BACHELOR OF MEDICAL LABORATORY TECHNOLOGY LAB MANUAL

3rd Semester

Prepared By

Paramedical & Allied Science Dept.

BMLT

MIDNAPORE CITY COLLEGE

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Department of Paramedical and Allied Health Sciences

BACHELOR OF MEDICAL LABORATORY TECHNOLOGY -BMLT (HONOURS) (under CCFUP, 2023)

SEMESTER-III

Paper Title: Parasitology (Practical)

Paper Code: MDC 3P: Parasitology (Practical)

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1. Identification of different disease-causing Helminth and Protozoan parasites.
2. Identification of different phases of life cycle of arthropods protozoa, helminth, having medical importance for causing disease.
3. Identification of microfilaria, *Taenia solium*, ascaris, ancylostoma and deferent stages of malaria.
4. Examination of stool for OPV (Ova parasite Cyst).
5. Laboratory Diagnosis of Various Parasites: Rapid Diagnostic Tests (RDTs), Malaria Rapid diagnostic test (MRDT), Leishmanin test.

Identification of *Entamoeba histolytica*

Introduction

Entamoeba histolytica is a protozoan parasite that primarily infects the human intestine, causing a disease known as amoebiasis or amoebic dysentery. It is transmitted through ingestion of cysts present in contaminated food or water. The organism exists in two forms: trophozoite (active stage) and cyst (infective stage). Microscopic examination of stool or stained smears is the most common method for identification.

Classification:

- **Kingdom:** Protista
- **Phylum:** Sarcomastigophora
- **Class:** Lobosea
- **Order:** Amoebida
- **Family:** Entamoebidae
- **Genus:** *Entamoeba*
- **Species:** *Entamoeba histolytica*

Microscopic Identifying Characters (in Fixed Stained Slide):

1. **Trophozoite Size:** 15–30 µm in diameter; round or irregular shape.
2. **Nucleus:** Single nucleus with a **centrally located karyosome** and evenly distributed **peripheral chromatin** visible with stains.
3. **Cytoplasm:** Finely granular; may show **ingested red blood cells (RBCs)** as dark dots—this is pathognomonic (seen only in *E. histolytica*).
4. **Cyst Size:** 10–20 µm; round with a clear cyst wall.
5. **Mature Cysts:** Contain **1 to 4 nuclei**; **chromatoid bodies** (rod-shaped with rounded ends) may be seen in the cytoplasm, often more visible in trichrome-stained smears.

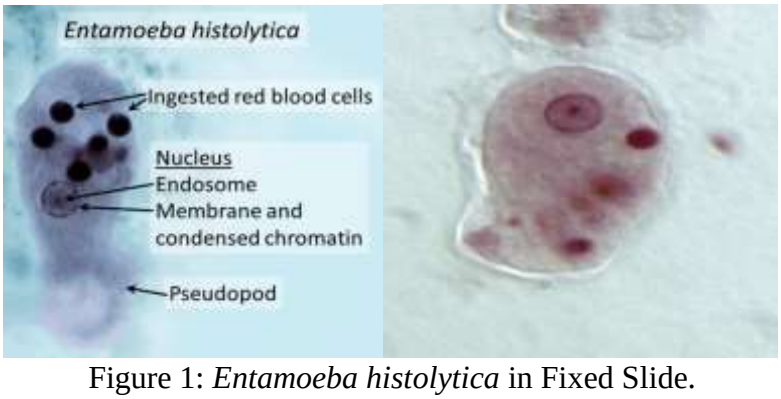


Figure 1: *Entamoeba histolytica* in Fixed Slide.

Conclusion:

Entamoeba histolytica can be reliably identified by its morphological characteristics under a microscope, especially its trophozoite form with ingested RBCs and cysts with multiple nuclei and chromatoid bodies. Accurate identification is crucial for diagnosing amoebiasis and initiating appropriate treatment.

Identification of *Giardia lamblia*

Introduction:

Giardia lamblia is a flagellated protozoan parasite that inhabits the small intestine and causes giardiasis, a diarrheal illness. It is transmitted through ingestion of cysts in contaminated water or food. Microscopic examination of fixed and stained slides is a key diagnostic tool for identifying both the trophozoite and cyst forms of *Giardia*.

Classification:

- **Kingdom:** Protista
- **Phylum:** Sarcomastigophora
- **Class:** Zoomastigophorea
- **Order:** Diplomonadida
- **Family:** Hexamitidae
- **Genus:** *Giardia*
- **Species:** *G. lamblia*

Microscopic Identifying Characters (in Fixed Stained Slide):

1. **Trophozoite Shape:** Pear- or teardrop-shaped, bilaterally symmetrical, 10–20 µm long.
2. **Nuclei:** Two large nuclei visible in stained slides, giving an "owl-eye" or "face-like" appearance.
3. **Flagella:** Four pairs (not always visible in fixed slides but located symmetrically).
4. **Adhesive Disc:** Concave ventral sucking disc may be seen as a clear zone or depression in stained slides.
5. **Cyst Form:** Oval, 8–12 µm in length, with 4 nuclei in mature cysts and presence of axonemes and median bodies.

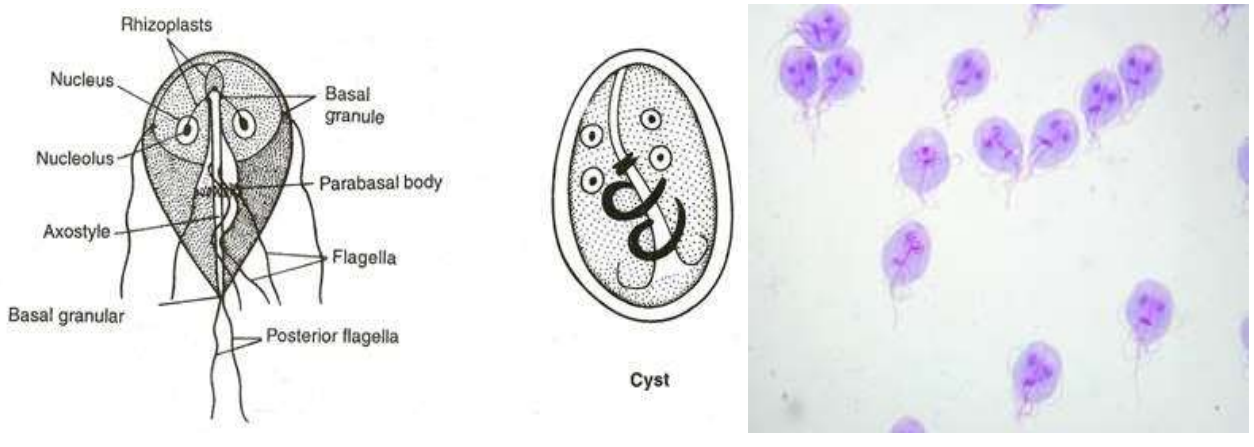


Figure 2: *Giardia lamblia* in Fixed Stained Slide.

Conclusion:

Microscopic examination of fixed stained slides allows for the identification of *Giardia lamblia* based on its characteristic pear shape, two nuclei, and cyst morphology. These features are essential for diagnosing giardiasis, especially in clinical stool samples.

Identification of *Ascaris lumbricoides*

Introduction:

Ascaris lumbricoides is a large intestinal nematode and one of the most common parasitic infections worldwide. It is transmitted via ingestion of the eggs, often found in contaminated soil or food. The adult worms inhabit the small intestine, while the eggs are passed in the feces. Microscopic examination of fixed stool or tissue samples is used to identify the distinctive characteristics of *Ascaris* eggs and adult worms.

Classification:

- **Kingdom:** Animalia
- **Phylum:** Nematoda
- **Class:** Secernentea
- **Order:** Ascaridida
- **Family:** Ascarididae
- **Genus:** *Ascaris*
- **Species:** *A. lumbricoides*

Microscopic Identifying Characters (in Fixed Stained Slide):

1. **Egg Size:** Oval, 45–75 µm in length, with a thick, mammillated outer shell. The shell is **bile-stained** in fixed preparations.
2. **Egg Shape:** The egg has a **rough, mammillated outer surface**, with a **smooth inner layer**. It can be *unfertilized* (larger, irregular) or *fertilized* (rounder, smaller).
3. **Larvae in the Egg:** Unfertilized eggs may contain **embryo** or **larvae**, which can sometimes be seen under higher magnification in fixed preparations.
4. **Adult Worms:** Female *Ascaris* worms are typically 20–35 cm long, and males are smaller (15–20 cm). They have a **straight, cylindrical body** with **a blunt anterior end** and **a tapered posterior end**.
5. **Cross-Section of Adult Worm:** In cross-section, the adult *Ascaris* shows **3 prominent muscle layers** and a large **gut cavity** in the center. The female may also contain **eggs** within the uterus visible in stained slides.

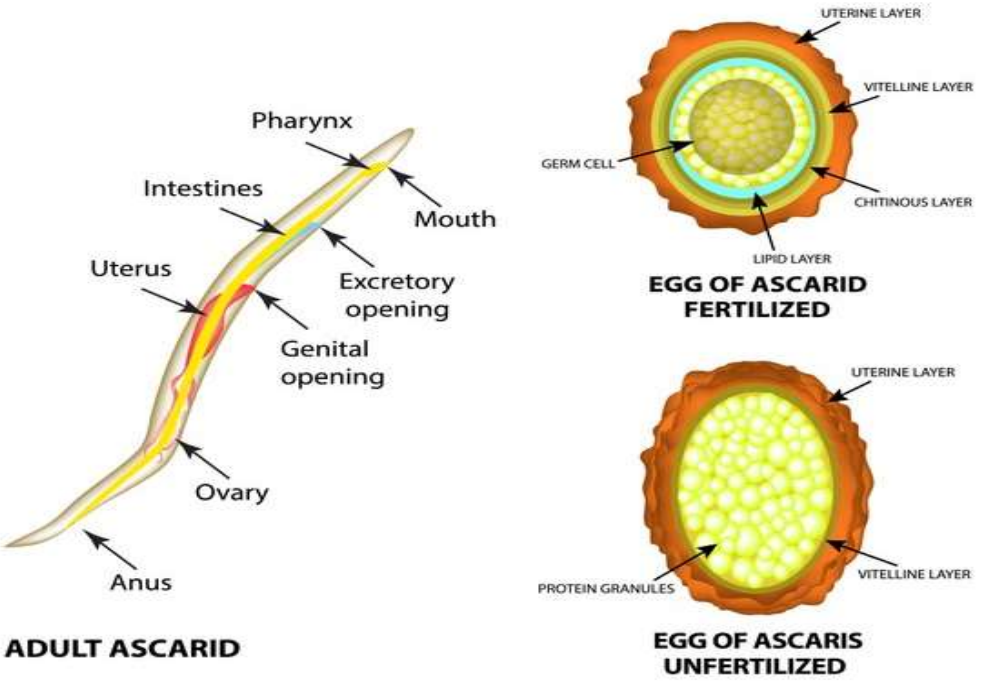


Figure 3: Ascaris lumbricoides Egg and Adult Worm in Fixed Stained Slide.

Conclusion:

Ascaris lumbricoides is identified in fixed and stained slides based on the characteristic features of the eggs, which are oval with a rough outer surface and the presence of adult worms with distinct cylindrical shapes. The eggs, when observed in stool samples, confirm the diagnosis of ascariasis, which can lead to a variety of gastrointestinal symptoms.

Identification of *Taenia solium*

Introduction:

Taenia solium, commonly known as the pork tapeworm, is a cestode parasite that infects humans through the ingestion of undercooked pork containing cysticerci (larval form). It causes taeniasis and, in some cases, cysticercosis if the eggs are ingested. Fixed and stained slides are useful for identifying the eggs, gravid proglottids, and scolex under the microscope.

Classification:

- **Kingdom:** Animalia
- **Phylum:** Platyhelminthes
- **Class:** Cestoda
- **Order:** Cyclophyllidea
- **Family:** Taeniidae
- **Genus:** *Taenia*
- **Species:** *T. solium*

Microscopic Identifying Characters (in Fixed Stained Slide):

1. **Egg Size and Shape:** Round, 30–40 µm in diameter, with a **thick, striated shell** (embryophore) enclosing the **oncosphere** (hexacanth embryo).
2. **Oncosphere:** Clearly visible **three pairs of hooklets** inside the egg in well-stained preparations.
3. **Gravid Proglottid:** Seen in stained mount; contains **15–20 lateral uterine branches** on each side of the uterus (used to differentiate from *T. saginata* which has 15–30).
4. **Scolex (if present):** Rounded head with **four suckers** and a **rostellum with a double row of hooks** (distinguishing feature of *T. solium*).
5. **Strobila (Body Segments):** Proglottids arranged in a chain-like manner; mature and gravid proglottids appear broader than long.

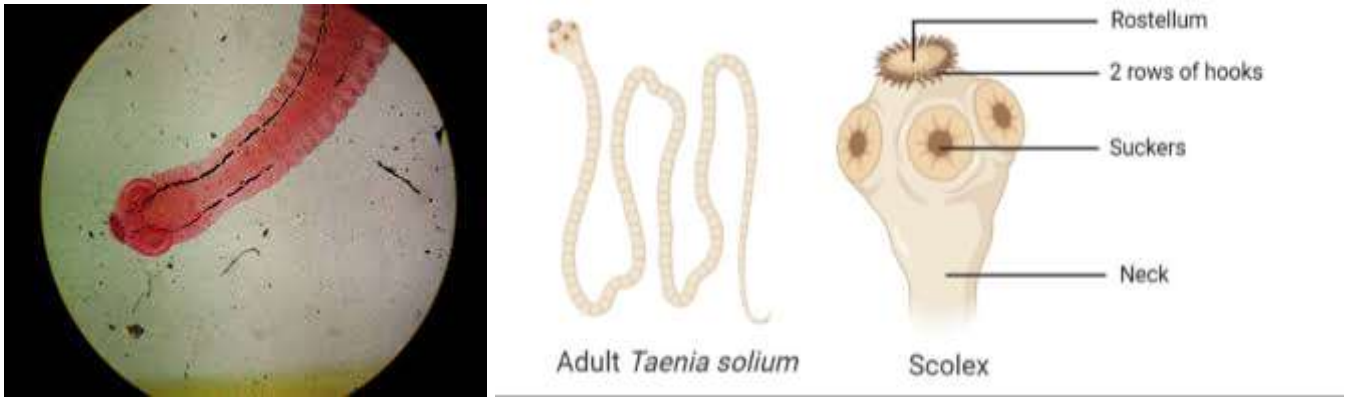


Figure 4: *Taenia solium*.

Conclusion:

Taenia solium is identified microscopically in fixed slides by its thick-shelled, striated eggs with hooklets, and the distinct morphological features of its gravid proglottids and scolex. Accurate identification is crucial to distinguish it from *T. saginata* and to prevent serious complications such as neurocysticercosis.

Identification of *Wuchereria bancrofti* (Microfilaria)

Introduction:

Wuchereria bancrofti is a filarial nematode responsible for lymphatic filariasis, commonly known as elephantiasis. The diagnostic stage is the **microfilaria**, which circulates in peripheral blood, typically at night (nocturnal periodicity). Fixed and stained blood smears allow for microscopic identification of the microfilarial stage.

Classification:

- **Kingdom:** Animalia
- **Phylum:** Nematoda
- **Class:** Secernentea
- **Order:** Spirurida
- **Family:** Onchocercidae
- **Genus:** *Wuchereria*
- **Species:** *W. bancrofti*

Microscopic Identifying Characters (in Fixed Stained Slide):

1. **Size:** Approximately 270–300 µm in length and 7–10 µm in width.
2. **Sheath:** Microfilaria is **sheathed**, and the sheath stains faintly in Giemsa-stained smears.
3. **Nuclei Pattern:** Numerous nuclei are present, but they **do not extend to the tip of the tail**—a key identifying feature.
4. **Shape:** Slender and gently curved, with a tapered tail.
5. **Staining:** Head space and tail are clear in Giemsa stain; body shows a column of nuclear granules not reaching the tail tip.

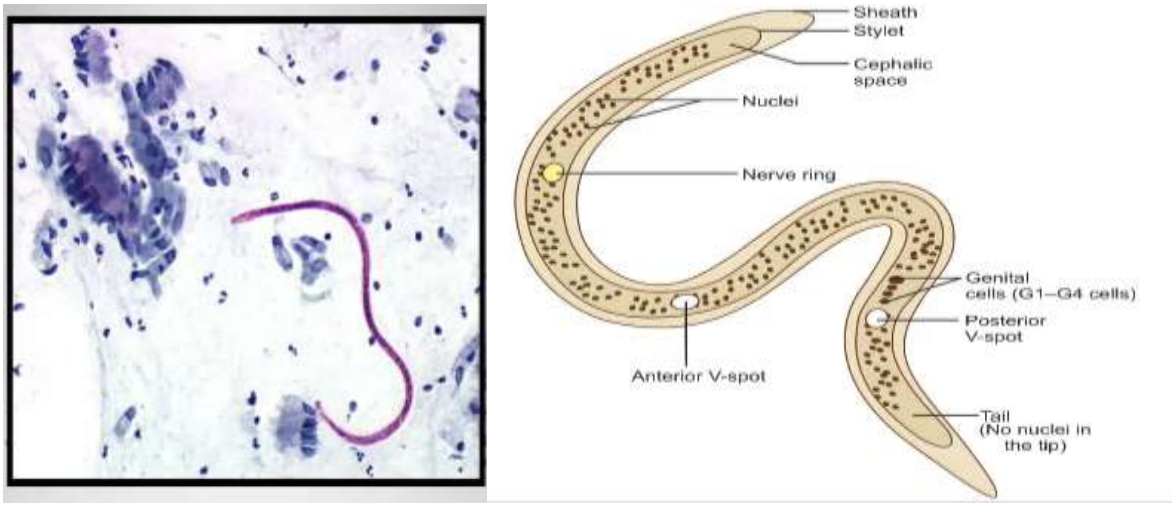


Figure 5: *Wuchereria bancrofti* Microfilaria

Conclusion:

Wuchereria bancrofti is identified microscopically by its sheathed body, absence of terminal nuclei, and nocturnal periodicity in peripheral blood. Its detection in stained smears is crucial for diagnosing lymphatic filariasis and initiating antiparasitic treatment.

