIONS OF METHODS

Routine urinalysis has several limitations. Reagent-strip results may be affected by urine concentration, pH, interfering substances, reagent-strip storage, and specimen age. Reagent strips should not be used beyond their expiration date. Dipstick protein testing primarily detects albumin and may not detect all urinary proteins. Microscopic findings may also be affected by delayed examination and inappropriate specimen handling. A normal urinalysis does not completely exclude disease, and results should be interpreted together with the patient's clinical findings and other laboratory results.

A TROUBLESHOOTING OR BACK-UP PLAN

When an unexpected result or instrument problem occurs, the specimen and testing system should first be evaluated. Patient identification, specimen condition, storage, reagent-strip expiration, reagent storage, and quality-control results should be checked. Testing may be repeated when appropriate. If an instrument is unavailable or malfunctioning, an approved manual or alternative method may be used when available. The problem and corrective action should be documented, and unresolved problems should be referred to the laboratory supervisor or other authorized personnel. Questionable results should not be released until the issue has been appropriately resolved.

TECHNICAL PROCEDURE:
SALIVA-BASED GENETIC TEST

Procedure Title: Saliva-Based Genetic Test
Department: Molecular Diagnostics
Procedure Type: Standard Procedure Type
Effective Date: August 28, 2026
Prepared by: Jhed Catherine Louise B. Baylon
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

A saliva genetic test uses cells found in saliva as a source of human DNA. The saliva sample is processed to release and purify the DNA. The purified DNA is then tested for specific genetic targets or variants using a validated method such as PCR, real-time PCR, genotyping, or DNA sequencing.

The accuracy of the test depends on proper specimen collection, storage, DNA extraction, and analysis. The test only detects the genetic targets included in the validated method.

SPECIMEN REQUIREMENTS

I. Collection
Collect human saliva containing buccal and other epithelial cells using an approved saliva collection tube or saliva DNA collection device.

II. Labeling
Label the specimen with the patient's name or unique laboratory identification number. Make sure the label matches the laboratory request.

III. Rejection
Reject the specimen according to laboratory policy if it is mislabeled, leaking, insufficient, contaminated, or stored incorrectly.

IV. Storage and Transport
Close the container securely. Transport and store the specimen according to the collection device instructions and the validated laboratory procedure.

V. Procedures for Submission to Central Laboratories

Before submission, check the specimen label against the laboratory request. Make sure the container is closed and placed in the approved transport system. Complete the required documentation and follow laboratory transport and safety procedures.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

I. Reagents/Media

- Saliva DNA collection or stabilization device/reagent
- DNA extraction and purification reagents
- Lysis buffer
- Wash buffers
- Elution buffer or nuclease-free water
- PCR reagents, when PCR is used
- Primers and probes for the genetic target
- Positive control
- Negative control
- No-template control (NTC)
- Internal or extraction control, when required
- DNA quantification reagents, when needed
- Sequencing reagents, when sequencing is performed

II. Supplies

- Sterile collection container
- Microcentrifuge tubes
- Aerosol-resistant pipette tips
- Disposable gloves
- Laboratory coat
- Tube racks
- Micropipette tips
- Biohazard waste containers
- Labels and specimen identification materials

III. Equipment

- Micropipettes
- Vortex mixer
- Microcentrifuge
- Heating block or incubator, when required
- DNA extraction equipment, when applicable
- PCR or real-time PCR instrument
- DNA quantification equipment, when applicable

- Electrophoresis equipment, when applicable
- DNA sequencing equipment, when applicable
- Refrigerator/freezer for proper reagent and specimen storage
- Required personal protective equipment (PPE)

The exact reagents and equipment depend on the genetic test and the manufacturer's validated procedure.

CALIBRATION

1. Calibrate pipettes, analyzers, thermal cyclers, and other equipment according to the laboratory schedule.
2. Follow the manufacturer's instructions for equipment calibration and maintenance.
3. Record calibration and maintenance results in the appropriate laboratory records.
4. Perform recalibration or verification after maintenance, repair, or unacceptable performance when required.
5. Use the calibration and performance requirements established during test validation or verification.

QUALITY CONTROL

1. Check that the saliva specimen meets the laboratory's acceptance criteria before testing.
2. Use the required positive and negative controls.
3. Include an extraction control when DNA extraction is performed.
4. Use a no-template control during amplification to check for contamination.
5. Review all QC results before reporting patient results.
6. Investigate any QC result that is outside the accepted range.
7. Record QC findings and corrective actions.
8. Do not release patient results if required QC has failed.
9. Keep specimen preparation and amplification areas separate when required to reduce contamination.

STEP-BY-STEP INSTRUCTIONS

I. Pre-Analytical Phase

1. Confirm the patient's identity and check the laboratory request.
2. Explain the saliva collection procedure to the patient.
3. Follow any preparation instructions given by the collection device manufacturer.
4. Collect the required amount of saliva using the approved collection device.
5. Close the collection container securely.
6. Label the specimen correctly.
7. Check the specimen for leakage, insufficient volume, wrong labeling, contamination, or other rejection criteria.
8. Transport and store the specimen according to the validated laboratory procedure.

II. Analytical Phase – DNA Extraction

1. Mix or prepare the saliva specimen according to the validated extraction procedure.
2. Transfer the required amount of saliva into the extraction tube or system.
3. Add the appropriate lysis reagent.
4. Break open the cells according to the manufacturer's instructions.
5. Separate the DNA from cell debris and other unwanted material.
6. Wash the extracted DNA using the required purification reagents.
7. Elute the purified DNA using the specified elution solution.
8. Include the required extraction control.
9. Check DNA quantity or quality when required by the test.