Identification of *Ancylostoma duodenale*

Introduction:

Ancylostoma duodenale is a hookworm that infects the human small intestine, causing iron-deficiency anemia and gastrointestinal discomfort. Infection occurs through skin penetration by the filariform larvae from contaminated soil. The diagnostic stage is the egg, found in stool samples. Fixed and stained slides allow microscopic identification of the egg and, occasionally, larval forms.

Classification:

- **Kingdom:** Animalia
- **Phylum:** Nematoda
- **Class:** Secernentea
- **Order:** Strongylida
- **Family:** Ancylostomatidae
- **Genus:** *Ancylostoma*
- **Species:** *A. duodenale*

Microscopic Identifying Characters (in Fixed Stained Slide):

1. **Egg Shape:** Oval or elliptical, symmetrical, thin-shelled.
2. **Size:** Approximately 60–75 µm in length and 35–40 µm in width.
3. **Shell:** Transparent, smooth, and colorless; clearly defined outer margin.
4. **Embryo:** Typically contains a segmented **4–8 cell stage** embryo inside (early cleavage).
5. **Larval Forms (if present):** In some preserved samples, **rhabditiform larvae** can be seen with a long buccal cavity and a prominent genital primordium.

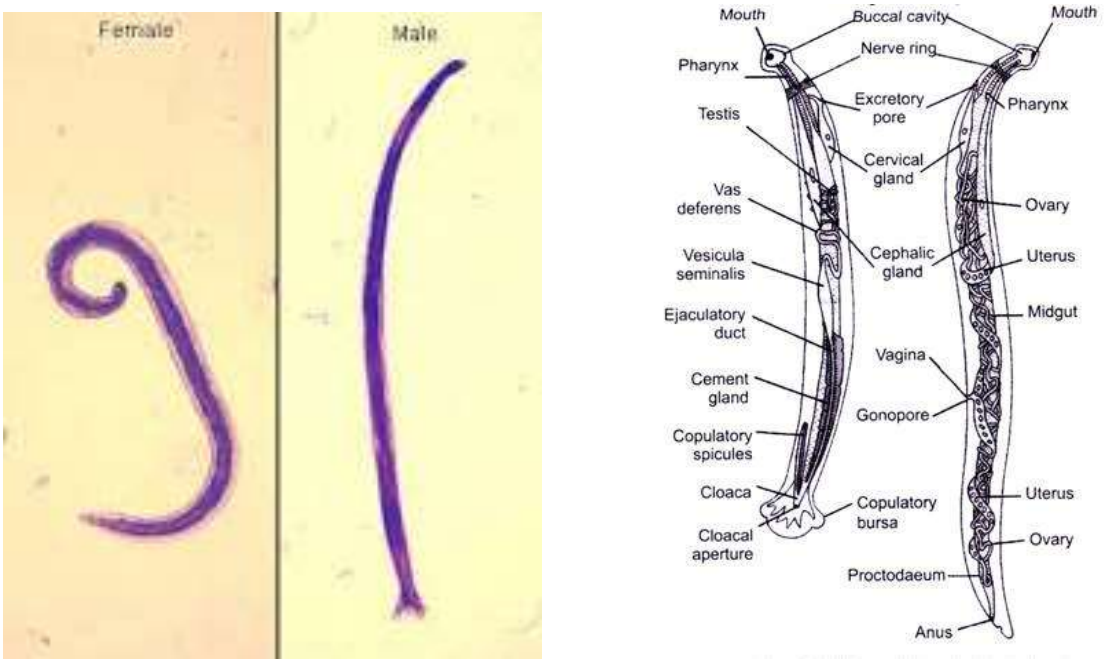


Figure 6: *Ancylostoma duodenale* Egg in Stool Sample

Conclusion:

Ancylostoma duodenale is identified by its characteristic oval, thin-shelled egg with a developing embryo inside. Early detection in stool samples through microscopy is essential for timely treatment of hookworm infection and prevention of anemia.

Identification of Different Species of Plasmodium

Introduction:

Malaria is a life-threatening disease caused by protozoan parasites of the genus *Plasmodium*. These parasites are transmitted to humans by the bite of infected female *Anopheles* mosquitoes. Five species of *Plasmodium* are known to infect humans—*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. Each species has distinct morphological characteristics and clinical features. Microscopic examination of Giemsa-stained thin and thick blood smears is the gold standard for identifying different stages and species of *Plasmodium*.

Microscopic Identifying Characters of Different *Plasmodium* Species (in Fixed Slide):

1. *Plasmodium falciparum*

- Multiple delicate **ring forms** in a single RBC.
- **Crescent-shaped gametocytes** (unique feature).
- **No Schizonts** usually seen in peripheral blood.
- RBCs are **not enlarged**.
- **No stippling** (no Schüffner's dots).
- May show **double chromatin dots** in ring forms.

2. *Plasmodium vivax*

- Large, **amoeboid trophozoites** occupying most of RBC.
- **Enlarged RBCs** with pale cytoplasm.
- **Schüffner's dots** present (pink granules).
- Schizonts with **12–24 merozoites**.
- Round gametocytes.

3. *Plasmodium malariae*

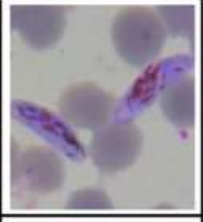
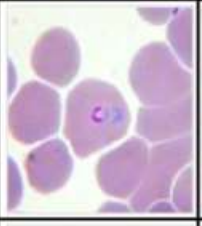
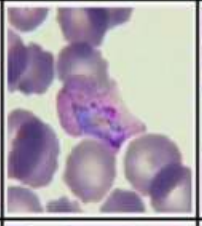
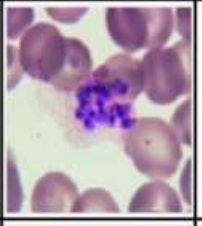
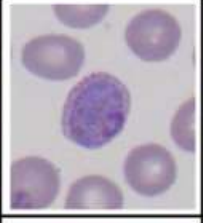
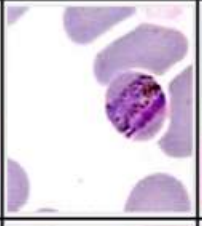
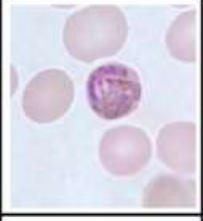
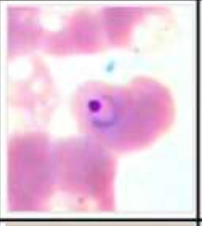
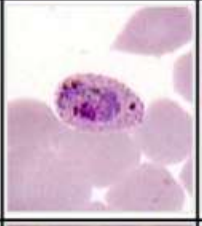
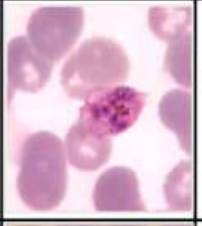
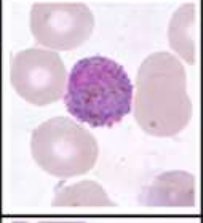
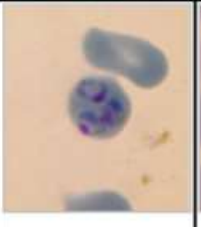
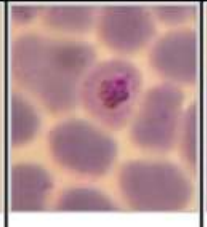
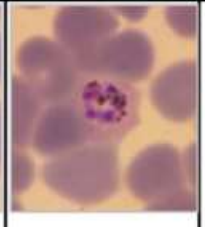
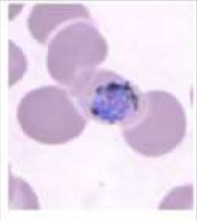
- **Band-shaped trophozoite** across the RBC.
- **Normal-sized RBCs**.
- Schizont with **6–12 merozoites**, arranged in **rosette** pattern.
- No Schüffner's dots.
- Compact gametocytes.

4. *Plasmodium ovale*

- **Oval-shaped RBCs** with **fimbriated edges**.
- **Schüffner's dots** present.
- Schizont with **6–12 merozoites**.
- Compact trophozoites and round gametocytes.
- RBCs moderately enlarged.

5. *Plasmodium knowlesi*

- Ring stages resemble *P. falciparum* (multiple rings per RBC).
- Late trophozoites and schizonts resemble *P. malariae*.
- **Daily (24-hour) replication cycle**.
- RBCs normal size.
- No Schüffner's dots.
- Rapid progression; can mimic severe malaria.

Human Malaria					
Stages / Species	Ring	Trophozoite	Schizont	Gametocyte	
P. falciparum					• *Parasitised red cells (pRBCs) not enlarged.* • *RBCs containing mature trophozoites sequestered in deep vessels.* • *Total parasite biomass = circulating parasites + sequestered parasites.*
P. vivax					• *Parasites prefer young red cells* • *pRBCs enlarged.* • *Trophozoites are amoeboid in shape.* • *All stages present in peripheral blood.*
P. malariae					• *Parasites prefer old red cells.* • *pRBCs not enlarged.* • *Trophozoites tend to have a band shape.* • *All stages present in peripheral blood*
P. ovale					• *pRBCs slightly enlarged and have an oval shape, with tufted ends.* • *All stages present in peripheral blood.*
P. knowlesi					• *pRBCs not enlarged.* • *Trophozoites, pigment spreads inside cytoplasm, like P. malariae, band form may be seen* • *Multiple invasion & high parasitaemia can be seen like P. falciparum* • *All stages present in peripheral blood.*

Conclusion:

The identification of *Plasmodium* species is crucial for appropriate malaria treatment and management. Each species shows distinct features under the microscope, particularly in the shape and size of trophozoites, schizonts, gametocytes, and the condition of infected RBCs. Accurate differentiation between species like *P. falciparum* and *P. vivax* is essential to avoid complications and ensure timely medical intervention.

Microscopic Examination of Stool for Ova and Parasites (O&P Test)

Principle:

The Stool Ova and Parasite (O&P) Test is based on the microscopic identification of parasitic stages—such as ova (eggs), cysts, trophozoites, and larvae—in a stool sample. Parasites that infect the human gastrointestinal tract often shed these stages in feces, making stool examination one of the most direct and effective diagnostic methods for intestinal parasitic infections.

The test uses wet mount preparations, typically with normal saline and iodine solution, to visualize these structures under a microscope. Saline wet mounts allow observation of motile trophozoites and larvae, whereas iodine wet mounts stain internal structures such as nuclei and glycogen vacuoles, making it easier to identify protozoan cysts.

In cases of low parasitic load, concentration techniques (such as flotation or sedimentation) may be used to increase the detection rate by concentrating the organisms into a smaller volume of the sample. The combination of direct and concentrated methods provides a comprehensive approach to diagnosis.

This test is crucial in diagnosing infections caused by protozoa (e.g., *Giardia lamblia, Entamoeba histolytica*) and helminths (e.g., *Ascaris lumbricoides, Taenia spp., Hookworms*). Proper identification requires attention to size, shape, internal structures, and staining characteristics of the observed organisms.

Procedure:

1. Sample Collection:

- Collect a fresh stool sample in a clean, dry, leak-proof container.
- Avoid contamination with urine or water.

2. Preparation of Wet Mounts:

- **Saline Wet Mount:**
  - Place a small amount of stool on a glass slide.
  - Add a drop of normal saline and mix gently.
  - Cover with a coverslip and examine under the microscope.
- **Iodine Wet Mount (Lugol's Iodine):**
  - On a separate slide, mix a small portion of the stool with a drop of iodine solution.
  - Place a coverslip and observe under the microscope.
  - Iodine stains glycogen and nuclei, enhancing visualization of cysts.

3. Microscopic Examination:

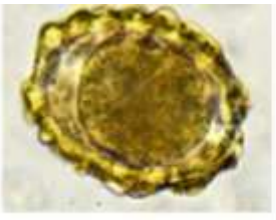
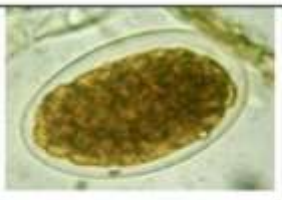
- Examine both mounts under low (10x) and high power (40x) objectives.
- Look for:
  - Ova (eggs) of helminths (e.g., *Ascaris*, *Taenia*)
  - Cysts and trophozoites of protozoa (e.g., *Giardia*, *Entamoeba*)
  - Larval forms if present

Result:

- **Positive:** Presence of parasitic ova, cysts, trophozoites, or larvae observed under the microscope.
- **Negative:** No parasitic forms detected in the stool sample.

Example:

Under high power field, *Entamoeba histolytica* cysts with four nuclei were observed, and *Ascaris lumbricoides* ova with a thick shell were identified in saline mount.

Round worm		Round or oval Bile stained, golden brown color An outer coarsely mammiliated albuminous coat, a thick transparent middle layer and the inner lipoidal vitelline membrane Large unsegmented Ovum at the centre, containing a mass of coarse lecithin granules.
Hook worm		Oval, non bile stained Thin transparent hyaline membrane 4-8 blastomeres Clear space between ovum and egg shell
Strongyloides stercoralis		Oval egg with rhabtidiform larvae
Trichuris trichuria		Barrel shaped, bile stained with mucus plug at both sides
Enterobius vermicularis		Colorless, non bile stained, plano convex, outer sticky albuminous layer, tad pole shaped embryo

Conclusion:

The microscopic examination of the stool sample revealed the presence of parasitic structures, confirming intestinal parasitic infection. Accurate identification aids in diagnosis and proper treatment. The O&P test remains a simple, cost-effective, and essential diagnostic tool in parasitology.

Formalin-Ethyl Acetate Stool Concentration Method

Principle:

The Formalin-Ethyl Acetate Concentration Technique is a type of sedimentation concentration method used to enhance the detection of protozoan cysts, helminth eggs, and larvae in stool specimens. Since these parasitic elements are often present in small numbers and mixed with large amounts of fecal debris, concentrating them improves their visibility and detection under a microscope.

The method operates on the principle of **differential specific gravity**:

Parasitic elements are denser than most of the debris and fat present in stool.

During centrifugation, heavier components (eggs, cysts, larvae) settle at the bottom, while lighter materials (fat, debris) remain in the upper layers or are dissolved by solvents.

Roles of Reagents:

Formalin (10% buffered formalin):

Acts as a fixative, preserving the morphology of parasitic stages.

Prevents degradation and distortion of delicate structures like protozoan cysts and helminth larvae.

Also serves as a preservative for the stool sample, allowing for delayed examination if necessary.

Ethyl Acetate:

A **solvent** that dissolves fats, plant materials, and debris present in stool.

Helps clarify the specimen by reducing background material, making parasitic elements easier to detect.

When mixed with the stool-formalin suspension, it creates a layered separation upon centrifugation.

Centrifugation Process:

Centrifugation uses **centrifugal force** to rapidly separate the components based on their specific gravities:

Heavier parasitic elements (eggs, cysts, larvae) move to the bottom forming a sediment.

Intermediate layer of formalin remains above the sediment.

Debris plug forms just above the formalin.

Ethyl acetate layer rises to the top, carrying dissolved fats and lighter materials.

This separation results in a **concentrated sediment** containing most of the parasites, which can then be isolated and examined microscopically.

Importance:

This concentration technique:

Increases diagnostic sensitivity, especially in **light infections** or samples with a low parasite load.

Provides **cleaner preparations** for microscopic examination by reducing background fecal debris.

Preserves the fine details of parasites, allowing for accurate identification and differentiation of species based on morphology.

Procedure:

1. The stool should be fixed in formalin for at least 30 minutes.

Mix 2 to 5 grams of the stool thoroughly in the 7ml 10% formalin.

2.Filter the above stool in the formalin. This can be done by two layers of gauze or a wire screen and collecting around 3 mL.

3. Add 10 to 12 mL of 0.85% saline and mix it well.

4. Centrifuge for 2 minutes at 2000 RPM (or 2500 RPM).

5. Discard the supernatant and leave 1 to 1.5 mL of the sediment. If the supernatant is cloudy, then repeat the above steps of saline.

6. Add 9 mL of 10% formalin to the sediment.

7. Now add 3 mL of ethyl acetate.

8. Cap the test tube and shake well for 30 seconds.

9. Centrifuge the tubes for 1 minute at 2000 RPM.

10. Four layers will form. The bottom is the sediment that is needed to prepare the smear.

Layer Separation:
After centrifugation, four layers form:

- **Top layer:** Ethyl acetate (containing fats and debris)
- **Second layer:** Plug of debris
- **Third layer:** Formalin
- **Bottom layer:** Sediment (containing the concentrated parasitic elements)

11. Remove the debris with a wooden applicator stick. Decant the upper three layers carefully and leave the sediments in the test tube.

12. Clean the sides of the test tube with a swab. Giardia cyst may stick to the side of the test tube.

13. Add a few drops of the formalin and mix the sediment thoroughly. This will preserve the sediment.

11. Now, we can make the smears in saline and iodine wet preparation.

14. Examine under the microscope at 10X and 40X magnification.

Results:

Positive: Identification of parasite cysts, eggs, or larvae in the sediment.

Negative: No parasites seen in the sediment.

Parasite morphology is generally well-preserved due to formalin fixation, allowing for clear identification.

Conclusion:

The Formalin-Ethyl Acetate Concentration Method is a simple, efficient, and reliable method for concentrating and detecting intestinal parasites in stool samples. It improves the chances of detecting low-density infections and is especially useful in epidemiological surveys and clinical diagnostics.

Stool preservation

Formalin Preservation

Principle:

Formalin (usually 5%–10%) acts as a fixative and preservative, killing and preserving parasite eggs, cysts, and larvae while maintaining their morphology for later identification under a microscope.

5% is ideal for protozoan cysts.

10% preserves eggs and cysts.

Materials:

10% Formalin solution (prepared in water)

Clean, dry, leak-proof specimen container

Applicator stick or spoon

Procedure:

1. Collect stool sample in a clean, dry container.
2. Mix approximately 1 part of stool with 3 parts of 10% formalin (for liquid stool, use equal volumes of stool and formalin).
3. Stir thoroughly with an applicator stick to evenly distribute the stool in the formalin.
4. Seal the container tightly and label with patient information.
5. Store the preserved sample at room temperature or refrigerated (if available).
6. Use for direct wet mounts, concentration methods (like Formalin-Ethyl Acetate method), or staining (e.g., iodine wet mounts).

Advantages:

Simple and inexpensive.

Preserves parasite morphology well.

Compatible with concentration techniques.

Limitations:

Not ideal for permanent staining methods (e.g., trichrome stains) as it may harden or distort protozoan trophozoites.

Polyvinyl Alcohol (PVA) Preservation

Principle:

PVA is a plastic resin-based fixative combined with mercuric chloride, Schaudinn's solution, or other preservatives to fix and preserve protozoan trophozoites, cysts, eggs, and larvae. It is especially valuable for preserving delicate trophozoites for permanent stained smears.

Materials:

PVA fixative (commercial or lab-prepared)

Clean, dry, leak-proof specimen container

Applicator stick or spoon

Procedure:

1. Collect stool specimen in a clean, dry container.
2. Mix a portion of the stool (about 2 parts stool to 3 parts PVA fixative) using an applicator stick until a uniform suspension is achieved.
3. Transfer the mixture to a properly labeled container with a tight-fitting lid.
4. Preserve at room temperature until laboratory examination.
5. Use preserved material for:

    Permanent stained smears (e.g., trichrome or iron hematoxylin stain)

    Wet mounts (though formalin is usually preferred for wet mounts)

Advantages:

Excellent preservation of protozoan trophozoites.

Allows for high-quality permanent stained smears.

Limitations:

Involves mercury-based fixatives (in some formulas), which are toxic and environmentally hazardous.

Not suitable for concentration techniques.

Requires careful handling and disposal.

Wheatley Trichrome Stain for Ova, Cyst and Parasite Identification

Principle

Chromotrope 2R has an affinity for chromatin material. Nuclear chromatin, chromatoid bodies, karyosomes, parasite eggs and larvae, bacteria, and ingested erythrocytes stain red to purple-red. Light green and fast green dyes stain the cytoplasm of preserved cysts, trophozoites, and cellular constituents blue-green. The Trichrome Stain results in excellent contrast and visualization of cellular details that aid in the identification of protozoa.

The Wheatley's Trichrome Stain is a permanent staining technique that differentiates parasitic elements (protozoa, cysts, trophozoites) from background material in stool samples by using multiple dyes with differing affinities for cellular structures.

- Chromatin material (like nuclei) stains red to purple.
- Cytoplasm of protozoan trophozoites and cysts stains blue-green to green.
- Background debris and fecal material pick up a pale green or blue color, providing contrast.

This method allows clear visualization of:

- Internal structures of protozoan trophozoites (nuclei, chromatin, karyosomes)
- Cyst walls
- Helminth ova morphology

It's particularly valuable for identifying delicate **protozoan trophozoites**, which are often missed in wet mounts or concentration methods.

Requirements:

Reagents:

- 70%, 90%, and absolute ethanol
- Wheatley's Trichrome Stain (contains chromotrope 2R, light green SF yellowish, and fast green FCF)
- Acid alcohol (90 mL absolute ethanol + 3 mL glacial acetic acid)
- Xylene or xylene substitute (for clearing)
- Coverslips
- Mounting medium (e.g., DPX or synthetic resin)

Equipment:

- Microscope slides
- Applicator sticks
- Centrifuge (if using concentrated samples)

- Microscope (with 10X and 40X objectives)

Procedure:

1. **Smear Preparation:**

   Prepare a thin smear of the stool specimen (preferably preserved in PVA) on a clean glass slide.

   Allow to air dry.

2. **Fixation (if required):**

   If not already PVA-preserved, fix the smear with Schüaudinn's fixative or equivalent.

3. **Staining Process:**

Stain the fixed smear in Wheatley's Trichrome Stain for 10 minutes.

Rinse briefly in acid alcohol (to differentiate stain and remove excess dye) for 1–3 seconds.

Quickly dehydrate in 95% ethanol (two changes).

Clear the smear in xylene or xylene substitute.

Mount with a coverslip using a synthetic resin mounting medium.

4. **Microscopic Examination:**

   Examine under 10X for scanning and 40X for detailed identification.

Results:

Component	**Staining Reaction**
Protozoan nuclei and chromatin	Red to purple
Protozoan cytoplasm (trophozoites & cysts)	Blue-green to green
Helminth eggs (ova)	Usually green to red, depending on species
Background debris	Pale green to bluish

Trophozoites and cysts can be clearly distinguished by their internal structures like nuclei, karyosomes, and chromatin bodies, thanks to this multichromatic contrast.

Conclusion:

The Wheatley's Trichrome Stain is a highly sensitive and reliable staining technique for the identification of intestinal protozoa and helminths in stool specimens. It provides excellent visualization of nuclear and cytoplasmic details, particularly for protozoan trophozoites, making it a critical tool in the diagnosis of parasitic infections.

This method is especially recommended when:

Trophozoites are suspected (as they are fragile and poorly visible in wet mounts)

Detailed morphological study is required for species identification

Confirmatory identification following concentration methods

Iron Hematoxylin Stain for Ova, Cyst, and Parasite Identification

Principle:

The Iron Hematoxylin Stain is a permanent staining technique that provides sharp contrast and high detail of the internal structures of protozoan cysts, trophozoites, and helminth ova in stool specimens.

- Hematoxylin acts as the primary dye, staining chromatin and nuclear material a dark blue to black.
- The stain is mordanted with ferric ammonium sulfate (iron), which intensifies and fixes the color.
- Background debris and cytoplasm appear pale gray or light blue, providing contrast against the deeply stained nuclei and chromatin structures.

This technique is particularly prized for its ability to show fine details of nuclear structures, enabling accurate identification of protozoan species.

Requirements:

Reagents:

- Schaudinn's fixative (or PVA-fixed slides)
- Hematoxylin stain (iron hematoxylin)
- Mordant (ferric ammonium sulfate)
- Differentiator (acid alcohol: 70% ethanol + a few drops of HCl)
- 70%, 90%, and absolute ethanol (for dehydration)
- Xylene or xylene substitute
- Mounting medium (e.g., DPX or synthetic resin)

Equipment:

Microscope slides

Applicator sticks

Centrifuge (if using concentrated samples)

Microscope (with 10X and 40X objectives)

Procedure:

1. Smear Preparation:

Prepare a thin, even smear of stool (preferably PVA-fixed) on a clean glass slide.

Allow to air dry.

2. Fixation (if needed):

If fresh or unfixed, immerse in Schaudinn's fixative.

3. Staining Process:

Stain the fixed smear in Iron Hematoxylin solution for 10 minutes.

Rinse in tap water.

Differentiate in acid alcohol for a few seconds until the background appears light.

Rinse again in water.

Dehydrate in graded alcohol series: 70%, 90%, and absolute ethanol.

Clear in xylene or xylene substitute.

Mount with a coverslip using synthetic resin mounting medium.

4. Microscopic Examination:

Examine under 10X for scanning and 40X or 100X oil immersion for detailed study.

Results:

Component	Staining Reaction
Protozoan nuclei and chromatin	Dark blue to black
Protozoan cytoplasm	Light blue to gray
Helminth eggs (ova)	Dark structures with pale background
Background debris	Light gray to pale blue

This stain highlights internal structures of protozoa (like karyosomes, peripheral chromatin, inclusions) more sharply than trichrome but provides less color differentiation for cytoplasm.

Conclusion:

The Iron Hematoxylin Stain is a highly sensitive and reliable permanent staining method for identifying protozoan trophozoites and cysts in stool specimens. It is particularly useful for detecting fine nuclear details and chromatin structures which are crucial for accurate protozoan species differentiation.

Although it requires more steps and time compared to Wheatley's Trichrome Stain, its superior nuclear detail makes it the gold standard for protozoan identification in many diagnostic parasitology laboratories.

Modified Acid-Fast Stain for Ova, Cyst, and Parasite Identification

Principle:

The Modified Acid-Fast Stain is a differential staining technique primarily used to detect acid-fast oocysts of intestinal coccidia, such as:

- *Cryptosporidium parvum*
- *Cyclospora cayetanensis*
- *Cystoisospora belli*

The method relies on the ability of the parasite's cell wall (rich in lipids and waxes) to retain the primary stain (carbol fuchsin) even after decolorization with a milder acid-alcohol solution compared to the traditional Ziehl-Neelsen method.

- Acid-fast organisms retain the red color of carbol fuchsin.
- Non-acid-fast background material and debris take up the counterstain (like methylene blue or malachite green), providing contrast.

This selective staining enhances the visibility of acid-fast oocysts against a colored background.

Requirements:

Reagents:

- Carbol fuchsin stain (strong or dilute depending on lab protocol)
- Acid-alcohol (usually **1% sulfuric acid** or **3% hydrochloric acid in 95% ethanol**)
- Counterstain (methylene blue, malachite green, or brilliant green)
- Distilled water

Equipment:

- Microscope slides
- Applicator sticks
- Microscope (with 10X and 40X objectives, sometimes oil immersion)
- Heating source (if using heat-assisted staining)

Procedure:

1. Prepare a smear with 1 to 2 drops of specimen on the slide and dry on a slide warmer at 60°C until dry. Do not make the smears too thick!
2. Fix with absolute methanol for 30 seconds.

3. Stain with Kinyoun's carbol fuchsin for one minute. Rinse briefly with distilled water and drain.
4. Destain with acid alcohol for 2 minutes. Rinse with distilled water and drain.
5. Counterstain with Malachite green for 2 minutes. Rinse briefly with distilled water and drain.
6. Dry on a slide warmer at 60°C for about 5 minutes. Mount with a coverslip using desired mounting media.
7. Examine 200 to 300 fields using 40× or higher objectives. To confirm internal morphology, use 100× oil immersion objective.

Results:

Component	Staining Reaction
Coccidian oocysts (e.g., Cryptosporidium, Cyclospora)	Bright red to pink (acid-fast positive)
Background debris and non-acid-fast organisms	Blue or green (depending on counterstain)
Other parasite ova and cysts	Typically unstained or counterstained

Cryptosporidium oocysts appear as small, round, red-stained bodies (4–6 µm**)** against a blue or green background.
Cyclospora and *Cystoisospora* are larger, with *Cyclospora* being spherical (8–10 µm) and *Cystoisospora* ovoid (20–30 µm).

Conclusion:

The Modified Acid-Fast Stain is a highly specific and sensitive diagnostic technique for detecting acid-fast intestinal coccidian parasites in stool specimens. It is particularly valuable in immunocompromised patients (e.g., HIV/AIDS) where infections with *Cryptosporidium*, *Cyclospora*, or *Cystoisospora* are common.

This method provides clear contrast and reliable identification of oocysts in mixed fecal backgrounds, making it an essential tool in parasitology diagnostics.

Collection, Preparation, and Identification of Haemoparasites

Principle:

Haemoparasites are blood-borne parasites that infect blood cells or plasma and are transmitted typically through insect vectors. Identification of these parasites is essential for diagnosing infections such as malaria, filariasis, trypanosomiasis, and babesiosis. The principle of this test lies in the microscopic examination of stained blood smears (thin and thick) to detect the presence of parasitic forms (e.g., trophozoites, gametocytes, microfilariae). Blood smears are stained using Romanowsky-type stains (commonly Giemsa stain), which highlight the internal structures of parasites and host blood cells, allowing for differentiation and identification based on morphology, size, and staining characteristics.

Sample Collection:

- **Specimen:** Peripheral blood
- **Timing:** Preferably collected during **febrile episodes** or when parasitemia is suspected to be high
- **Method:** Finger prick (capillary blood) or venipuncture (for larger samples) using sterile technique
- **Anticoagulant:** Not required for smears; EDTA tubes may be used for molecular or concentration techniques

Slide Preparation:

1. Thin Blood Smear:

- Place a small drop of blood near one end of a clean glass slide.
- Use another slide held at a 30–45° angle to spread the drop into a thin film.
- Air dry and fix with **methanol**.

2. Thick Blood Smear:

- Place a larger drop of blood at the center of a slide.
- Using the corner of another slide, spread it into a circular area about 1–2 cm wide.
- Allow to air dry thoroughly (do **not** fix with methanol).

Staining Procedure (Giemsa Staining):

1. Prepare a working Giemsa stain (1:20 dilution with buffered water, pH 7.2).
2. Immerse air-dried slides in the stain:
    - **Thin smear**: 10 minutes
    - **Thick smear**: 15–20 minutes
3. Rinse with buffered water and allow to air dry.

Microscopic Examination:

- Examine the smears under **oil immersion (100x objective)**.
- Observe for:
    - **Plasmodium species** (rings, trophozoites, schizonts, gametocytes)
    - **Trypanosomes** (flagellated extracellular forms)
    - **Microfilariae** (slender, thread-like organisms)
    - **Babesia** (ring forms inside RBCs, typically without pigment)

Result:

- **Positive:** Presence of haemoparasites such as:
    - *Plasmodium falciparum*: multiple ring forms, crescent-shaped gametocytes
    - *Plasmodium vivax*: enlarged RBCs, amoeboid trophozoites
    - *Wuchereria bancrofti*: microfilariae with sheath
    - *Trypanosoma*: flagellated parasites outside RBCs
    - *Babesia*: small rings, sometimes in tetrads (Maltese cross form)
- **Negative:** No parasitic forms observed.

Conclusion:

Through proper collection, smear preparation, and Giemsa staining, haemoparasites can be effectively identified under the microscope. This diagnostic approach remains essential in the detection and differentiation of malaria,

filariasis, trypanosomiasis, and other parasitic infections of the blood. Accurate identification aids in timely and targeted treatment, especially in endemic areas.

Whole Mount Preparation of Different Disease-Causing Arthropods

Principle:

Disease-causing arthropods play a significant role in the transmission of various pathogens, including viruses, bacteria, protozoa, and helminths. These arthropods may act as vectors (mechanical or biological) or directly cause disease through bites, stings, or infestations. Whole mount preparation involves placing the entire body of the arthropod on a slide for microscopic examination, enabling the study of morphological features crucial for identification and classification. Proper identification is essential for understanding the life cycles of vector-borne diseases, implementing control strategies, and promoting public health awareness.

Common Disease-Causing Arthropods:

- *Anopheles* mosquito (*Malaria* vector)
- *Aedes* mosquito (*Dengue, Zika, Yellow fever*)
- *Culex* mosquito (*Filariasis, Japanese encephalitis*)
- *Pediculus humanus* (Lice – causes Pediculosis, vector of Typhus)
- *Xenopsylla cheopis* (Rat flea – vector of Plague)
- *Phlebotomus* (Sandfly – vector of Leishmaniasis)
- *Ticks* (e.g., *Ixodes*, *Rhipicephalus* – vector of Lyme disease, Tick typhus)

Materials Required:

- Preserved arthropod specimens
- Clean glass slides and coverslips
- Forceps and dissecting needles
- Glycerin or mounting medium
- Dropper or pipette
- Labeling materials
- Stereomicroscope/compound microscope

Procedure:

1. Select a clean, whole arthropod specimen (e.g., mosquito, louse, flea, etc.).
2. Place a small drop of **glycerin** or mounting medium on a clean glass slide.
3. Using forceps or a needle, carefully place the specimen in the mounting medium.
4. Gently position the arthropod flat, with body parts spread to display legs, wings, antennae, etc.
5. Place a coverslip over the specimen without trapping air bubbles.
6. Label the slide with the name of the arthropod and date.
7. Examine the slide under a **stereomicroscope** or **compound microscope** depending on the size.