III. Genetic Analysis

1. Prepare the molecular test reaction according to the validated laboratory procedure.
2. Add the extracted DNA and required controls.
3. Perform PCR, real-time PCR, genotyping, or sequencing, depending on the test.
4. Analyze the amplification, genotype, or sequencing data.
5. Check that the positive, negative, extraction, and/or internal controls meet the acceptance criteria.
6. Interpret the patient's result using the validated assay criteria.

IV. Post-Analytical Phase

1. Review the result for accuracy and completeness.
2. Confirm that all required QC results are acceptable.
3. Investigate unexpected, questionable, or inconclusive results.
4. Repeat testing or use an approved confirmatory method when required.
5. Record the final result in the laboratory information system (LIS).
6. Report the result according to laboratory policy.
7. Communicate critical or clinically significant findings according to laboratory policy.
8. Store or dispose of the specimen according to laboratory requirements.

V. Quantitative Testing

Most qualitative genetic tests do not require manual mathematical calculations. The result is based on the validated assay's interpretation criteria.

If DNA concentration is measured, report the value produced by the validated DNA quantification method. For quantitative molecular tests, use the calculation method required by the validated procedure or manufacturer's instructions

VI. Qualitative Testing

Qualitative genetic testing determines whether the genetic target or variant is detected. The result is interpreted using the validated criteria of the specific test.

Possible Result	Meaning
Detected / Positive	The genetic target or variant was detected.
Not Detected / Negative	The tested target or variant was not detected
Invalid	The test did not meet the required analytical or QC criteria
Inconclusive / Indeterminate	The available result does not allow a reliable classification.

VII. Interpretation

Interpret the result according to the purpose and validated criteria of the genetic test. A negative result does not exclude a genetic condition when the condition or variant is not included in the test. Results should not be interpreted beyond the intended purpose of the assay.

CALCULATIONS

For most qualitative saliva genetic tests, no manual calculation is needed. If DNA concentration or a quantitative molecular result is required, follow the validated calculation method or manufacturer's instructions.

REPORTING RESULTS

I. Reference / Expected Reporting

The report should clearly state the result produced by the validated assay. Results may be reported as Detected/Positive, Not Detected/Negative, Invalid, or Inconclusive/Indeterminate.

II. Procedures for Reporting Abnormal or Unexpected Results

Review the specimen condition, test setup, QC results, and assay data. Repeat testing or use an approved confirmatory method when required. Do not release results when required QC criteria have not been met.

III. Reporting Format

- Patient identification
- **Specimen type**: saliva
- Genetic test performed
- Genetic target or variant examined
- Test result Interpretation
- Testing method
- Important limitations
- Date of testing
- Laboratory identification
- Authorized laboratory personnel or laboratory director

PROCEDURE NOTES

1. Use only saliva collection devices and DNA extraction procedures that are validated for the intended test.
2. Follow the manufacturer's instructions for the collection and extraction system.
3. Keep correct specimen identification throughout the entire procedure.
4. Avoid contamination during saliva collection, DNA extraction, and amplification.
5. Use aerosol-resistant pipette tips when appropriate.
6. Keep pre-amplification and post-amplification activities properly separated.
7. Include all required QC materials with patient testing.
8. Do not report patient results when required QC criteria have not been met.
9. Document specimen rejection, repeat testing, QC failures, and corrective actions.
10. Maintain patient confidentiality throughout testing and reporting.
11. Interpret genetic results only within the purpose and limits of the test.

I. Special Precautions

Use appropriate PPE and follow laboratory safety procedures when handling specimens, reagents, and waste. Treat human saliva specimens as potentially infectious. Prevent contamination and properly dispose of biohazard waste.

II. Possible Sources of Error

Possible Error	**Possible Effect**
Insufficient saliva	Not enough DNA for testing
Poor saliva collection	Low DNA yield or poor DNA quality
Mislabeled specimen	Patient identification error; testing should not proceed
Contaminated specimen or reagents	Incorrect or unreliable molecular result

Improper storage or transport	Reduced specimen quality
PCR inhibition	Invalid or inaccurate amplification result
Poor DNA extraction	Low DNA yield or failed testing
QC failure	Patient result should not be released

LIMITATIONS OF METHODS

- The amount of DNA obtained from saliva can vary between specimens.
- Poor saliva collection may produce too little DNA.
- Contamination may affect the accuracy of the result.
- Improper storage or transport may reduce specimen quality.
- Substances in the specimen may interfere with DNA extraction or amplification.
- PCR inhibition may cause an invalid or inaccurate result.
- A negative result does not exclude a condition if the tested variant is not included in the assay.
- The test detects only the targets or genomic regions covered by the method.
- Some variants may not be detected because of limits in the assay design or analytical sensitivity.
- An inconclusive result may require repeat testing or another validated method.
- Results should not be interpreted beyond the intended purpose of the test.
- Genetic results may need clinical correlation when used for health-care or diagnostic purposes.

A TROUBLESHOOTING OR BACK-UP PLAN

Problem	**Corrective Action**
Saliva specimen is insufficient	Reject or request recollection according to specimen acceptance criteria.
Specimen is mislabeled	Do not continue until the identification problem is resolved according to laboratory policy.
DNA yield is too low	Review collection and extraction. Repeat extraction when appropriate or request a new specimen.
DNA quality is inadequate	Review storage and extraction conditions and repeat extraction if enough specimen remains.

Positive control fails	Do not release patient results. Check reagents, instrument performance, and test setup before repeating the run.
Negative or no-template control is positive	Suspect contamination. Stop testing, find and correct the contamination source, prepare fresh reagents when needed, and repeat the affected test.
Internal/extraction control fails	Check for extraction failure or inhibition and repeat extraction/testing according to the validated procedure.
Genetic result is inconclusive	Repeat the analysis or use an approved confirmatory method when required.

TECHNICAL PROCEDURE:
TOTAL CHOLESTEROL TEST

Procedure Title: Quantitative Determination of Total Cholesterol in Serum by Enzymatic Colorimetric CHOD-PAP Method
Department: Clinical Chemistry
Procedure Type: Enzymatic Colorimetric Method
Effective Date: August 21, 2026
Prepared by: Tricia Myrmel I. Bacli
Reviewed by Chief Medical Technologist: Princess Mekaella S. Araneta
Approved by Laboratory Director: Wendy Summer Faye P. Austero

PRINCIPLE

The Total Cholesterol Test is an enzymatic colorimetric method used for the quantitative determination of cholesterol in serum. In this method, cholesterol esters present in the specimen are hydrolyzed by the enzyme cholesterol esterase to form free cholesterol and fatty acids. The free cholesterol is then oxidized by cholesterol oxidase to produce cholest-4-en-3-one and hydrogen peroxide (H_2O_2).

The hydrogen peroxide produced reacts with 4-aminophenazone and a phenolic compound in the presence of peroxidase, forming a colored quinoneimine dye. The intensity of the color produced is directly proportional to the concentration of cholesterol present in the specimen. The absorbance of the colored product is measured photometrically. This final color-forming reaction is known as the Trinder reaction.

I. Chemical Reactions

$$Cholesterol\ ester + H_2O \rightarrow Cholesterol + Fatty\ acid$$
$$Cholesterol + O_2 \rightarrow Cholest\text{-}4\text{-}3\text{-}one + H_2O_2$$
$$H_2O_2 + 4\text{-}aminophenazone + phenol \rightarrow Quinoneimine\ dye + H_2O$$

The color intensity formed during the reaction is proportional to the total cholesterol concentration in the patient's specimen.

II. Clinical Reasons for Performing the Test

Cholesterol is a lipid that is essential for normal physiological functions, including the formation of cell membranes and the production of certain hormones. It is synthesized mainly in the liver and intestinal tissues, while some cholesterol is also obtained from dietary sources.

Measurement of total cholesterol is performed to assess a patient's lipid status and to help evaluate the risk of atherosclerotic cardiovascular disease. It may also be used in the diagnosis and management of disorders associated with elevated cholesterol and lipid or lipoprotein metabolism.

Total cholesterol is generally evaluated together with other lipid measurements, such as HDL cholesterol, LDL cholesterol, and triglycerides, when assessing cardiovascular risk.

SPECIMEN REQUIREMENTS

I. Specimen

The preferred specimen for this procedure is serum. Plasma may also be acceptable when specifically validated by the laboratory and permitted by the reagent manufacturer's instructions. CDC laboratory methods have used serum or plasma for enzymatic cholesterol measurement.

II. Specimen Collection

1. Collect the patient's blood using standard venipuncture technique.
2. Use an appropriate blood collection tube for serum.
3. Properly identify and label the specimen immediately after collection.
4. Allow the blood to clot when serum is required.
5. Centrifuge the specimen according to laboratory procedure.
6. Separate the serum from the blood cells as soon as practical.
7. Transfer the specimen to an appropriately labeled container when necessary.

III. Specimen Labeling

The specimen must be properly labeled with the required patient identifiers, including:

- Patient name or identification number
- Date of collection
- Time of collection, when required
- Specimen type
- Other identifiers required by laboratory policy

Unlabeled or improperly identified specimens should not be tested.

IV. Specimen Rejection

A specimen may be rejected when:

- It is unlabeled or improperly identified.
- An incorrect specimen type was submitted.
- There is insufficient specimen volume.
- The specimen is contaminated.
- The specimen has exceeded the laboratory's established stability period.
- The specimen does not meet the laboratory's acceptance criteria.

Specimens with significant hemolysis, icterus, or lipemia should be evaluated according to the interference limitations of the specific analytical method.

V. Specimen Storage and Transport

Serum should be separated from the cells and stored according to the laboratory's validated specimen-stability requirements and the reagent manufacturer's instructions.

If specimens must be transported to another laboratory, they should be properly identified, securely packaged, and maintained under the required temperature conditions.

REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

I. Reagents

The following reagents are required for the enzymatic CHOD-PAP method:

- Cholesterol reagent
- Cholesterol esterase
- Cholesterol oxidase
- Peroxidase
- 4-aminophenazone or 4-aminoantipyrine
- Phenolic chromogen
- Appropriate buffer system
- Cholesterol standard or calibrator
- Quality control materials

The exact reagent composition and concentration depend on the specific commercial reagent system used by the laboratory.

II. Supplies

The following supplies may be required:

- Test tubes or reaction cuvettes
- Micropipettes
- Pipette tips
- Disposable gloves
- Laboratory coat
- Distilled or deionized water
- Absorbent material
- Biohazard waste containers

III. Equipment

The following equipment is required:

- Spectrophotometer or automated chemistry analyzer
- Centrifuge
- Calibrated micropipettes
- Timer
- Refrigerator for reagent and specimen storage

IV. Preparation of Reagents

1. Check the reagent label before use.
2. Verify the reagent name, lot number, expiration date, and storage condition.
3. Do not use expired, contaminated, or visibly deteriorated reagents.
4. Prepare reagents according to the manufacturer's instructions.
5. Allow reagents to reach the required working temperature when specified.
6. Mix reagents appropriately before use.
7. Avoid contamination of reagent bottles and working solutions.
8. Record the reagent lot number and expiration date as required by laboratory policy.