Observation (What to Look For):

- **Body segmentation** (head, thorax, abdomen)
- **Wings and antennae** structure (in insects)
- **Legs** (jointed, clawed, number of pairs)
- **Mouthparts** (piercing, sucking, chewing)

- **Presence of sensory hairs or bristles**
- **Size and color patterns**
- Specific identifiers (e.g., palps in *Anopheles*, notched abdomen in *Culex*)

Result:

- The prepared slides clearly displayed the morphological characteristics of the arthropod specimens.
- Examples:
  - *Anopheles* showed long palps and spotted wings.
  - *Pediculus humanus* had clawed legs adapted for clinging to hair.
  - *Ixodes* tick showed a hard dorsal shield (scutum) and four pairs of legs (arachnid).

Conclusion:

The whole mount preparation of disease-causing arthropods enables detailed examination of external morphology, aiding in accurate identification. This is crucial in understanding the epidemiology and transmission cycles of many vector-borne diseases. Practical knowledge of vector biology supports diagnosis, prevention, and control strategies in medical and public health settings.

Rapid Diagnostic Tests (RDTs) for Parasitic Infections

Principle:

Rapid Diagnostic Tests (RDTs) are immunochromatographic assays designed to detect specific parasitic antigens or antibodies in blood, serum, or other body fluids. These tests utilize a lateral flow assay system where a sample (blood or urine) is applied to a test device. The device contains specific antibodies or antigens immobilized on a membrane. When the sample is added, if the target antigen or antibody is present, it binds to the corresponding antibody or antigen on the test strip, leading to the formation of a visible colored line due to a conjugated enzyme or dye. This reaction is specific, rapid, and simple, providing diagnostic results in minutes without the need for sophisticated laboratory equipment.

RDTs are particularly useful for disease screening in remote or resource-limited areas, where advanced laboratory facilities may not be available. They are commonly used for diagnosing malaria, filariasis, leishmaniasis, and other parasitic infections.

Procedure:

1. Sample Collection: A blood, serum, or urine sample is collected from the patient.
2. Application of Sample: A small amount of the sample is placed onto the sample pad of the RDT device.
3. Buffer Addition: Add the tris-buffered saline (TBS) or phosphate-buffered saline (PBS) solution provided with the test kit to the sample pad.
4. Wait for Results: Wait for 10–20 minutes for the test to process.
5. Result Observation: Observe for the appearance of a visible colored line. If the test line appears, the result is positive; if it does not appear, the result is negative.

Result:

- **Positive Result:** A visible colored line appears at the test region of the RDT device, indicating the presence of the target antigen or antibody. This suggests that the patient is infected with the parasite detected by the RDT.
    - **Example:** For malaria, the appearance of a colored line at the test region indicates the presence of malaria-specific antigens (such as **HRP-2** or **pLDH**).
- **Negative Result:** No visible colored line appears at the test region, indicating that the target antigen or antibody was not detected in the sample. This suggests that the patient does not have the parasitic infection detected by the test.
    - **Example:** A malaria RDT without a test line would indicate that no malaria antigens were present in the sample.
- **Invalid Result:** If the **control line** does not appear, regardless of the test line, the result is invalid. This typically indicates an error in the test procedure or an issue with the test device itself (e.g., improper sample application or expired test kit). The test should be repeated with a new kit.

Conclusion:

Rapid Diagnostic Tests (RDTs) are **rapid, easy-to-use**, and **specific** diagnostic tools for detecting parasitic infections. These tests are particularly useful in **remote** and **resource-limited areas**, where laboratory testing is not available. RDTs offer the advantage of quick results (in **15–20 minutes**) and can be performed at the point of care without requiring specialized equipment.

Despite their effectiveness, RDTs may have limitations, such as **false-negative** or **false-positive** results under certain conditions. Therefore, **positive results** should be confirmed with more advanced diagnostic

techniques, such as **microscopy** or **PCR**, and **negative results** should be interpreted carefully, particularly if clinical symptoms suggest the presence of infection.

Malaria Rapid Diagnostic Test (MRDT)

Principle:

The Malaria Rapid Diagnostic Test (MRDT) is an immunochromatographic test designed to detect malaria parasite antigens in the blood. Specifically, MRDTs are used to detect HRP-2 (Histidine-rich protein 2), pLDH (Plasmodium lactate dehydrogenase), and aldolase antigens, which are released by malaria parasites into the bloodstream during the infection. When a blood sample is applied to the test device, these antigens bind to antibodies that are immobilized on the test membrane, causing the formation of a visible colored line. The test is simple, rapid, and can provide results in 15–20 minutes without the need for laboratory equipment.

Procedure:

1. Sample Collection: Collect a fingerstick or venous blood sample from the patient.
2. Application of Sample: Apply a small amount of blood (usually around 5–10 μL) to the sample pad on the test device.
3. Buffer Addition: Add PBS buffer or the buffer provided with the test kit to the sample pad to facilitate proper movement of the sample and antigen-antibody interaction.
4. Wait for Results: Allow the test to develop for 15–20 minutes.
5. Result Observation: Observe the test device for the appearance of colored lines at the control and test regions.

Result:

- **Positive Result:**
  - Control Line + Test Line(s): The appearance of the control line is a required result in all tests to show that the test is working correctly.
  - If a test line appears along with the control line, the result is positive, indicating the presence of malaria antigens (e.g., HRP-2, pLDH, or aldolase) in the patient's blood.
  - Example: If both the control line and test line (e.g., HRP-2) appear, the result is positive for *Plasmodium falciparum.*
- **Negative Result:**
  - **Control Line Only:** If only the **control line** appears and no test line forms, the result is **negative**, indicating the absence of malaria antigens in the sample.
  - **Example:** A malaria RDT without a test line would indicate no malaria antigens were detected, and the patient is likely not infected with malaria.

- **Invalid Result:**
  - No Control Line: If the control line does not appear, the test is considered invalid, regardless of whether a test line appears or not. This suggests a problem with the test procedure, sample

application, or a malfunction in the test device. A new test should be performed with a fresh kit.

Conclusion:

- The Malaria Rapid Diagnostic Test (MRDT) provides a rapid, simple, and effective method for diagnosing malaria in individuals. It detects Plasmodium antigens (such as HRP-2, pLDH, and aldolase) directly from the blood, offering results in 15–20 minutes. This test is particularly useful in remote and resource-limited settings, where microscopic examination or other laboratory-based methods are unavailable.
- While MRDTs are valuable tools for early diagnosis and screening of malaria, they may still produce false-negative or false-positive results due to factors like low parasite density or antigen variations. Therefore, positive results should be confirmed with microscopy or PCR, and negative results should be interpreted cautiously, particularly in areas with high malaria transmission.

Leishmanin Skin Test (Montenegro Test) for Leishmaniasis

Principle:

The Leishmanin skin test, also known as the Montenegro test, is a delayed-type hypersensitivity (DTH) test used to assess cell-mediated immune response to *Leishmania* antigens. It helps identify prior exposure or immune response to *Leishmania* infection, especially in cases of cutaneous or mucocutaneous leishmaniasis. In this test, killed promastigotes of *Leishmania* spp., suspended in a physiological saline buffer, are injected intradermally. In individuals previously exposed to *Leishmania*, sensitized T lymphocytes recognize the antigen and trigger an inflammatory response, leading to induration (a raised, hardened area) at the injection site within 48–72 hours. The test is not useful in active visceral leishmaniasis, where the immune response is suppressed.

Buffer Used:

The antigen is prepared in 0.9% normal saline (sodium chloride solution) as a physiological buffer to maintain antigen stability and pH for safe intradermal injection.

Procedure:

1. Preparation of Site: Clean the inner aspect of the forearm with alcohol.
2. Injection: Inject 0.1 mL of the Leishmanin antigen intradermally using a sterile syringe.
3. Monitoring Period: Advise the subject not to scratch or apply pressure to the site.
4. Reading the Result: After 48 to 72 hours, measure the diameter of induration (not erythema) using a transparent ruler or caliper.

Result:

- Positive Result:

  - Induration ≥ 5 mm after 48–72 hours indicate a positive result.
  - Suggests previous exposure or immune sensitization to *Leishmania* spp.
  - Common in cutaneous and mucocutaneous leishmaniasis or recovered visceral cases.
- Negative Result:
  - Induration < 5 mm or no reaction indicates a negative result.
  - May occur in naïve individuals or in those with active visceral leishmaniasis (due to immunosuppression).

Conclusion:

The Leishmanin skin test is a simple, cost-effective method to detect delayed-type hypersensitivity to *Leishmania* antigens. It is particularly helpful for diagnosing cutaneous and mucocutaneous leishmaniasis in endemic areas. A positive test indicates previous exposure and functioning cell-mediated immunity, while a negative test does not exclude disease, especially in immunocompromised individuals or cases of visceral leishmaniasis.

Course Outline:

1. Donor selection and blood collection,
2. Forward grouping, reverse grouping, Coomb's test: direct and indirect, cross matching of blood, compatibility test, Rho typing.
3. Demonstration of apheresis technique.
4. Preparation of reagents and preservatives in blood bank.
5. Preparation of packed red cells.
6. HIV-testing, hepatitis B and C testing, Syphilis testing.
7. Pooled cell preparation, Weak-D test
8. Blood group subtypes determination

CRITERIA FOR SELECTION OF DONORS

Following guidelines should be observed in order to determine that the blood donation will not be detrimental to the donors/recipients.

Physical Examination

A medical officer should certify the donor fit for blood donation.

General Appearance The prospective donor should appear to be in good health.

Age - Donors should be between the age of 18 and 65 years.

Haemoglobin or packed Cell Volume (Haematocrit): The haemoglobin should be not less than 12.0 gm/dl or the packed cell volume (haematocrit) should be not less than 36%. The screening should be carried out by using any appropriate and validated methodology. A more sensitive method of hemoglobin testing should be available as a reference or control.

Weight - Blood collection from donors weighing 45-55 Kg should be 350 ml blood and from those weighing 55 Kg and above should be 450 ml.

Blood Pressure - The systolic blood pressure should be between 100 and 160 mm of mercury and the diastolic pressure should be between 60-90 mm of mercury.

Temperature - Temperature should not exceed 37.50 C/ 99.5o F

Pulse - Pulse should be between 60 to 100 beats per minute and regular.

Donor Skin - The skin at the venipuncture site should be free of any skin lesion or scar indicative of addiction to narcotics or infection as well as marks of repeat venipuncture.

MEDICAL HISTORY

Conditions that affect safety of donors: Before each donation questions should be asked to determine that the donor is in normal health and has not suffered or is not suffering from any serious illness e.g. malignant

disease, epilepsy, bronchial asthma, diabetes, excessive menstrual bleeding, cardio-vascular conditions, renal disease, allergic diseases, abnormal bleeding tendency.

Pregnancy:

Prospective donor should not be accepted during period of pregnancy and till 12 months after full term delivery and also during lactation. Donors who have abortions should be deferred till 6 months after 2nd and 3rd trimester abortion. Menstruation in itself should not be a cause for deferral. Any donor who appears to be under the influence of alcohol or any drug abuse and who does not appear to be providing reliable answers to questions on their medical history should not be accepted. .

Any donor on antibiotic therapy or other medications should be deferred after evaluating his/her suitability as donor.

Infectious disease

- Donors having history of malaria should be accepted after 3 months.Donors having history of jaundice should be deferred up to 1 year.
- Donors having history of being HIV, HBsAg / HCV antibody positive should be permanently deferred. Donors having intimate contact with HIV, HBsAg / HCV antibody positive individual should be deferred for 1 year.
- Donors having history of measles/mumps/chickenpox should be deferred for 8 weeks.
- Donors having history of influenza and URTI (Upper Respiratory Tract Infections) should be deferred till 1 week after treatment. Donors having history of diarrhoea in preceding week particularly if associated with fever should be deferred.
- For emerging potential transfusion transmissible infections, the guidelines published from central health authorities should be followed.
- Private interview of each donor is essential to assess the risk of HIV infection due to high risk sexual behavior and unsafe sexual practice.

Blood Collection

Venipuncture should only be undertaken by authorized and trained personnel. Items used for venipuncture must be sterile, single-use and disposable. If the dry outer wrapping of sterile packs becomes wet the contents must not be used. Prior to use, session staff must ensure that the materials used for venipuncture are sterile, in date and suitable for the procedure to be undertaken. The sterile donor needle should not be uncovered and its tamper-proof cover should be checked for integrity immediately prior to the venipuncture. As soon as the venipuncture has been performed, the clamp on the bleed line must be released. It is important that a clean, skilful venipuncture is carried out to ensure the collection of a full, clot-free unit of blood suitable for the preparation of labile blood components. The tubing attached to the needle should be taped to hold the needle in place during the donation.

Site Selection: A suitable vein, typically in the antecubital fossa, is selected for venipuncture.

Tourniquet Application: A tourniquet is applied to make the vein more prominent.

Skin Disinfection: The venipuncture site is thoroughly disinfected to prevent infection.

Needle Insertion: A needle is carefully inserted into the vein, and blood is collected into a sterile bag containing an anticoagulant and preservative.

Post-Collection Procedures:

Labeling and Storage:

The collected blood bag is labeled with the donor's information and stored in a controlled environment to maintain its quality.

Testing:

The blood is tested for various infectious diseases and for compatibility with potential recipients.

Blood Component Separation:

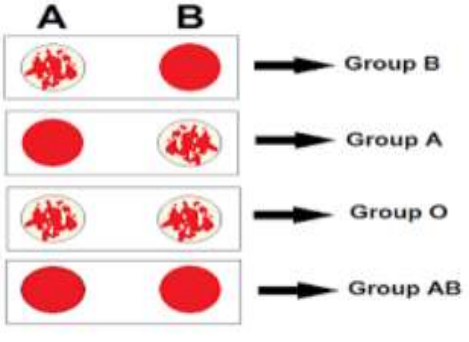
Whole blood is often separated into its components (e.g., red blood cells, plasma, platelets).

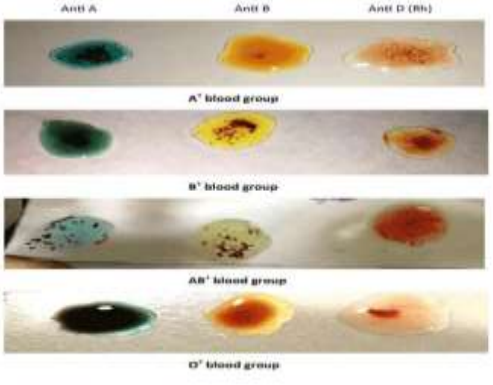
Forward grouping and Reverse Grouping

PRINCIPLE

Testing with both Anti-A and Anti-B is necessary to determine if red blood cells possess or lack A and/or B blood group antigens. Absence of agglutination is a negative test result, which indicates the corresponding antigen is not demonstrable. Agglutination of red blood cells with a given reagent is a positive test result, which indicates the presence of the corresponding antigen on the red blood cells. (Forward Type) Direct agglutination of A or B reagent red cells with the patient serum/plasma indicates the Presence of the appropriate ABO antibody. (Reverse type).

1- Directions: **Front Grouping (forward**): Steps
   1. prepare a 3-5% suspension of red blood cells to be tested in isotonic saline. 2. Place 1 drop of Anti-A and Anti-B respectively, in two small, properly labeled test tubes 3. Add one drop of RBC suspension into the tube and mix. 4. Centrifuge the test tube 5. completely resuspend cells and examine macroscopically for agglutination. 6. Grade and record results
2- **Reverse method**: Reagent: A cell, B cell. / Sample: plasma Steps 1. Label a test tube for each RBC reagent to be tested. 2. Place two (2) drops of the plasma into each labeled tube. 3. Add one (1) drop each of A cells and B cells to the appropriate tube. 4. Mix gently. 5 Completely resuspend cells and examine macroscopically for agglutination. 6. record result





ACCUCARE™

DIRECT AND INDIRECT COOMB'S TESTS
Blood Grouping

Qualitative test for determination of human anti-IgG and anti-C3 on red blood cells. For In Vitro Diagnostic Test use only.

ORDER INFORMATION

REF	Pack Size
AHG 10	1 X 10mL
AHG 100	10 X 10 mL
AHG 1000	1 X 1000mL

CLINICAL SIGNIFICANCE

In 1945, Coombs, Mourant and Race described the use of anti-human globulin serum for detecting red cell-bound non-agglutinating antibodies. In 1957, Dacie et al showed that the antibodies present in antiglobulin sera were directed against certain components of complement.

Antihuman globulin reagents detect non-agglutinating antibody molecules as well as molecules of complement attached to red cells following *in vivo* or *in vitro* antigen-antibody reactions. Accordingly, Anti-Human Globulin is used for compatibility testing, antibody detection, antibody identification, testing for the variant of the Rho (D) antigen (D^U tests), and umbilical cord red blood cell testing.

METHOD

Hemagglutination technique.