The exact preparation procedure should follow the package insert for the specific CHOD-PAP reagent used.

V. Storage Requirements

Reagents, calibrators, controls, and specimens must be stored according to their respective manufacturer's instructions and laboratory policy.

Storage conditions must be monitored to maintain reagent integrity. Reagents should not be exposed to temperatures outside their specified storage range.

Expired or improperly stored reagents must not be used for patient testing.

CALIBRATION

Calibration is performed to establish the relationship between absorbance and the concentration of cholesterol.

1. Use the appropriate cholesterol calibrator or standard.
2. Verify the assigned concentration and expiration date of the calibrator.
3. Prepare the calibrator according to the manufacturer's instructions.
4. Perform calibration according to the analytical system's procedure.
5. Verify that the calibration is acceptable before reporting patient results.
6. Recalibrate when required by the laboratory's calibration policy, such as after an applicable reagent lot change or when QC results indicate a possible calibration problem.
7. Document calibration results.

The exact calibration procedure depends on whether the laboratory uses a manual spectrophotometric method or an automated chemistry analyzer.

QUALITY CONTROL

Quality control is performed to ensure that the analytical system is producing accurate and reliable results.

I. Control Materials

Appropriate commercial cholesterol control materials should be used. At least two control levels are recommended when applicable:

- Normal-level control
- Abnormal/high-level control

II. Preparation, Handling, and Storage

Control materials must be prepared, handled, and stored according to the manufacturer's instructions. Controls must not be used beyond their assigned stability period.

III. Frequency of Testing

Quality control should be performed according to the laboratory's established QC policy and before patient results are released when required.

Additional QC testing may be required after:

- Calibration
- Reagent lot changes
- Instrument maintenance
- Troubleshooting
- QC failure

IV. Expected Results

Control results must fall within the laboratory's established acceptable ranges before patient results are reported.

V. Corrective Actions

If QC results are outside the acceptable range:

1. Do not report patient results affected by the QC failure.
2. Check the reagent expiration date and storage conditions.
3. Check the control material.
4. Verify the calibration status.
5. Check pipettes and equipment.
6. Repeat the QC material when appropriate.
7. Recalibrate the instrument if necessary.
8. Perform additional troubleshooting.
9. Notify the laboratory supervisor when required.
10. Document the corrective action.

VI. QC Records

QC records should include:

- Date and time of QC testing
- Control identification
- Control level
- Result obtained
- Acceptable range
- Reagent lot number
- Calibration information
- Instrument identification
- Corrective action, if applicable

STEP-BY-STEP INSTRUCTIONS

The following describes a general manual enzymatic colorimetric procedure. Exact reagent volumes, incubation time, temperature, and wavelength must follow the package insert of the specific reagent kit being used.

I. Preparation

- Bring the reagents and specimens to the required working temperature.
- Label the reaction tubes or cuvettes appropriately.
- Prepare tubes for:
  - Reagent blank
  - Cholesterol standard/calibrator
  - Patient specimen
- Ensure that the spectrophotometer is ready for use.
- Verify that acceptable QC results have been obtained.

II. Reaction

1. Pipette the required volume of reagent into the appropriate reaction tube or cuvette.
2. Add the appropriate volume of cholesterol standard to the standard tube.
3. Add the appropriate volume of patient serum to the sample tube.
4. Mix the contents thoroughly.
5. Incubate for the time and temperature specified by the reagent manufacturer.
6. Measure the absorbance using the wavelength specified for the reagent system.
7. Record the absorbance obtained for the standard and patient specimen.
8. Calculate the total cholesterol concentration.
9. Review the result and verify that QC is acceptable.
10. Report the result using the laboratory's approved reporting format.

CDC reference methods for enzymatic cholesterol testing have used measurement near **500–505 nm**, with some analyzer-specific methods also using a secondary wavelength of 700 nm. The exact wavelength must therefore be taken from the method being used.

III. Quantitative Testing

The Total Cholesterol Test is a **quantitative test** because it determines the concentration of cholesterol present in the patient's specimen.

The intensity of the colored reaction product is directly proportional to the concentration of cholesterol in the specimen. The absorbance measured by the spectrophotometer is therefore used to determine the patient's cholesterol concentration.

The final result is generally expressed in **mg/dL** or **mmol/L**.

IV. Qualitative Testing

The Total Cholesterol Test is primarily a quantitative laboratory test. Qualitative interpretation may be performed by categorizing the numerical result according to the laboratory's established reference or clinical decision limits.

V. Interpretation

Common adult categories for total cholesterol are:

Total Cholesterol	**Interpretation**
Less than 200 mg/dL	Desirable
200–239 mg/dL	Borderline high
240 mg/dL or higher	High

These categories are commonly used for interpretation, but the laboratory's established reference or decision intervals should be used for reporting.

Total cholesterol should not be interpreted independently. Other lipid measurements and the patient's clinical history should be considered when assessing cardiovascular risk.

CALCULATIONS

For a single-point standard/calibrator method, the cholesterol concentration may be calculated using:

$$Total\ Cholesterol\ (mg/dL) = Absorbance\ of\ Patient\ /\ Absorbance\ of\ Standard \times Concentration\ of\ Standard$$

Example:

If:

- Absorbance of patient = 0.400
- Absorbance of standard = 0.500
- Standard concentration = 200 mg/dL

Then:

- Total Cholesterol = (0.400 ÷ 0.500) × 200
- **Total Cholesterol = 160 mg/dL**

Therefore, the patient's total cholesterol concentration is **160 mg/dL**.

For automated analyzers using multipoint calibration, the instrument calculates the result using the established calibration curve.

To convert cholesterol from mg/dL to mmol/L:
$mmol/L = mg/dL \times 0.02586$

REPORTING RESULTS

The result should be reported using the laboratory's approved format.

The report should contain:

- Patient identification
- Specimen identification
- Test name
- Total cholesterol result
- Unit of measurement
- Reference or decision interval
- Date of testing
- Appropriate abnormal-result indicator, when applicable
- Authorized laboratory personnel

Results must be reviewed for analytical validity before release.

I. Reference Intervals

For adults, commonly used total cholesterol categories are:

- **Desirable:** less than 200 mg/dL
- **Borderline high:** 200–239 mg/dL
- **High:** 240 mg/dL or higher

These values should be treated as clinical decision categories rather than automatically substituting for the laboratory's validated reference interval.

Reference intervals or decision limits should be clearly stated on the laboratory report.

II. Procedures for reporting abnormal results

Results that fall outside the laboratory's established reference or decision limits should be appropriately flagged according to the laboratory's reporting system.

When a result is considered critical according to laboratory policy, the responsible healthcare provider should be notified following the laboratory's established critical-result reporting procedure.

The notification should be documented when required.

III. Reporting Format

A typical report may be presented as follows:

- **Test:** Total Cholesterol
- **Specimen:** Serum
- **Result:** _____ mg/dL
- **Reference/Decision Interval:** _____
- **Interpretation:** _____
- **Date/Time:** _____
- **Performed by:** _____
- **Verified by:** _____

The actual reporting format should follow the laboratory's approved format.

PROCEDURE NOTES

The Total Cholesterol Test should be performed using standardized procedures to ensure consistency and reliability.

Important notes include:

1. Verify patient identification before testing.
2. Verify specimen acceptability.
3. Check reagent and calibrator expiration dates.
4. Follow the manufacturer's instructions.
5. Use properly calibrated pipettes.
6. Ensure proper incubation conditions.
7. Use the correct wavelength for the analytical method.
8. Perform QC according to laboratory policy.
9. Do not report patient results when QC is unacceptable.
10. Document all necessary testing information.

I. Special Precautions

1. Treat all blood specimens as potentially infectious.
2. Wear appropriate personal protective equipment, including laboratory coat and gloves.
3. Avoid contact with biological specimens.
4. Dispose of contaminated materials in appropriate biohazard containers.
5. Prevent contamination of reagents and specimens.
6. Avoid bubbles in reaction cuvettes because they may interfere with photometric measurement.
7. Handle reagents according to their safety instructions.
8. Clean spills immediately according to laboratory safety procedures.
9. Follow the laboratory's standard precautions and waste-disposal procedures.

II. Possible Sources of Error

Possible sources of error include:

1. Pre-analytical Errors:

- Incorrect patient identification
- Improper specimen collection
- Incorrect specimen type
- Improper specimen labeling
- Improper specimen storage
- Delayed specimen processing
- Insufficient specimen volume

2. Analytical Errors:

- Incorrect pipetting
- Incorrect reagent volume
- Expired reagents
- Improper reagent storage
- Incorrect incubation time
- Incorrect incubation temperature
- Incorrect wavelength
- Improper calibration
- Instrument malfunction
- Contaminated reagents
- Poor-quality control performance

3. **Post-analytical Errors:**

   - Incorrect calculation
   - Incorrect transcription
   - Incorrect interpretation
   - Failure to review abnormal results
   - Incorrect reporting

III. Answers to Common Problems

1. Problem 1: QC Result Is Outside the Acceptable Range

- **Possible causes**:

  - Reagent deterioration
  - Expired control
  - Calibration error
  - Instrument problem
  - Pipetting error

- **Corrective action**:

  1. Stop reporting patient results.
  2. Check reagents and controls.
  3. Verify storage conditions.
  4. Check calibration.
  5. Repeat QC.
  6. Recalibrate if necessary.
  7. Check instrument performance.
  8. Document the corrective action.

2. Problem 2: Unexpected Patient Result

1. Verify patient identification.
2. Check specimen integrity.
3. Review QC results.
4. Verify calibration.
5. Repeat the test when appropriate.
6. Review previous results when available.
7. Consult the laboratory supervisor if necessary.

3. **Problem 3: Instrument Failure**
    1. Stop testing on the affected instrument.
    2. Document the instrument problem.
    3. Notify the appropriate laboratory personnel.
    4. Perform troubleshooting according to the instrument manual.
    5. Use a validated backup analyzer or method if available.
    6. Perform calibration and QC on the backup system before reporting patient results.

LIMITATIONS OF METHODS

1. The test determines total cholesterol and does not independently determine HDL cholesterol, LDL cholesterol, or triglycerides.
2. Results may be affected by substances that interfere with the specific analytical method.
3. Hemolysis, icterus, and lipemia may interfere with some methods depending on their degree and the analytical system used.
4. Different reagent systems may have different analytical specifications.
5. The wavelength, reagent volume, incubation conditions, and calibration procedure may vary among manufacturers.
6. The result should be interpreted together with other lipid measurements and clinical information.
7. Results are dependent on proper specimen handling, reagent performance, calibration, and quality control.
8. Reference or decision intervals may vary between laboratories and patient populations.