PRINCIPLE

The procedures used with this reagent are based on the principle of heteroagglutinins directed against components of human serum as originally described by Moreschin and agglutination as described by Landsteiner. Normal human red blood cells, in the presence of antibody directed toward an antigen they possess, may become sensitize but fail to agglutinate due to the particular nature of the antigen and antibody involved. Anti-Human Serum will react with immunoglobulins and/or complement attached to the red cell surface, resulting in agglutination (clumping) of adjacent sensitized cells. Cells not sensitized will not be agglutinated (See **Limitations**).

REAGENT

Anti-Human Globulin Elite Green reagents contain anti-IgG derived from rabbits with nonspecific activity removed by absorption and mouse monoclonal IgM anti-C3d, Clone BRIC-8. The antibodies are diluted in a buffered solution containing bovine albumin. Each reagent is supplied at optimal dilution, for use with all the recommended techniques stated below without need for further dilution or addition.

Reagent	Colour	Dye Used
Coomb's Sera (AHG)	Green	Patent Blue + Tartrazine

REAGENT PREPARATION

The reagent supplied is ready to use. Protect from Bright Light.

REAGENT STORAGE AND STABILITY

This product will be well-preserved within utility limit till the expiry date, if stored at temperature between +2°C and +8°C.

Caution: Do not freeze.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- If the reagent vial is cracked or leaking, discard the contents immediately.
- Do not use the reagents if a precipitate is present.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagents have been filtered through a 0.2µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- Consider Blood specimen as potentially infectious, handle and dispose it as per national applicable guideline.
- For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- Test tubes (8X50mm),
- Pipettes,
- Centrifuge
- (0.9% NaCl) saline.
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

- Blood should be drawn by an aseptic technique with an anticoagulant. The specimen should be tested as soon as possible after collection.
- If delay in testing should occur, the specimen must be stored at 2°C to 8°C. Bacterial contamination may cause false test results.
- Blood drawn into EDTA should be use within 24 hours. If EDTA is unavailable, samples drawn into ACD, CPD or CPDA-1 are preferable to clotted ones. If only clotted samples are available, do not refrigerate them before testing. All blood samples should be washed at least twice with PBS before being tested.

ASSAY PROCEDURE

Direct Antiglobulin Technique (DAT)

1. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
2. Add 2 volumes of Anti-Human Globulin to each dry cell button.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination

Indirect Antiglobulin Technique (NISS IAT)

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labeled test tube: 2 volumes of test serum and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37oC for 15 minutes.
4. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant saline after last wash.
5. Add 2 volumes of Anti-Human Globulin to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000rcf or for a suitable alternative time and force.
7. Gently resuspend red cell button and read macroscopically for agglutination

LISS Indirect Antiglobulin Technique (LISS IAT)

1. Prepare a 1.5-2% suspension of washed test red cells in LISS.
2. Place in a labeled test tube: 2 volumes of test serum and 2 volumes of test red cell suspension.

Labcare

ACCUCARE™
DIRECT AND INDIRECT COOMB'S TESTS
Blood Grouping

3. Mix thoroughly and incubate at 37oC for 15 minutes.
   Follow steps 4 to 7 of **NISS IAT** above.

NOTES

1. It is recommended a positive control (weak Anti-D 0.1 IU/ml) and a negative control (an inert serum) be test in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. The antiglobulin techniques can only be considered valid if all negative tests react positively with IgG sensitized red cells.
3. In the techniques, here mentioned, one volume is approximately 40µl when using the vial dropper provided.
4. Use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use. User must determine the suitability of the reagents for use in other techniques.

Stability of the reactions

1. Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent.
2. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

INTERPRETATION

Positive: Agglutination of test red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates the presence of IgG and/or complement (C3) on the test red cells.

Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of IgG and/or complement (C3) on the test red cells.

PERFORMANCE CHARACTERISTICS

1. The reagents have been characterized by all the procedures here described.
2. Prior to release, each lot of Accucare's Anti-Human Globulin is tested, by the techniques here mentioned, against red cells coated with Anti-D, Anti-K and Anti-Fya to check suitable reactivity.
3. Potency of anti-IgG and anti-C3d have been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-AHG reference standard 96/666
4. Anti-C3d potency is demonstrated in tests employing cells coated with C3.
5. The presence of contaminating heterospecific agglutinins or antibodies to C4d has been excluded in tests employing red cells of all ABO groups and cells coated with C4d.
6. The reactivity of any Anti-IgM, Anti-IgA or Anti-light chain components that might be present has not been established.
7. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.
8. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

DISCLAIMER

- Each facility should verify the optimum spin time for the specific centrifuge in use.
- Manual techniques are to be performed according to the manufacturer's instructions.
- Each deviation from these instructions is the sole responsibility of the user.
- Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

LIMITATION OF THE PROCEDURE

- Red cells that have a positive DAT due to a coating of IgG cannot be typed by the Indirect Antiglobulin Techniques.
- A positive DAT due to complement sensitization may not reflect *in vivo* complement fixation if test cells are from a refrigerated clotted specimen.
- Inadequate washing of red cells in the indirect antiglobulin techniques may neutralize the anti-human globulin reagent.
- Following completion of the wash phase excess residual saline may dilute the anti-human globulin, reducing its potency.
- A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO Hemolytic Disease of the Newborn or Auto Immune Hemolytic Anemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.
- False positive or false negative results may also occur due to:
  - Improper storage, cell concentration, incubation time or temperature
  - Improper or excessive centrifugation
- The user is responsible for the performance of the reagents by any method other than those here mentioned.
- Any deviations from the techniques here recommended should be validated prior to use Contamination of test materials.

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GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155. Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
Email: accucarediagnostics.com; Website: www.labcarediagnostics.com

Major and minor Cross match

Principle: • Cross-matching will detect incompatibilities between the donor and recipient that will not be evident on blood typing. There are two types of cross-matches: Major cross-match and Minor crossmatch.

• **The major crossmatch**: involves testing the patient's serum with donor cells to determine whether the patient has an antibody which may cause a hemolytic transfusion reaction or decreased cell survival of donor cells. This is the most important cross-match.

• **The minor crossmatch**: involves testing the patients cells with donor plasma to determine whether there is an antibody in the donor's plasma directed against an antigen on the patient's cells.

Method

Major Cross Match

1. Put 2 drops of recipient serum in a labeled test tube. 2. Add 1 drop of 2-5%donor red cells suspended in saline to the tube. 3. Mix and incubate for 5-10min at room temperature. 4. Centrifuge at1000rpm for 1min. 5. Observe for hemolysis or agglutination. 6. Confirm all negative results under the microscope. 7. Alternatively, the tube may be incubated for 30-60min at room temperature and the result read. Centrifugation is optional. Interpretation Agglutination or hemolysis indicates incompatibility of donor and recipient blood.

Minor Cross Match

(See IAT)

Rho (D) typing

Rho (D) typing is a blood test that determines whether an individual's red blood cells have the D antigen, also known as the Rh factor. This test classifies individuals as Rh positive or Rh negative. Rho (D) typing is crucial for blood transfusions, prenatal care, and in some cases, certain medical procedures.

Here's a more detailed explanation:

Rh Factor:

The Rh factor is a protein found on the surface of red blood cells. If the D antigen (Rh factor) is present, the individual is Rh positive (Rh+), and if it's absent, they are Rh negative (Rh-).

Importance:

Rh typing is vital for:

Blood Transfusions:

Rh positive individuals can receive Rh+ and Rh- blood, while Rh negative individuals can only receive Rh- blood.

Pregnancy: Rh negative mothers who carry Rh positive fetuses may develop antibodies that could harm future Rh+ pregnancies. RhoGAM, an anti-D immunoglobulin, can prevent this.

Other Medical Procedures: In some cases, Rh typing may be necessary for certain medical procedures.

Testing:

Rh (D) typing typically involves adding anti-D serum to the individual's blood sample. If the red blood cells agglutinate (clump), it indicates the presence of the D antigen, meaning the individual is Rh positive.

Apheresis

Apheresis, also known as therapeutic plasma exchange (TPE), is a medical procedure that separates blood components, allowing for the removal of specific elements and the return of others. It can be used to treat various diseases by removing harmful substances from the blood and replenishing it with necessary components.

What is Apheresis?

Apheresis involves taking whole blood from a donor or patient, separating it into its components (like red blood cells, white blood cells, platelets, and plasma), and then selectively removing or returning specific components.

- **How does it work?**

A centrifuge machine draws blood, spins it to separate the elements, and then returns the remaining blood components to the body. This process can also involve using filters or other methods to separate blood components.

Apheresis procedures typically involve inserting a needle into a vein and connecting it to a machine. The blood is drawn, processed, and then returned to the body. A temporary apheresis catheter may be used for repeated treatments.

Apheresis is used for various medical purposes, including:

Collecting blood components for transfusion: Apheresis can be used to collect specific blood components like platelets, red blood cells, or white blood cells for transfusion to patients in need.

Removing harmful substances: Apheresis can be used to remove harmful substances from the blood, such as antibodies, toxins, or proteins that contribute to certain diseases.

Therapeutic Plasma Exchange (TPE): TPE involves removing plasma and its soluble constituents, often used in kidney diseases to remove harmful antibodies.

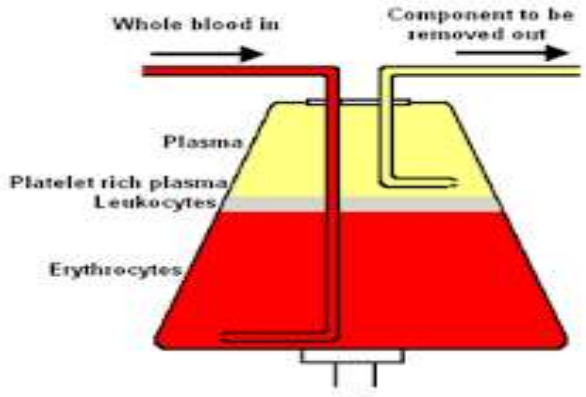
Different types of apheresis:

Leukapheresis: Removes white blood cells.

Plasmapheresis: Removes plasma.

Plateletpheresis: Removes platelets.

Red Blood Cell (RBC) Exchange: Removes red blood cells.



Additives that inhibit blood and/or plasma from clotting ensuring that the constituent to be measured is non-significantly changed prior to the analytical process. Anticoagulation occurs by binding calcium ions (EDTA, citrate) or by inhibiting thrombin activity (heparinates, hirudin).
The following solid or liquid anticoagulants are mixed with blood immediately after sample collection:
EDTA -Salt of ethylene diamine tetraacetic acid. Dipotassium (K2), tripotassium (K3) (41) and disodium (Na2) salts are used (86); concentrations: 1.2 to 2.0 mg/mL blood (4.1 to 6.8 mmol/L blood) based on anhydrous EDTA.
Citrate- Trisodium citrate with 0.100 to 0.136 mol/L citric acid. Buffered citrate with pH 5.5 to 5.6: 84 mmol/L tris odium citrate with 21 mmol/L citric acid. Differences were noticed between 3.2% and 3.8% (v/v) citrate when reporting results in INR (1, 145, 192, 210). WHO and NCCLS recommend 0.109 mol/L (3.2%) citric acid. The International Society for Thrombosis and Hemostasis (ISTH) recommends the use of Hepes buffered citrate for all investigations of haemostastic functions.
a. A mixture of one part citrate with nine parts blood is recommended for coagulation tests .
b. One part citrate mixed with four parts blood is recommended to determine the erythrocyte sedimentation rate.

Heparinates- 12 to 30 IU/mL of unfractionated sodium, lithium or ammonium salt of heparin with a molecular mass of 3 to 30 kD is recommended to obtain standardized heparinized plasma (86). Calcium-titrated heparin at a concentration of 40 to 60 IU/mL blood (dry heparinization) and 8 to 12 IU/mL blood (liquid heparinization) is recommended for the determination of ionized calcium.

Hirudin- Hirudin is an antithrombin extracted from leeches or prepared by a genetic engineering process. Hirudin inhibits thrombin by forming a 1:1 hirudin-thrombin complex. Hirudin is used at a concentration of 10 mg/L.

The colour codes of anticoagulants described in ISO/DIS 6710 are:

EDTA = lavender/red;♣ citrate 9 + 1 = light blue/green;♣ Citrate 4 + 1 = black/mauve;♣ Heparinate = green/orange;♣ No additives (for serum) = red/white.♣

Acid citrate dextrose (ACD) - The ACD is hardly used as a blood preservative anymore in any modern setup, because of its shorter storage time (21 days) and poor viability of cells (70%). It was used with the glass bottles. Citrate phosphate dextrose (CPD) It was discovered by Gibson, et al that addition of phosphate in the ACD anticoagulant increases the post-transfusion survival of cells to 80% compared to ACDs 70% after 21 days of storage.

The composition of **CPD s**olution is given below: •

Trisodium citrate (dihydrate) 26.30 gm • Sodium dihydrogen phosphate 2.28 gm • Dextrose 25.50 gm • Citric acid 3.27 gm • Distilled water 1.0 L The pH of the solution is 5.6-5.8. The citrate in the solution acts as the anticoagulant (0.2%) citrate can prevent clotting of 2.5 litres of blood. Phosphate helps in maintaining the pH of the blood. Dextrose provides nourishment to the cells and helps in the synthesis of ATP. The post-transfusion survival of cells depends on ATPs.

Citrate phosphate dextrose-adenine (CPDA-1) - It was observed by Simon in 1962 that addition of adenine to the CPD solution increases the post-transfusion survival of cells to 80% and the storage period to 35 days. The ATP concentration is higher and the viability is more in adenine blood.

The composition of CPDA -1 is given below: • Trisodium citrate (dihydrate) 26.30 gm • Sodium dihydrogen phosphate 2.22 gm • Dextrose (monohydrate) 31.8 gm • Adenine 0.275 gm • Citric acid 3.27 gm • Distilled water 1 L The ratio of preservative to blood is 14 ml of CPDA-1 solution for 100 ml of blood. The amount of preservative present in 350 ml and 450 ml capacity bags is 49 ml and 63 ml respectively.

The other preservative **CPDA-2** in which the amount of dextrose is increased to 44.6 gm, improves the viability of cells further.

Additive solutions - The commonly used additive solutions are CPD-SAG, CPD-SAGM and CPD-Adsol. CPD-SAGM The SAGM stands for Saline-Adenine-Glucose-Mannitol.

The four-bag system introduced by Hogman, et al in 1978, contains preservative solution in two bags. The bag 1 contains 63 ml of CPD solution while the bag 2 contains 100 ml of SAGM solution.

The composition of the SAGM is as follows: • Sodium chloride 8.77 gm • Adenine 0.16 gm • Glucose 8.99 gm • Mannitol 5.25 gm • Distilled water 1 L 450 ml of blood is collected in the primary bag containing CPD solution, the plasma is expressed after centrifugation to the empty bag and then the 100 ml of the SAGM solution is expressed in the primary bag containing red cells. The red cells can be stored for 42 days in SAGM. The posttransfusion survival of cells in SAGM is more than 80%.

Packed Red Blood Cells preparation

Red Cells are obtained by removal of a major portion of the plasma from Whole Blood. Red Cells also contain the greater part of the Whole Blood leucocytes (about 2.5 to 3.0 × 109 cells) and a variable content of platelets, depending on the method of centrifugation. Preparation Packed Red blood Cells is prepared by removing 200 ml ± 50 ml of supernatant plasma from Whole Blood unit by centrifugation. The entire red blood cell content is then suspended in the remaining 100 ml ± 50 ml of plasma depending on the type of blood bag (350ml / 450ml capacity) in which whole blood was collected.

Quality Control Parameters

Parameter	Specification	Frequency of testing
Appearance	No haemolysis, No turbidity, No Visible clots, no frothing, discoloration	All units
Volume	250 ml ± 10 per cent (for initial 450 ml blood in bag) 150 ml ± 10 per cent (for initial 350 ml blood in bag) (including anticoagulant-preservative solution)	1 per cent of all units or 4 units /month whichever is higher
Hematocrit	65 per cent -70 per cent when stored in in CPDA	1 per cent of all units or 4 units/month whichever is higher
Hemolysis at the end of storage period	< 0.8 per cent of red cell mass	1 per cent of all units or 4 units/month whichever is higher
Sterility (2.2.11)	Complies with the tests for sterility.	1 per cent of all units or 4 units/month whichever is higher

Packed RBCs may be warmed using a designated/approved blood warmer if indicated.

Packed red blood cells resuspended in ABO compatible thawed Fresh frozen plasma (final Hematocrit - 0.5-0.6) are recommended for Exchange transfusion.

General requirements shall be referred regarding labeling, storage and transportation requirements.

HIV (1/2 TRILINE) CARD (WB)

IMMUNOPAK

(Whole Blood/Serum/Plasma)

Last update 10-2020

Ref. RDT-HIC.103W, 50 Test

INTENDED USE

The HIV-1/2 test is a solid phase immunochromatographic assay for the qualitative detection of antibodies against HIV-1 and HIV-2 in human whole blood/serum/plasma.

INTRODUCTION

HIV-1 has been isolated from patients with AIDS and AIDS related complex, and from healthy persons with high potential risk of developing AIDS. Patients with HIV-2 are found primarily in parts of West Africa.HIV-1 and HIV-2 are similar in their morphology, cell tropism, host interaction and genetic structure Serological studies have determined that HIV-1 and HIV-2 have multiple common epitopes in core antigens but much less in the envelope antigens.