A TROUBLESHOOTING OR BACK-UP PLAN

If problems occur during testing, the following procedure should be followed:

1. Stop testing if the analytical system is producing unreliable results.
2. Check reagent expiration dates and storage conditions.
3. Check calibrator and QC status.
4. Verify pipette performance.
5. Check the spectrophotometer or analyzer.
6. Repeat QC testing.
7. Recalibrate when necessary.
8. Repeat affected patient specimens when appropriate.
9. Notify the laboratory supervisor if the problem cannot be resolved.
10. Use a validated backup method or analyzer when available.
11. Document the problem and all corrective actions taken.

Patient results should not be released until the analytical problem has been resolved and acceptable QC results have been obtained.

REFERENCES

Abadie, J. (2026). *Laboratory reference ranges*. Merck Manual Professional Edition. https://www.merckmanuals.com/professional/resources/normal-laboratory-values/laboratory-reference-ranges

Adewoyin, A. S., & Nwogoh, B. (2014). Peripheral blood film—A review. *Annals of Ibadan Postgraduate Medicine, 12*(2), 71–79. https://pubmed.ncbi.nlm.nih.gov/25960697/

American College of Gastroenterology. (2024a). *ACG Clinical Guideline: Treatment of Helicobacter pylori infection*. *The American Journal of Gastroenterology, 119*(9), 1730–1753. https://journals.lww.com/ajg/fulltext/10.1038/ajg.2016.563~acg-clinical-guideline-treatment-of-helicobacter-pylori

American College of Gastroenterology. (2024b). *ACG guideline on treatment of Helicobacter pylori: New recommendations*. https://gi.org/journals-publications/ebgi/schoenfeld_sep2024/

Aryal, S. (2022). *Gram staining: Principle, procedure, interpretation, examples and animation*. Microbiology Info. https://microbiologyinfo.com/gram-staining-principle-procedure-interpretation-examples-and-animation/

Becton Dickinson. (n.d.). *BD BBL™ quality control slides*. https://www.bd.com/en-us/products-and-solutions/products/product-families/bd-bbl-quality-control-slides

Centers for Disease Control and Prevention. (n.d.-a). *Collection and transport of patient specimens.* https://www.cdc.gov/cholera/media/pdfs/2024/07/3-Collection-and-Transport-of-Patient-Speciments-Laboratory-methods-for-the-diagnosis-of-Vibrio-cholerae_ENG.pdf

Centers for Disease Control and Prevention. (n.d.-b). *Facilitator guide for routine microscopy procedures: Laboratory exercises*. https://reach.cdc.gov/sites/default/files/job-aids-resources/Facilitator-Guide-Routine-Microscopy-508.pdf

Centers for Disease Control and Prevention. (n.d.-c). *Gram stain procedure* [Transcript]. https://reach.cdc.gov/sites/default/files/video-transcripts/Gram%20Stain%20Procedure_508.pdf

Centers for Disease Control and Prevention. (n.d.-d). *Specimen storage and shipping guidance*. https://www.cdc.gov/laboratory/specimen-submission/pdf/Specimen-Packing-and-Shipping-Guidance-Infectious-Diseases-Laboratories.pdf

Centers for Disease Control and Prevention. (n.d.-e). *Stool specimens – Detection of parasite antigens*. CDC DPDx. https://www.cdc.gov/dpdx/diagnosticprocedures/stool/antigendetection.html

Centers for Disease Control and Prevention. (2006). *Gastroenteritis and transmission of Helicobacter pylori infection in households*. *Emerging Infectious Diseases, 12*(11). https://wwwnc.cdc.gov/eid/article/12/11/06-0086_article

Centers for Disease Control and Prevention. (2009–2010). *Laboratory procedure manual: Total cholesterol in serum*. National Center for Health Statistics. https://wwwn.cdc.gov/nchs/data/nhanes/public/2017/labmethods/BIOPRO-J-MET-Total-Cholesterol-508.pdf

Centers for Disease Control and Prevention. (2015–2016). *Laboratory procedure manual: Total cholesterol in serum*. National Center for Health Statistics https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/labmethods.aspx?Cycle=2015-2016

Centers for Disease Control and Prevention. (2017). *Total cholesterol (P_TCHOL): Laboratory methodology*. National Center for Health Statistics. https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2017/DataFiles/P_TCHOL.htm

Centers for Disease Control and Prevention. (2021–2023). *Laboratory procedure manual: Total cholesterol (frozen)*. National Center for Health Statistics. https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/labmethods.aspx?Cycle=2021-2023

Centers for Disease Control and Prevention. (2024). *Testing for diabetes*. https://www.cdc.gov/diabetes/diabetes-testing/index.html

Centers for Disease Control and Prevention. (2026). *Collecting and handling specimens for monkeypox PCR testing*. https://www.cdc.gov/monkeypox/hcp/diagnosis-testing/collecting-specimens.html

Centers for Medicare & Medicaid Services. (n.d.). *§493: Subpart K—Quality system for nonwaived testing*. https://www.cms.gov/files/document/apcsubk1pdf

Centers for Medicare & Medicaid Services. (2004). *State operations manual: Appendix C—Survey procedures and interpretive guidelines for laboratories and laboratory services*. https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/App-C_Survey-Procedures-IGs-for-Labs-Labs-Svcs-Final.pdf

Centers for Medicare & Medicaid Services. (2024). *Revisions to State Operations Manual (SOM), Appendix C—Survey procedures and interpretive guidelines for laboratories and laboratory services (Clinical Laboratory Improvement Amendments [CLIA])—Advance copy*. https://www.cms.gov/files/document/qso-25-10-clia.pdf

Chen, B., Mei, J., Kalman, L., Shahangian, S., Williams, I., Gagnon, M., Bosse, D., Ragin, A., Cuthbert, C., & Zehnbauer, B. (2012). Good laboratory practices for biochemical genetic testing and newborn screening for inherited metabolic disorders. *Morbidity and Mortality Weekly Report, 61*(RR-02), 1–37. https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6102a1.htm

Chey, W. D., Howden, C. W., Moss, S. F., Morgan, D. R., Greer, K. B., Grover, S., & Shah, S. C. (2024). *ACG Clinical Guideline: Treatment of Helicobacter pylori infection*. *The American Journal of Gastroenterology, 119*(9), 1730–1753. https://webfiles.gi.org/links/journals/ACG-Hpylori-Guidelines-Highlights-2024-FINAL.pdf

Chung, P. H. (2026). *Urinalysis and urine culture*. Merck Manual Consumer Version. https://www.merckmanuals.com/home/urinary-tract-disorders/diagnosis-of-urinary-tract-disorders/urinalysis-and-urine-culture

Cleveland Clinic. (2022a). *Gram stain*. https://my.clevelandclinic.org/health/diagnostics/22612-gram-stain

Cleveland Clinic. (2022b). *Pregnancy tests*. Cleveland Clinic https://my.clevelandclinic.org/health/diagnostics/9703-pregnancy-tests

Clinical and Laboratory Standards Institute. (n.d.). H20: *Reference leukocyte (WBC) differential count profile.* https://clsi.org/standards/products/hematology/documents/h20/

Clinical and Laboratory Standards Institute. (2017). *Collection of diagnostic venous blood specimens (7th ed.; CLSI standard GP41).* https://clsi.org/standards/products/general-laboratory/documents/gp41/

Code of Federal Regulations. (2026a). *42 C.F.R. § 493.1232: Standard: Specimen identification and integrity*. eCFR. https://www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493/subpart-K/subject-group-ECFR6b8923402db3f2f/section-493.1232

Code of Federal Regulations. (2026b). *42 C.F.R. § 493.1242: Standard: Specimen submission, handling, and referral*. eCFR. https://www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493/subpart-K/subject-group-ECFR5f8f0b6639946fd/section-493.1242

Code of Federal Regulations. (2026c). *42 C.F.R. § 493.1251: Standard: Procedure manual*. eCFR. https://www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493/subpart-K/subject-group-ECFRc96daead380f6ed/section-493.1251

Cole, L. A., & Khanlian, S. A. (2009). The need for a quantitative urine hCG assay. *Clinical biochemistry*, *42*(7-8), 676–683. https://doi.org/10.1016/j.clinbiochem.2008.10.018

Deeg, R., & Ziegenhorn, J. (1983). Kinetic enzymic method for automated determination of total cholesterol in serum. *Clinical Chemistry, 29*(10), 1798–1802 .https://pubmed.ncbi.nlm.nih.gov/6577981/.

DePristo, M. A., et al. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics, 43*(5), 491–498. https://doi.org/10.1038/ng.806

Grenache D. G. (2020). Current Practices When Reporting Quantitative Human Chorionic Gonadotropin. Test Results. *The journal of applied laboratory medicine*, *5*(5), 850–857. https://doi.org/10.1093/jalm/jfaa082

Greene, D. N., Schmidt, R. L., Kamer, S. M., Grenache, D. G., Hoke, C., & Lorey, T. S. (2013). Limitations in qualitative point of care hCG tests for detecting early pregnancy. *Clinica chimica acta; international journal of clinical chemistry*, *415*, 317–321. https://doi.org/10.1016/j.cca.2012.10.053

Gronowski A. M. (2020). Why Is It So Hard to Report Quantitative Human Chorionic Gonadotropin Results? *The journal of applied laboratory medicine*, *5*(5), 847–849. https://doi.org/10.1093/jalm/jfaa095

Goode, M. R., et al. (2014). Collection and extraction of saliva DNA for next generation sequencing. *Journal of Visualized Experiments, 90*. https://doi.org/10.3791/51697

Gulati, G., Uppal, G., & Gong, J. (2013). Purpose and criteria for blood smear scan, blood smear examination, and blood smear review. *Annals of Laboratory Medicine, 33*(1), 1–3. https://doi.org/10.3343/alm.2013.33.1.1

Hansen, T. V. O., et al. (2007). Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: Comparison of the response rate and quality of genomic DNA. *Cancer Epidemiology Biomarkers & Prevention, 16*(10), 2072–2076. https://doi.org/10.1158/1055-9965.epi-07-0611

Healthline. (n.d.). *Quantitative hCG blood pregnancy test: Procedure and results*. Healthline. https://www.healthline.com/health/hcg-blood-test-quantitative#other-uses-for-the-test

Jones, G. L., & Dever, S. M. (1984). *The Gram stain: A new look at an old tool*. Centers for Disease Control. https://stacks.cdc.gov/view/cdc/7646

Kindly MedTech. (2025). *Blood glucose tube : Types, additives, and how they work*. KDL. https://www.kdlnc.com/blood-glucose-tube-guide/

Labcorp. (n.d.-a). *Urinalysis, routine with microscopic examination on positives information*. https://www.labcorp.com/tests/003038/urinalysis-routine-with-microscopic-examination-on-positives

Labcorp. (n.d.-b). *Urine Specimen Collection Guidelines for lab testing*. https://www.labcorp.com/test-menu/resources/urine-specimens

Lynch, E. C. (1990). *Peripheral blood smear.* In H. K. Walker, W. D. Hall, & J. W. Hurst (Eds.), Clinical methods: The history, physical, and laboratory examinations (3rd ed.). Butterworths. https://www.ncbi.nlm.nih.gov/books/NBK263/