PRINCIPLE

Rapid HIV 1 & 2 Triline card employs chromatographic lateral flow test in a cassette format. Colloidal gold conjugated recombinant antigens (Au-Ag) corresponding to HIV-1 (gp120 + gp41) and HIV-2 (gp-36) are dry-immobilized at the end of nitrocellulose membrane strip. HIV 1 & 2 antigens are bound at the Test region (T1 & T2) respectively. Goat Anti-Mouse IgG antibodies are bound at the Control region(C). When the sample is added, it migrates by capillary diffusion rehydrating the gold conjugate. If there are HIV- 1 or HIV-2 antibodies in sample, they will bind with the gold conjugated antigens forming an antibody-antigen-colloidal gold complex. These complexes will continue to migrate along the strip until the Test region (T1 or T2) where they are captured by the HIV 1 or 2 antigens generating a visible pink-purple line. If there are no HIV 1 or HIV 2 antibodies in sample, no pink-purple line is formed in the Test region (T1 or T2).The gold conjugate will continue to migrate alone until it is captured in the Control region (C) by the Goat Anti Mouse IgG antibodies aggregating in a pink-purple line, which indicates the validity of the test.

PRESENTATION

	50 Test
HIV (1/2 Triline) Test cards	50 Cards
Assay buffer	1 Vial

PRECAUTION

1. The HIV-1/2 test card is sensitive to humidity as well as to heat. Perform the test immediately after removing the test device from the foil pouch. Do not use it beyond the expiration.
2. Specimen with extremely high concentrations of red blood cells, fibrin should be re-centrifuged before use.

STORAGE AND STABILITY

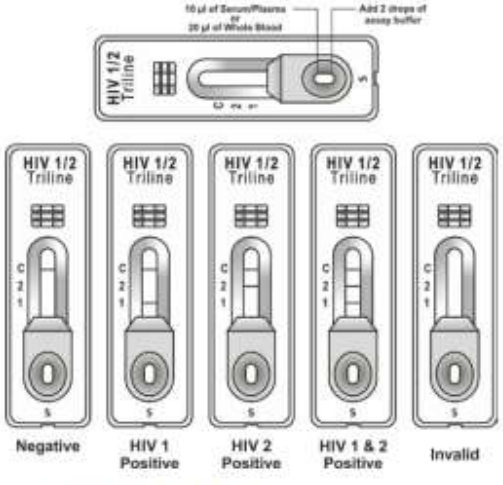
HIV 1/2 test card should be stored at 2°C-40°C. The card may be stored at room temperature but not exceeding 40°C in the original sealed pouch. The shelf life or expiry of the card is printed on Pouch as well as carton label. The test kit should be kept away from direct sunlight, moisture and heat.

SPECIMEN COLLECTION AND STORAGE

1. The test must be performed using human whole blood/ serum/plasma.
2. **Whole blood as specimen**
   Fresh blood from finger prick/puncture may be used as a test specimen for collection of whole blood as a test specimen; EDTA, heparin or oxalate can be used as a suitable anticoagulant. the specimen should be collected in a clean glass or plastic test tube, if immediate testing is not possible, then the specimen may be stored at 2°C-8°C for upto 72 hours before testing. Do not use hemolysed clotted or contaminated blood samples for performing the test.
3. **Serum / Plasma as specimen**
   For Serum, collect blood into a test tube without anticoagulant. Allow the blood to clot and separate the serum from the clot. Use the serum for testing.
4. Specimens containing precipitates may yield inconsistent test results. Such specimens must be clarified prior to assaying.

TEST PROCEDURE

1. Allow the test, specimen and / or control to room temperature prior to testing.
2. Remove the test card from the pouch and place it on a clean, flat surface.
3. With the help of the **Dropper** provided, add one drop of serum/plasma (approx. 10µl) or 2 drops of whole blood (approx. 20µl) into the sample well.
   For Whole Blood: - Wait for few seconds till the Whole blood absorbed by sample pad (approx. 30 second).
4. Add 2 drops (60 µl) of assay buffer (Diluent) into the sample well of the test device. **(If needed add one more drop of assay buffer).**
5. As the test begins to work, you will see pink-purple color moving across the result Window in the center of the Test cassette.
6. **Interpret test results at 10 to 15 minutes. Do not interpret test result beyond 20 minutes.**



INTERPRETATION OF RESULTS

- **Negative:** Only one pink-purple line appears on the control (C) region. No apparent line on the test (T1 and T2) region.
- **HIV 1 Positive:** In addition to a pink-purple control (C) line, a distinct pink-purple line will appear in the test (T1) region

POCT HBsAg (HEPA™ CARD)

IMMUNOPAK

(Whole Blood / Serum / Plasma)

Last update 10-2020

Ref. POCT-HEC.73W, 50 Test

INTENDED USE

CARD test for detection of Hepatitis B (HBsAg) in whole blood, serum or plasma.

INTRODUCTION

Hepatitis B surface antigen ("Australia Antigen") consists of lipid, carbohydrate and protein elements; the protein moiety provides a marker for identification of chronic, infectious HBV infections. Hepatitis B is transmitted sexually or intravenously and has an incubation period of six months. If not diagnosed properly and in time, it can develop into acute or chronic infection, liver cirrhosis and fulminant hepatitis.

This test is very useful for screening blood donors, to find out whether they are HBsAg positive before collection of blood.

PRINCIPLE

HBsAg Card is a qualitative test based on immunochromatography sandwich principle. The test card includes a combination of monoclonal anti-body gold conjugate (colloidal gold) and polyclonal solid phase antibodies which selectively binds Hepatitis B surface antigen with high degree of sensitivity.

The HBsAg test is a one-step immunochromatographic assay based on the antigen capture, or "Sandwich" principle. The method uses monoclonal antibody conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced into well and flows laterally through an absorbent pad where it mixes with the signal reagent. If the sample contains HBsAg, the colloidal gold-antibody (mouse) conjugate binds to the antigen, forming an antigen-antibody-colloidal gold complex. The complexes then migrate through the nitrocellulose strip by capillary action, which are stopped by an immobilized antibody zone forming a pink-purple line. The formation of the first pink-purple line (T zone) is indicative of hepatitis positive. To serve as a procedural control, an additional line of Goat anti-mouse IgG has been immobilized on the card. If the test is performed correctly, this will result in the formation of pink-purple line upon contact with the conjugate as a control line.

PRESENTATION

	50 Tests
HBsAg (WB) (Hepa™ Card)	50 Cards
Assay Buffer	1 Bottle
Sample Dropper (5µl)	50 Droppers
Sterile Lancet	50 Nos.
Alcohol Pad	50 Nos.

PRECAUTION

- HBsAg Card is for in vitro diagnostic use only.
- Handle all specimens as if they contain infectious agents. After the completion of assay procedure, treat the glass wares with 0.5% to 1% solution of sodium hypochlorite for 1 hour before disposal.
- Avoid any contact between hands and eyes or nose during (specimen) collection and testing.

STORAGE AND STABILITY

HBsAg card should be stored at 2°C-40°C. The card may be stored at room temperature but not exceeding 40°C in the original sealed pouch. The shelf life or expiry of the card is printed on Pouch as well as carton label. The test kit should be kept away from direct sunlight, moisture and heat.

SPECIMEN COLLECTION AND STORAGE

• Whole blood as specimen

Fresh blood from finger prick/puncture may be used as a test specimen for collection of whole blood as a test specimen; EDTA, heparin or oxalate can be used as a suitable anticoagulant. the specimen should be collected in a clean glass or plastic test tube, if immediate testing is not possible, then the specimen may be stored at 2°C-8°C for upto 72 hours before testing. Do not use hemolysed clotted or contaminated blood samples for performing the test.

• Serum / Plasma as specimen

For Serum, collect blood into a container without anticoagulant. Allow the blood to clot and separate the serum from the clot. Use the serum for testing.

It the specimen cannot be tested on the day of collection, store the serum specimen in a refrigerator or freezer. Stir and bring the specimen to room temperature before testing. Do not freeze and thaw the specimen repeatedly.

TEST PROCEDURE

1. Bring the specimen and pouch containing the HBsAg Card to room temperature prior to testing.
2. Remove one test card from the pouch and place it on a clean flat surface.

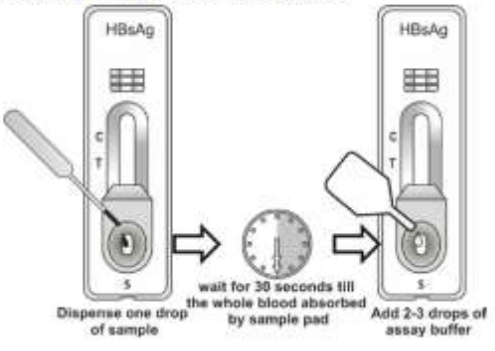
WHOLE BLOOD AS SPECIMEN

3. With the help of the dropper provided add one drop (approx. 25µl) of anticoagulated or finger prick blood to the sample well, **Wait for few seconds till the Whole blood absorbed by sample pad (approx. 30 second),** alternatively 25 µl of whole blood specimen may be delivered in the sample well using a micropipette.(see the figure)
4. Add 2-3 drops (60-90 µl) of assay buffer (Diluent) into the sample well of the test device. **(If needed add one more drop of assay buffer).**

SERUM/PLASMA AS SPECIMEN

5. Using the dropper provided add 2 to 3 drops of serum sample into the sample well. Avoid overflowing.
6. Let the reaction to proceed until the appearance of positive line and control line or upto 20 minutes.
7. Read results within 20 minutes. Strong positive reaction may visible within 5 minutes.
8. If negative or questionable results are obtained, and HBV infection is suspected, the test should be repeated on a fresh specimen.

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but such result should be interpreted only after all clinical and laboratory findings have been evaluated.



POCT HBsAg (HEPA™ CARD)

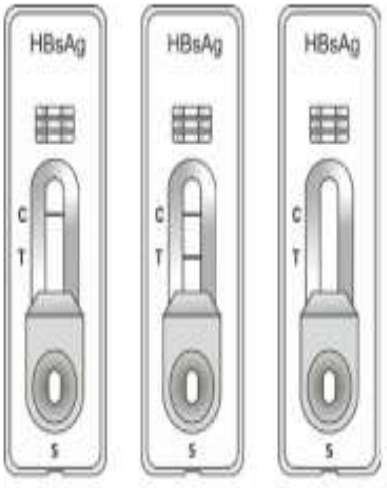
IMMUNOPAK

(Whole Blood / Serum / Plasma)

Last update 10-2020

INTERPRETATION OF RESULTS

- **Negative:** If a distinct pink-purple line is formed only at the control zone marked 'C' (control line) the test result is negative.
- **Positive:** If a distinct pink-purple line is formed at the test zone marked 'T' (test line) and the control zone marked 'C' (control line) the test result is positive, indicating that the sample contains Hepatitis B Antigen. The interpretation of test result (+ve for hepatitis) remains unchanged even if there is a difference in intensity of color in positive line and control line as is many times found.
- **Invalid:** A total absence of pink-purple line in both regions or no pink-purple line appears on the control (C) region is an indication of procedure error and / or the test reagent deterioration. Repeat the test with a new test kit.



Negative Positive Invalid

HCV CARD

Serum/Plasma

IMMUNOPAK

Last update 10-2020

Ref. RDT-HCC.105, 50 Test

INTENDED USE

HCV Card Test is a rapid Chromatographic Immunoassay for the Qualitative detection of antibodies generated against proteins that are encoded by conserved sequence of core,NS3, NS4, NS5 parts of HCV genome in human serum/plasma.

INTRODUCTION

Hepatitis C Virus (HCV) is a small, enveloped, positive-sense, single-stranded RNA virus. Antibody to HCV is found in over 80% of patients with well-documented non-A, non-B hepatitis. Conventional methods failed to isolate the virus in cell culture or visualize it by electron microscope. Cloning the viral genome has made it possible to develop serologic assays that use recombinant antigens. Compared to the first generation HCV EIAs using single recombinant antigen, multiple antigens using recombinant protein and/or synthetic peptides have been added in new serologic tests to avoid nonspecific cross-reactivity and to increase the sensitivity of the HCV antibody tests.

PRINCIPLE

HCV Rapid Test (Serum/Plasma) is a lateral flow chromatographic immunoassay based on the principle of the double antigen-sandwich technique. The membrane is coated with recombinant HCV antigen (core, NS3, NS4, NS5) on the test region of the device.

During testing, the serum or plasma specimen reacts with the HCV antigen (core, NS3, NS4, NS5) gold conjugate. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a pink-purple line at test region. Presence of this pink-purple line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, an additional line of Goat anti-mouse IgG has been immobilized on the card. If the test is performed correctly, this will result in the formation of pink-purple line upon contact with the conjugate as a control line.

PRESENTATION

	50 Tests
HCV Test Cards	50 Cards
Assay Buffer	1 Vail

PRECAUTION

- HCV CARD is for in-vitro diagnostic use only.
- Handle all specimens as if they contain infectious agents. After the completion of assay procedure, treat the glasswares with 0.5% to 1% solution of sodium hypochlorite for 1 hour before disposal.
- Avoid any contact between hands and eyes or nose during (specimen) collection and testing.

STORAGE AND STABILITY

HCV test card should be stored at 2°C-40°C. The card may be stored at room temperature but not exceeding 40°C in the original sealed pouch. The shelf life or expiry of the card is printed on the pouch as well as on the carton label. The test kit should be kept away from direct sunlight, moisture and heat.

SPECIMEN COLLECTION AND STORAGE

HCV CARD TEST is performed on human serum or plasma. It is recommended that the test should be carried out immediately after the collection of blood and separation of serum. Serum specimen can be stored at 2°C-8°C following collection upto 3 days or for longer storage the specimen should be frozen (-20°C).

Specimen containing precipitates, can cause a problem, is well known in chromatography procedures, and hence should be clarified either by centrifugation or by filtration.

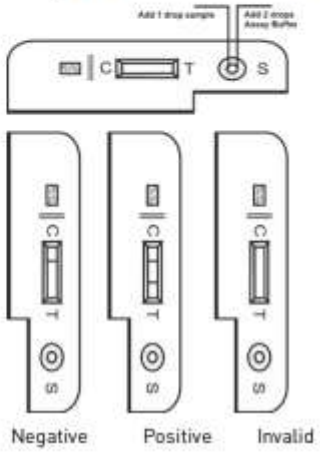
If your card test is showing stagnant flow on chromatography it is most likely due to problem in the sample. Retest with a fresh fasting sample or a diluted sample.

TEST PROCEDURE

1. Bring the test, specimen and /or control to room temperature prior to testing.
2. Remove one test card from the pouch and place it on a clean flat surface.
3. **Using the dropper provided add one drop of serum/plasma sample (Approx. 30µl) then two drops of buffer (Approx. 60µl) immediately into the sample well. Avoid overflowing.**
4. Read results within 15 minutes. Strong positive reaction may visible within 5 minutes. Do not read result after 20 minutes.
5. If negative or questionable results are obtained, and HCV infection is suspected, the test should be repeated on a fresh serum specimen.

INTERPRETATION OF RESULTS

- **Negative:** If a distinct pink-purple line is formed only at the control region marked 'C' (control line) the test result is negative.
- **Positive:** If a distinct pink-purple line is formed at the test region marked 'T' (test line) and the control region marked 'C' (control line) the test result is positive, indicating that the sample contains Hepatitis C Antibody.
  The interpretation of test result (+ve for hepatitis) remains unchanged even if there is a difference in intensity of colour in positive line and control line which is found many times.
- **Invalid:** A total absence of pink-purple line in both regions or no pink-purple line appears on the control (C) region is an indication of procedure error and / or the test reagent deterioration. Repeat the test with a new test cassette.



SYPHILIS CARD (WB)

(Whole Blood/Serum/Plasma)

IMMUNOPAK

Last update 10-2020

Ref. RDT-SYC.108W, 50 Test

INTENDED USE

CARD test for detection of Syphilis in human Whole blood, serum or plasma.

INTRODUCTION

Ultra-sensitive One Step Anti-Syphilis Cassette Test is a rapid and immunochromatographic procedure for the qualitative detection of *Treponemal antibodies* (IgA, IgM, IgG) generated against *Treponema pallidum* antigens (17KDa, 15KDa, 47KDa) in human whole blood /serum or plasma with high sensitivity and specificity. Test results are read visually without any instrument. Purified recombinant syphilis antigens are employed to identify anti-Syphilis antibodies specifically and it also used in detection of congenital syphilis.

PRINCIPLE

Syphilis Rapid Test Device is a qualitative membrane based immunoassay for the detection of TP antibodies (IgA, IgM, IgG) in whole blood / serum or plasma. In this procedure, recombinant syphilis antigen (17KDa, 15KDa, 47KDa) is immobilized in the test line region of the device. After the specimen is added to the specimen well of the device, it reacts with syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized Syphilis antigen. The double antigen test format can detect IgA, IgG and IgM in specimens. If the specimen contains TP antibodies a pink-purple line will appear in the test line region, indicating a positive result. If the specimen does not contain TP antibodies, a pink-purple line will not appear in the test region, indicating a negative result. To serve as a procedural control, an additional line of Goat anti-mouse IgG has been immobilized on the card. If the test is performed correctly, this will result in the formation of pink-purple line upon contact with the conjugate as a control line.

PRESENTATION

	50 Tests
Syphilis Cards	50 Cards
Assay Buffer	1 Vial

PRECAUTION

- For in vitro diagnostic use only.
- Do not use test kit beyond expiry date.
- The test device should not be reused.
- Keep out of the reach of children.
- Do not freeze the Kits.
- Specimen with extremely high concentrations of red blood cells, fibrin should be re-centrifuged before use.

STORAGE AND STABILITY

Syphilis test card should be stored at 2°C-40°C. The card may be stored at room temperature but not exceeding 40°C in the original sealed pouch. The shelf life or expiry of the card is printed on the pouch as well as on the carton label. The test kit should be kept away from direct sunlight, moisture and heat

SPECIMEN COLLECTION & STORAGE

• Whole blood as Specimen

Fresh blood from finger prick/puncture may be used as a test specimen for collection of whole blood as a test specimen; EDTA, heparin or oxalate can be used as a suitable anticoagulant.The specimen should be collected in a clean glass or plastic container, if immediate testing is not possible, then the specimen may be stored at 2°C-8°C for upto 72 hours before testing. Do not use hemolysed clotted or contaminated blood samples for performing the test.

• Serum / plasma as Specimen

For Serum, collect blood into a container without anticoagulant. Allow the blood to clot and separate the serum from the clot. Use the serum for testing.

If the specimen is not tested on the day of collection, store the serum specimen in a refrigerator or freezer. Stir and bring the specimens to room temperature before testing. Do not freeze and thaw the specimen repeatedly.

TEST PROCEDURE

1. Bring the specimen and pouch containing the SYPHILIS CARD to room temperature prior to testing.
2. Remove one test card from the pouch and place it on a clean flat surface.

WHOLE BLOOD AS SPECIMEN:

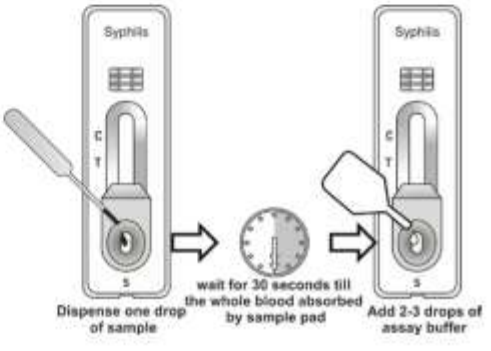
3. With the help of the dropper provided dispense one drop (Approx. 25µl) of anticoagulated or finger prick blood to the sample well, **Wait for few seconds till the Whole blood absorbed by sample pad (Approx. 30 second)**, alternatively 25 µl of whole blood specimen may be delivered in the sample well using a micropipette.(see the figure)
4. Add 2 to 3 drops (60-90 µl) of assay buffer (Diluent) into the sample well of the test device. **(If needed add one more drop of assay buffer).**

SERUM/PLASMA AS SPECIMEN

5. Using the dropper provided, put 2 to 3 drops of serum sample into the sample well. Avoid overflowing.
6. Let the reaction to proceed until the appearance of positive line and control line or upto 20 minutes.
7. Read results within 20 minutes. Strong positive reaction may visible within 5 minutes.

IMPORTANT NOTE

Do not read results after 30 minutes since serum back flow may cause false results.

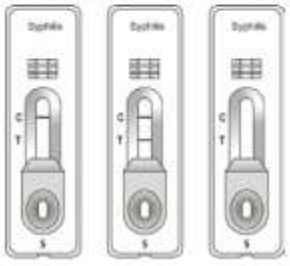


SYPHILIS CARD (WB) **IMMUNOPAK**

(Whole Blood/Serum/Plasma) Last update 10-2020

INTERPRETATION OF RESULTS

1. **Negative:** Only one pink-purple line appears on the control (C) region. No apparent line on the test (T) region.
2. **Positive:** In addition to a pink-purple control (C) line, a distinct pink-purple line will also appear in the test (T) region.
3. **Invalid:** A total absence of pink-purple line in both regions or no pink-purple line appears on the control (C) region is an indication of procedure error and / or the test reagent deterioration. Repeat the test with a new kit.



Preparation of Pooled Cell Suspension

Principle: The ratio of serum to red cells may dramatically affect the sensitivity of agglutination tests. Consistent preparation of either 2 to 5% red cell suspension is critical to any agglutination test.

Preparation of Pooled Cell Suspension:

1. Label tubes with A, B, & O groups.
2. Place 1 drop of red cells each from 3 of A group sample tubes or segment into the A labelled tube.
3. Place 1 drop of red cells each from 3 of B group sample tubes or segment into the B labelled tube
4. Place 1 drop of red cells each from 3 of O group sample tubes or segment into the O labelled tube
5. Fill the tube ¾ full with 0.9% saline to resuspend the cells.
6. Centrifuge the tubes for at least 2 to 3 minutes on high speed. Decant the supernatant fluid.
7. Remove any debris or fibrin with the pipette. Add enough saline to produce a cherry red colour comparable to that of the reagent red cell suspension.

8. If the colour is too dark, add additional isotonic saline to the tube until the suspension colour is right.
9. If the colour is too light, repeat steps 6 and 7.
10. Test the pooled cells prepared using the antisera (anti-A, B, AB & D) in use.

Proceed to use the same procedure to prepare cell suspension of particular donor or patient sample for grouping and cross matching.

Du Test

Some red cells possess the D antigen but it is expressed so weakly that the cells are not agglutinated directly by anti-D sera. An indirect antiglobulin test is necessary to identify patients with the Weak D (formerly known as $D^{u)}$ phenotype. Weak D testing is done on all prenatal patients and candidates for Rh immune globulin. Weak D testing is also done on Rh negative donors to ensure they are truly D negative. It may or may not be done routinely on Rh negative candidates for transfusion, depending on the policy of the transfusing institution. If routine weak D testing is done, weak D positive patients should receive Rh positive blood.

1. PROCEDURE:

1. Prepare a washed, 3% suspension of patient cells.
2. Take one 1 drop of anti D (Ig G) in a cleaned labelled test tube.
3. Add 1 drop of 2-5% labelled test RBCs suspension.
4. Mix & incubate at 37^0 C x 45 min.
5. If still negative, add 1 drop of AHG reagent and centrifuge at 1000 rpm x I min.
6. Record results in the appropriate column on the worksheet
7. Record results. Confirm all negative reactions microscopically.
8. Confirm all negative results by adding one drop Coombs control cells to all tubes showing no agglutination and centrifuge 15-30 seconds at high speed in the serofuge.
9. Gently resuspend and examine for agglutination. Agglutination should be present in this step or the test is invalid.

Interpretation :

No agglutination- D^U negative

Agglutination - D^U positive

A true weak D should give at least a 2+ positive result. Weaker results may be due to mixed field agglutination in an Rh negative individual who received Rh positive blood, or vice-versa. Obtain a recent transfusion history on patients who give inconclusive weak D results.

Atlas Medical

Anti-A1 LECTIN Blood Grouping Reagents

For Tube Techniques

IVD *For in -vitro diagnostic use only*

Store at 2-8 ºC

SUMMARY

A1 antigen is a subgroup of A and was discovered in 1910. Anti-A1 is usually non-reactive at 37ºC, however examples reactive at 37°C and predominately IgM can cause *in vivo* red blood cell destruction. About 78% of group a people are A1 and 22% are A2, similar proportions apply among AB people.

PRINCIPLE

The reagent will cause agglutination (clumping) of test red cells that carry the A1 antigen, after centrifugation. No agglutination generally indicates the absence of the A1 antigen (see **Limitations).**

REAGENT

Atlas Anti-A1 Lectin blood grouping reagent is prepared from an extract of *Dolichos biflorus* seeds, diluted with a sodium chloride solution containing bovine albumin. The reagent is supplied at optimal dilution for use with all recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see **Vial Label.**

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8°C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity.

SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed then store specimens at 2-8°C. EDTA and citrate samples should be typed within 48 hours. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS before being tested.

PRECAUTIONS

- The reagent is intended for *in vitro* diagnostic use only.
- If a reagent vial is cracked or leaking, discard the contents immediately.
- Do not use the reagent past the expiration date (see Vial Label).
- Do not use the reagent if a precipitate is present.
- Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
- The reagent has been filtered through a 0.2 µm capsule to reduce the bio burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
- The reagent contains < 0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
- No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

CONTROLS AND ADVICE

- It is recommended a positive control (ideally group A1B cells) and a negative control (group A2 cells) be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
- In the Recommended Techniques one volume is approximately 50 µl when using the vial dropper provided.
- The use of the reagent and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagent is in use.
- User must determine suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- PBS solution (pH 6.8–7.2) or isotonic saline solution (pH 6.5–7.5).
- Positive (group A1B) and negative (group A2) control red cells.
- Test tube centrifuge.
- Volumetric pipettes.

RECOMMENDED TECHNIQUES

Tube Technique:

1. Prepare a 2-3% suspension of washed test red cells in PBS or Isotonic saline.
2. Place in a labeled test tube: 1 volume Atlas Anti-A1 reagent and 1 volume test red cell suspension.
3. Mix thoroughly and then centrifuge all the tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination

INTERPRETATION OF TEST RESULTS

1. Positive: Agglutination of test red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates the presence of A1 antigen on the test red cell.
2. Negative: No agglutination of test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of A1 antigen on the test red cells.
3. Discrepancies: If the results obtained with reverse group don't correlate with forward group, further investigation is required.

STABILITY OF THE REACTIONS

1. Tube tests must be read immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes leading to false negative, or weak positive reactions.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. Anti-A1 may react with Tn-polyagglutinable or

Paper Title: Histology and Cytology (Practical)

Paper Code: MAJOR (MJ)-3P

Practical contents:

1. Tissue collection and fixation.
2. Dehydration of collected tissue sample in the graded alcohol.
3. Embedding & preparation of blocks, Section cutting, use & care of microtome
4. Stain preparation – Haematoxylin, eosin, PAS, Trichrome, iron haematoxylin, and Giemsa stain
5. Staining techniques using above stains.
6. Preparation of specimen for cytological evaluation by Papanicolaou (PAP) stain, crystal violet staining.
7. Characterization of benign and malignant cells.

Introduction to histopathological technique:

Histopathology is the branch of pathology which concerns the demonstration of minute structural alterations in tissues as a result of the disease. Most histopathological techniques simulating to those applied to study normal histological structures. For the demonstration of minute histological changes, the tissue must be processed in such a manner that it will provide
maximum information. Most convenient way to study morbid tissue is to use a permanent section. A section is prepared by cutting a thin slice from a small piece of fixed tissue. Thin slice is mounted upon a glass slide in a medium of suitable refractive index and covered with
a glass slide.

Histopathology: It is a branch of pathology that deals with the study of disease in a tissue section. The tissue undergoes a series of steps before it reaches the examiner's desk to be thoroughly examined microscopically to arrive at a particular diagnosis. To achieve this it is important that the tissue must be prepared in such a manner that it is sufficiently thick or thin
to be examined microscopically and all the structures in a tissue may be differentiated.

Histochemistry: The term histochemistry means the study of the chemical nature of tissue components by histological methods.

Cytology: The cell is the single structural unit of all tissues. The study of the cell is called cytology.

Scope of Histopathology:

The histopathological techniques are labor intensive, cumbersome, and time-consuming, particularly when there is automation equipment is not available. However, their use in the diagnosis of diseases is unequivocal. Some of the areas where the histopathological diagnosis is helpful are as follows:

• Useful in establishing the pathogenesis and pathology of any disease caused by bacteria, viruses, chlamydia, rickettsia, mycoplasma, parasite, toxin, poisons, etc.

• Certain diseases in which histopathological examination of tissues is the only alternative to diagnose the disease. e.g. Bovine spongiform encephalopathy. The agent of this disease takes a very long incubation period and is very difficult to isolate and there is no immune response and inflammation in an animal. Therefore, histopathology remains the only alternative for confirmatory diagnosis.

Samples for histopathology:

1. Small piece of tissue (as early as possible) is removed with a sharp knife in a particular orientation.
2. Size of piece=1cm to achieve better penetration of fixative.
3. Wash the specimen with normal saline to achieve maximum penetration of the fixative.
4. At the time of tissue collection, the representative tissue piece should include the part of the lesion and a part of normal tissue. The representative tissue should not be collected from damaged portions.
5. Tissue pieces for the histopathological examination should be collected from all the organs.
6. Tissues should be collected directly in the fixative and not in any other pot or water.
7. The tissue pieces from hollow organs like intestines, oviducts, etc should be cut transversely and placed on hard paper.
8. The tissues from encapsulated organs should be collected along with a capsule or covering.

Type of sample obtained in the laboratory:

The human tissue comes from the surgery and the autopsy room surgery two types of tissue is obtained:

1. As biopsy- A small piece of lesions or tumor is sent for diagnosis before the final removal of the lesion or the tumor (Incisional biopsy).
2. If the whole of the tumor or lesion is sent for examination and diagnosis by the pathologist, it is called an excisional biopsy.
3. Tissues from the autopsy are sent for the study of disease and its course, for the advancement of medicine.

Types of Histological preparation:

1. Whole mounts- These are prepared for entire animals eg. fungi, and parasites. These preparations should be no more than 0.2-0.5 mm in thickness.

2. Sections- The majority of the preparations in histology are sections. The tissue is cut in about 3-5 mm thick pieces processed and 5 microns thick sections are cut on a microtome. These are then stained and permanently mounted. Microtomes are special instruments that have an automatic mechanism for cutting very thin sections. To cut the sections on the microtome; the tissue must be made hard enough to not get crushed. There are 2 methods of hardening the tissues. One is by freezing them and the other is by embedding them in a hard material such as
paraffin wax or gelatin.

3. Smears- Smears are made from blood, bone marrow, or any fluid such as pleural or ascitic fluid. These are immediately fixed in alcohol to present the cellular structures that are then stained. Smears are also made by crushing soft tissue between two slides or an impression smear is made by pressing a clean slide in contact with the moist surface of a tissue. By doing this, the cells are imprinted on the slide and these may be stained for cytological examination.

Responsibility of a laboratory technician:

1. Specimen preservation.
2. Specimen labeling, logging, and identification.
3. Preparation of the specimen to facilitate their gross and microscopy.
4. Record keeping.

EMBEDDING

Embedding is the process in which the tissues or the specimens are enclosed in a mass of the embedding medium using a mould. Since the tissue blocks are very thin in thickness, they need a supporting medium in which the tissue blocks are embedded. This supporting medium is called embedding medium. Various embedding substances are paraffin wax, celloidin,
synthetic resins, gelatine, etc.

Embedding Media:

The choice of embedding media depends upon 1.Type of microscope 2. Type of microtome 3. Type of tissue eg. hard tissue like bone or soft tissue like liver biopsy Paraffin wax with a higher melting point (56

to 62$_o$C) is used for embedding. The molten wax is filtered inside the oven through a course filter paper into another container. This will protect the knife edge.

Method of Embedding:

1. Open the tissue cassette, check requisition form entry to ensure the correct number of issue pieces is present.
2. Select the mould; there should be sufficient room for the tissue with allowance for at least a 2 mm surrounding margin of wax. Leuckhart mould method-This is the traditional embedding method. The "L moulds are adjusted according to the shape and size of the tissue. Glycerine may be applied to the L pieces and also to the metal or glass plate on which the moulds are placed for embedding. Simple glossed wall or floor tiles may also be used in place of glass plate.
3. Fill the mould with paraffin wax.
4. Using warm forceps select the tissue, taking care that it does not cool in the air; at the same time.
5. Place the tissue in the mould according to the side to be sectioned. This side should be facing down against the mould. A small amount of pressure may be used in order to have more even embedding.
6. Chill the mould on the cold plate, orienting the tissue and firming it into the wax with warmed forceps. This ensures that the correct orientation is maintained and the tissue surface to be sectioned is kept flat.
7. Insert the identifying label or place the labelled embedding ring or cassette base onto the mould
8. Add more paraffin into the mould to fill the cassette and mould.
9. Cool the block on the cold plate.
10. Remove the block from the mould.
11. Cross check block, label and requisition form.

Some general considerations are as follows:

Elongate tissues are placed diagonally across the block. Tubular and walled specimens such as vas deferens, cysts and gastrointestinal tissues are embedded so as to provide transverse sections showing all tissue layers. Tissues with an epithelial surface such as skin, are embedded to provide sections in a plane at right angles to the surface (hairy or keratinized epithelia are oriented to face the knife diagonally).

Multiple tissue pieces are aligned across the long axis of the mould, and not placed at random. Incorrect placement of tissues may result in diagnostically important tissue elements being missed or damaged during microtomy. In circumstances where precise orientation is essential, tissue should be marked or agar double embedded. Usually, tissues are embedded with the surface to be cut facing down in the mould.

HEMATOXYLIN-EOSIN STAINING

Properties of Hematoxylin:

1. Hematoxylin has no staining property.
2. Hematin with mordant such as ammonium or potassium alum forms lake which functions as cationic dye and stains anionic tissue components.
3. Hematin in an aqueous solution can be acidic or an alkaline dye depending on pH.
4. Hematin has affinity for several tissues with an appropriate mordant.

Progessive staining - When tissue is left in the stain just long enough to reach the proper end point. The slides have to be examined at different interval to find out when the staining is optimum.