Maddukuri, G. (2026). *Evaluation of the patient with renal issues*. Merck Manual Professional Edition. https://www.merckmanuals.com/professional/nephrology/approach-to-the-patient-with-renal-issues/evaluation-of-the-patient-with-renal-issues

Malfertheiner, P., Megraud, F., Rokkas, T., Gisbert, J. P., Liou, J.-M., Schulz, C., Gasbarrini, A., Hunt, R. H., Leja, M., O'Morain, C., Rugge, M., Suerbaum, S., Tilg, H., Sugano, K., & El-Omar, E. M. (2022). Management of *Helicobacter pylori* infection: The Maastricht VI/Florence Consensus Report. *Gut, 71*(9), 1724–1762. https://gut.bmj.com/content/gutjnl/early/2022/08/08/gutjnl-2022-327745.full.pdf

Mayo Clinic Staff. (n.d.). *Complete blood count (CBC).* Mayo Clinic. *https://www.mayoclinic.org/tests-procedures/complete-blood-count/about/pac-20384919#*

MedlinePlus. (n.d.). *Pregnancy test*. U.S. National Library of Medicine https://medlineplus.gov/lab-tests/pregnancy-test/

MedlinePlus. (2024). *Blood smear.* U.S. National Library of Medicine. https://medlineplus.gov/lab-tests/blood-smear/

Meiattini, F., Prencipe, L., Bardelli, F., Giannini, G., & Tarli, P. (1978). The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. *Clinical Chemistry, 24*(12). ,https://pubmed.ncbi.nlm.nih.gov/719864/

Miller, J. M. (1983). *Handbook of specimen collection and handling in microbiology*. Centers for Disease Control, Laboratory Program Office. https://stacks.cdc.gov/view/cdc/7700

National Center for HIV, STD and TB Prevention (U.S.), Division of Sexually Transmitted Diseases. (2001). *Program operations guidelines for STD prevention: Medical and laboratory services*. Centers for Disease Control and Prevention. https://stacks.cdc.gov/view/cdc/40224

National Cholesterol Education Program Expert Panel. (1988). Report of the National Cholesterol Education Program Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults. *Archives of Internal Medicine, 148*(1), 36–69. https://pubmed.ncbi.nlm.nih.gov/3422148/

National Library of Medicine. (n.d.). *Gram stain*. MedlinePlus. https://medlineplus.gov/lab-tests/gram-stain/

Peterson, P. E., McNeill, S., & Gulati, G. (2012). *Cellular morphologic analysis of peripheral blood.* In Laboratory hematology practice. Wiley. https://doi.org/10.1002/9781444398595.ch2

Quinque, D., et al. (2006). Evaluation of saliva as a source of human DNA for population and association studies. *Analytical Biochemistry, 353*(2), 272–277. https://doi.org/10.1016/j.ab.2006.03.021

Shaker, G., & Swift, C. J. (2023). *Peroxidase-coupled glucose method*. StatPearls Publishing. PubMed. https://www.ncbi.nlm.nih.gov/books/NBK594277/

Snyder, J. A., Haymond, S., Parvin, C. A., Gronowski, A. M., & Grenache, D. G. (2005). Diagnostic considerations in the measurement of human chorionic gonadotropin in aging women. *Clinical chemistry*, *51*(10), 1830–1835. https://doi.org/10.1373/clinchem.2005.053595

Testing.com. (n.d.). *Pregnancy test (hCG)*. Testing.com. https://www.testing.com/tests/pregnancy-test-hcg/

Themes, U. F. O. (2017). *Clinical chemistry laboratory*. Basicmedical Key. https://basicmedicalkey.com/clinical-chemistry-laboratory/

Tripathi, N., Zubair, M., & Sapra, A. (2025). Gram staining. In *StatPearls*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK562156/

University of Minnesota Advanced Research and Diagnostic Laboratory (ARDL), Li, D., Peters, J. (n.d.). *Laboratory Procedure Manual.* https://wwwn.cdc.gov/nchs/data/nhanes/2017-2018/labmethods/TCHOL-J-MET-508.pdf

U.S. Food and Drug Administration. (2001). *BAM R32: Gram stain*. https://www.fda.gov/food/laboratory-methods-food/bam-r32-gram-stain

Washington State Department of Health. (2004). *Gram stain self-assessment checklist.* https://doh.wa.gov/sites/default/files/legacy/Documents/2700/gram.pdf

World Health Organization. (1993). *Blood Grouping Reagents*. World Health Organization Regional Office for the Eastern Mediterranean Alexandria, Egypt. https://applications.emro.who.int/dsaf/dsa24.pdf

World Health Organization. (2002). Model Standard Operating Procedures for Blood Transfusion Service. https://iris.who.int/server/api/core/bitstreams/374fe4f8-321f-4b92-ba7e-3aac4938f3c8/content

World Health Organization. (2003). *Manual of basic techniques for a health laboratory* (2nd ed.). https://www.who.int/publications/i/item/9241545305

World Health Organization. (2010a). *WHO best practices for injections and related procedures toolkit*. https://www.who.int/publications/i/item/9789241599252

World Health Organization. (2010b). *WHO guidelines on drawing blood: Best practices in phlebotomy*. https://www.who.int/publications/i/item/9789241599221

World Health Organization. (2009). Blood Group Serology. 10-82. https://cdn.who.int/media/docs/default-source/blood-transfusion-safety/guidelines-and-principles-for-safe-blood-transfusion-practice-module-3-.pdf?sfvrsn=e942e4c9_1&utm