Regressive staining - In this method the tissue is overstained and then destained (differentiate) until the proper endpoint is reached. Harris hematoxylin is a regressive stain; the overstaining is removed by acid - alcohol. The removal of this excess dye is called differentiation. The hematoxylin alum gives a reddish hue to the tissues because of acidic pH. To convert this colour to the final blue, alkaline pH is required. This process is called “blueing”. It is done either by tap water or by ammonium hydroxide.

Preparation of Harris’s hematoxylin

Ingredients:

Hematoxylin 5gm
Absolute alcohol 50ml
Ammonium alum 100gm
Distilled water 1000ml
Mercuric oxide 2.5gm
Glacial acetic acid 40ml

Method - Dissolve the hematoxylin in absolute alcohol and ammonium alum in hot water. Mix the two solutions and heat to boiling. Remove from flame, and add mercuric oxide and cool rapidly. Glacial acetic acid if added gives brisk nuclear staining, but life of the solution is reduced. Hence if acetic acid is to be added, it should be added in working solution.

Preparation of Mayer's hematoxylin

Ingredients:

Hematoxylin 1.0gm

Distilled water 1000ml

Ammonium alum 50gm

Sodium iodate 0.2gm

Citric acid (reduces pH) 1.0gm

Chloral hydrate (preservative) 50gm

Method - Hematoxylin is dissolved in distilled water using gentle heat. Then alum is added and dissolved. Then sodium iodate, citric acid and chloral hydrate are added respectively.

EOSIN

Eosin is used as the counterstain that stains the cytoplasm rose coloured. The intensity of the eosin is individual choice. The most widely used eosin is "eosin Y". The "Y" stands for yellowish. It is available in either water soluble or alcohol soluble form. Most laboratories use the water-soluble form of eosin Y in an alcohol-water solution which is described here.

Eosin Y (water soluble) 1.0gm

Distilled water 80ml

95% alcohol 320ml

Glacial acetic acid 0.4ml

Method of H-E Staining:

1. Deparaffinize sections in xylene, 10-20 minutes. Filter Hematoxylin.
2. Rehydrate sections: 100% alcohol for 1-2 minutes 95% alcohol for 1-2 minutes
3. Rinse in tap water 4. Rinse in distilled water 5. Stain with Hematoxylin for 3-5 minutes
6. Wash in tap water
7. Differentiate section with 1% HCl in 70% alcohol 1-2 dips and check under microscope. If necessary, return slides to HCl for further differentiation.
8. Wash slides in running tape water for 15 minutes
9. Stain slides in Eosin for 1-4 minutes
10. Dehydration and Differentiation: 95% alcohol 5-6 dips 100% alcohol 5-6 dips
11. Clear slides in xylene 2 times
12. Mount slides with mounting media (Permount or DPX).

PERIODIC ACID – SCHIFF (PAS) STAIN

Periodic acid causes oxidation of 1:2 glycol groups in the tissues to di-aldehydes. The dialdehyde reacts with fuchsin – sulfurous acid solution (Schiff's) to form a magenta-colored compound.

Aim: To demonstrate glycogen, epithelial mucin, fungi, amoeba and basement membrane.

Control: Liver and intestine

Reagents:

Periodic acid 1%

Distilled water 100 mL

Schiff's reagent

Basic fuchsin 1 gm

Distilled water 200 ml

1N hydrochloric acid 20 ml

Sodium or Potassium metabitesulfite 1gm

Activated charcoal 2gm

Preparation:

1.Dissolve basic fuchsin in boiling distilled water

2.Shake for 5 minutes and cool to 50°C z Filter and add 1N solution.

3.Cool further and add sodium or potassium metabisulfite.

4.Keep for 18 hours in dark.

5.Add activated charcoal, shake well, filter and store the solution at 40°C.

Procedure:

Bring the sections to water. z Dip the slide in Periodic acid solution for 5-10 minutes.

Wash in tap water and rinse in distilled water. Z

Put Schiff's reagent on the section for 20 minutes.

Wash thoroughly in running water.

Counerstain with Hematoxylin, dehydrate, clear and mount in DPX.

Results:

Glycogen (except non-sulfated acid mucopolysaccharide), basement membrane, fungi, parasites and other positive substances – magenta z Nucleus – blue or violet.

STAIN FOR RETICULIN FIBRES

Aim: To identify reticulin fibers in sections.

Principle: Reticulin fibers are treated with potassium permagnate to produce sensitized sites

for silver deposition. Silver is in a form readily able to precipitate as metallic silver. Formalin, a reducing agent causes deposition of metallic silver at pH 9.0.Excess silver is removed by sodium thiosulphate solution .Treatment with gold chloride produces permanent precipitate.
Control: Normal liver.

Reagents :

Acidified potassium permagnate

0.5% potassium permagnate 95ml

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3% sulfuric acid 5ml

Solution should be made fresh.

Silver nitrate solution

To 5 ml of 10% aqueous silver nitrate, add strong ammonia drop by drop until the precipitate which has formed initially is dissolved. Add 5ml of 3% sodium hydroxide. Again add strong ammonia drop by drop till the precipitate is completely dissolved. Add distilled water to make it 50ml and keep it in a jar.

2% Oxalic acid

Oxalic acid 2gm

Distilled water 100ml

4% aqueous iron alum

Ferric ammonium sulphate

Distilled water 100ml

10% Formalin

Formaldehyde 10ml

Distilled water 90ml

0.2% Gold chloride

Gold chloride 0.2%

Distilled water 100ml

Store in refrigerator

2% Sodium thiosulphate

Sodiun thiosulphate 2gm

Distilled water 100ml

Neutral red (acidified)

Neutral red 1gm

Distilled water 100ml

Glacial acitic acid 1ml

Dissolve the dye in distilled water. Add the acid, mix, filter and store.

Procedure:

1. Deparaffinize and bring the sections to water.
2. Oxidize in acidified potassium permagnate for 3 minutes.
3. Rinse in distilled water.
4. Decolorize with 2% oxalic acid for 1 minute.
5. Rinse in distilled water.
6. Put iron alum for 10 minutes.
7. Rinse in distilled water.
8. Put ammonical silver solution for 10 seconds.
9. Rinse in distilled water.
10. Immediately reduce with formalin for 2 minutes.
11. Wash in running tap water for 2 minutes.
12. Tone in 0.2% gold chloride for 2 minutes.
13. Rinse in distilled water.
14. Fix in 2% thiosulphate for 2 minutes.
15. Wash in water for 2 minutes.
16. Counter-stain with neutral red for 2 minutes.
17. Dehydrate, clear in xylene and mount in DPX.

Results:

Reticulin fibres -black

Nuclei – red

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VERHEOFF STAIN FOR COLLAGEN

Aim: To identify collagen and elastic tissue in the same section.

Principle: In the presence of ferric salts (oxidizers) elastic fibers stain with hematoxylin, along with the nuclei.

Control: skin

Reagents

1. Verhoeff's solution: Freshly prepared solution gives best result.

Solution A

Hematoxylin 5gm

Absolute alcohol 100ml

Dissolve hematoxylin with the aid of heat, cool and filter.

Solution B

Ferric chloride 10gm

Distilled water 100ml

Solution C

Iodine 2gm

Potassium iodide 4gm

Distilled water 100ml

Add 8ml of solution B into 20ml of solution A and then add 8ml of solution C.

2. 2% Ferric chloride solution

3. 1% aqueous solution of acid fuchsin

4. Saturated aqueous solution of picric acid

5. Van Gieson's stain

Acid Fuchsin 1% (aqueous) 5ml

Saturated aqueous solution of picric acid 100ml

6. Sodium thiosulphate, 5% (aqueous solution)

Procedure:

1. Deparaffinize and take the section to water.

2. Stain in Verhoeff solution until the section is black.

3. Wash in distilled water.

4. Differentiate in 2% Ferric chloride with agitation for few minutes. Check differentiation by rinsing in distilled water. Under the microscope the elastic fibers and nuclei should stain black and rest of the tissue should be light grey.

5. Put in 5% sodium thiosulphate for 1 minute.

6. Wash in tap water for 5minutes.

7. Counter-stain with Van Gieson's stain for 1-2 minutes.

8. Differentiate in 95% alcohol.

9. Dehydrate in absolute alcohol two times.

10. Clear in xylene and mount in DPX.

Result: Elastic fibres- black ; Nuclei – black; Collagen- red; Other tissues- yellow

LEISHMAN-GIEMSA STRAINING

Aims: It is a Romanisky group of stains mainly used for home topological purposes. It differentiates acidophilic and basophilic cells and recognized different types of abnormal calls

Principle: It consists of both acidic and basic dyes. Basic dyes are positively changed which binds with the anionic site and impart blue-grey color of basophilic cells and nuclei. The acidic

dye is negatively charged and binds with the catatonic site and imparts orange color to the hemoglobin and eosinophil granules.

Methods:

Take one air-dried smear (FNAC) and stained it with Leishman stain for 2 mins.

Add the double amount of butter below and mix well form

Then add the dilution of Giemsa stain.

Then stain the smear with Giemsa stain and wait for ford 10 minutes.

Wash in running tap water.

Air-dried and examined under oil immersion

Results:

Nucleus →Bluish red

Acidophilic granules → pink to red

Basophilic granules → Blue

RBC → salmon pink.

MAY-GRÜNWALD GIEMSA STAIN

This is one of the common Romanwsky stains used in cytology. It is useful for studying cell morphology in air-dried smears. It is superior to Papanicolaou to study the cytoplasm, granules, vacuoles, basement membrane material etc. For nuclear staining Papanicolaou is superior.

Staining reagents:

May-Grünwald solution 0.2%

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Methanol 99 %

May-Grünwald´s eosin-methylene blue 0.2 %

Contains: Eosin G, Methylene blue

Giemsa solution

Methanol 73 %

Glycerol 26 %

Giemsa´s Azur-Eosin-Methylene blue 0.6 %

Contains: Azur I, Eosin G, Methylene blue Phosphate buffer Potassium dihydrogen phosphate/ disodium hydrogen phosphate x 2H2O 67.0 mmol/l

Storage Giemsa solution,

May-Grünwald solution: protected from light at 2-25°C. Unopened reagents may be used until the expiry date on the label.

Phosphate buffer: at 2-8°C. Unopened reagents may be used until the expiry date on the label.

Preparation of working solutions

1. Buffered water: Dilute phosphate buffer with deionised or distilled water 1:20, e.g. 30 ml phosphate buffer + 570 ml deionised or distilled water.

2. Giemsa working solution : Mix 84 ml of Giemsa solution into 516 ml of buffered water.

3. May-Grünwald working solution: Mix 360 ml of May-Grünwald solution into 240 ml of buffered water.

Staining method

1. Fix the air-dried smear specimen in methanol for 10 -20 minutes
2. Stain with May-Grünwald working solution for 5 minutes
3. Stain with Giemsa working solution for 12 minutes
4. Wash with clean buffered water for 2, 5 and 2 minutes
5. Dry the slides in upright position at room temperature
6. Mount the slides with a coverslip using DPX Any modifications to the staining procedure/working solutions may affect the staining result, and are subject to precise method validation.

Results: May Grunwald Giemsa stained smear detect the pleomorphic adenoma. It demonstrates the extracellular substance of adenoma.

Papanicolaou stain

Introduction:

Dr. George Papanicolaou, the father of cytopathology, first introduced this stain. Papanicolaou's stain (PAP stain) is the most important stain in cytology and is used in all cervical smear and non-gynaecologic exfoliative smears.

This stain has the following excellent properties:

• Cytoplasmic differentiation: It helps in the assessment of cellular differentiation.

• Nuclear details seen.

• Transparent stain.

• Demonstrates intracytoplasmic keratin.

Papanicolaou stain

1. Harris' hematoxylin Hematoxylin 5g Ethanol 50ml Potassium alum 100g Distilled water (50°C) 1000ml Mercuric oxide 2-5g Glacial acetic acid 40ml

2. Orange G 6 Orange G (10% aqueous) 50ml Alcohol 950ml Phosphotungstic acid 0-15g

3. EA 50 0.04 M light green SF 10ml 0.3M eosin Y 20ml Phosphotungstic acid 2g Alcohol 750ml Methanol 250ml Glacial acetic acid 20ml Filter all stains before use.

Papanicolaou staining method:

1. 96% ethyl alcohol 15 seconds
2. 70% ethyl alcohol 15 seconds
3. 50% ethyl alcohol 15 seconds
4. Distilled water 15 seconds
5. Harris hematoxylin 6 minutes
6. Distilled water 10 dips
7. Hydrochloric acid 0.5% solution, 1-2 quick dips
8. Distilled water 15 seconds
9. Few dips in 0.1% ammoniated water. The smear turns to blue.
10. 50% ethyl alcohol 15 seconds
11. 70% ethyl alcohol 15 seconds
12. 96% ethyl alcohol 15 seconds
13. OG-6 (orange) 2 minutes
14. 96% ethyl alcohol 10 dips
15. 96% ethyl alcohol 10 dips
16. EA 50 eosin yellowish 3 minutes
17. 96% ethyl alcohol (10 dips)
18. 100% ethyl alcohol (10 dips) 19. Xylene (10 dips)
20. Mount: in DPX using coverslip

Results: The nuclei should appear blue/black The cytoplasm (non-keratinising squamous cells) – blue/green Keratinising cells- pink/orange.

Course No: MJ-4P

Clinical Biochemistry (Lab)

Practical contents:

1. Preparations of plasma, serum, and protein free filtrate from blood.

2. Determination of Blood glucose, glucose tolerance test, HbA1c

3. KFT: urea, uric acid and creatinine in blood, determination of Na +, K , Ca++

4. LFT: total bilirubin, direct-indirect, ACP, ALT, AST, gamma-GT, ALP, total protein in serum, albumin-globulin ratio

5. Lipid profile: serum TG, blood cholesterol, HDL,LDL, VLDL, Troponin T, TroponinI

6. Amylase, lipase

1.Preparations of plasma, serum, and protein free filtrate from blood for biochemical analysis

Serum

Serum is the specimen used for most clinical biochemistry analyses. Serum is the fluid portion that remains after blood has been allowed to clot. Hence, blood is collected in a dry plain tube without any anticoagulant, allowing the blood to clot, centrifuging the clotted specimen, and removing the liquid (serum). Some blood collection tubes are especially made by the manufacturer to speed up the clotting process. Many modern laboratories are now using serum separator tubes that contain a special gel that forms a barrier between the serum and cells during centrifugation. Blood can also be collected in serum separator tubes, which contains special gel. In these tubes, during centrifugation, the red blood cells displace the gel in the bottom of the tube.

Plasma, on the other hand, is obtained by removing the liquid portion of anticoagulated blood (whole blood) following centrifugation. It the laboratory is using a modern Vacutainer tube, the colour or the cap of the collection tube denotes the anticoagulant present in the tube as inner coating.

Plasma separating tubes are available; these contain a gel that becomes displaced by red blood cells and plasma.

Preparation of plasma for biochemical analysis:

1.**Blood Collection**

- Collect blood in a sterile tube containing an anticoagulant (e.g., EDTA, citrate, or heparin).

- Ensure proper venipuncture technique to prevent hemolysis.

2. **Centrifugation**

- Centrifuge the blood sample at 3,000 x g for 5 minutes.

- Separate the plasma from blood cells and platelets.

3. **Plasma Transfer**

- Carefully transfer the plasma to a clean, sterile tube.

- Avoid contaminating the plasma with blood cells or platelets.

4. **Storage**

- Store the plasma at the appropriate temperature:

- Room temperature (15-25°C) for short-term storage (<4 hours).

- Refrigerated (2-8°C) for short- to medium-term storage (<24 hours).

- Frozen (-20°C or -80°C) for long-term storage (>24 hours).

5. **Sample Preparation**

- Allow frozen plasma to thaw at room temperature or in a water bath (37°C).

- Mix the plasma gently to ensure uniform distribution of analyte

6.**Sample Treatment (if necessary)**

- Add stabilizers or preservatives to prevent degradation or contamination.

- Perform protein precipitation or other sample treatments as required for specific tests.

7. **Quality Control**

- Verify sample integrity and purity.

- Check for hemolysis, lipemia, or icterus, which can interfere with test results.

8: **Analysis**

- Proceed with biochemical analysis using various techniques (e.g.,spectrophotometry, chromatography, or immunoassays).

Preparation of serum specimen for biochemical analysis:

Serum must be separated promptly from the clotted blood. This is easy when the blood is collected in a centrifuge tube.

1. Allow the blood to clot for 15 min at room temperature (do not refrigerate). If the clotting period is prolonged (1 h) it produces a greater quantity of serum and minimizes haemolysis.

2. Remove the clot that is adhering to the wall of the centrifuge tube by 'ringing'. Ringing is a common laboratory term that means a gentle sweep around the inside walls of the tube with an applicator stick or glass rod in order to dislodge the clot from the wall. Excessive ringing is not necessary.

3. Centrifuge at 3000 rpm tor 5 min.

4. Separate the supernatant (serum) within 2 h from the time of specimen collection. If the serum is not separated promptly, intracellular fluid is excreted, which produces erroneous findings for such analyses as potassium. Serum glucose level is also found to fall more rapidly due to glycolysis, if the cells are in contact with the serum. While separating the serum avoid contamination with red cells.

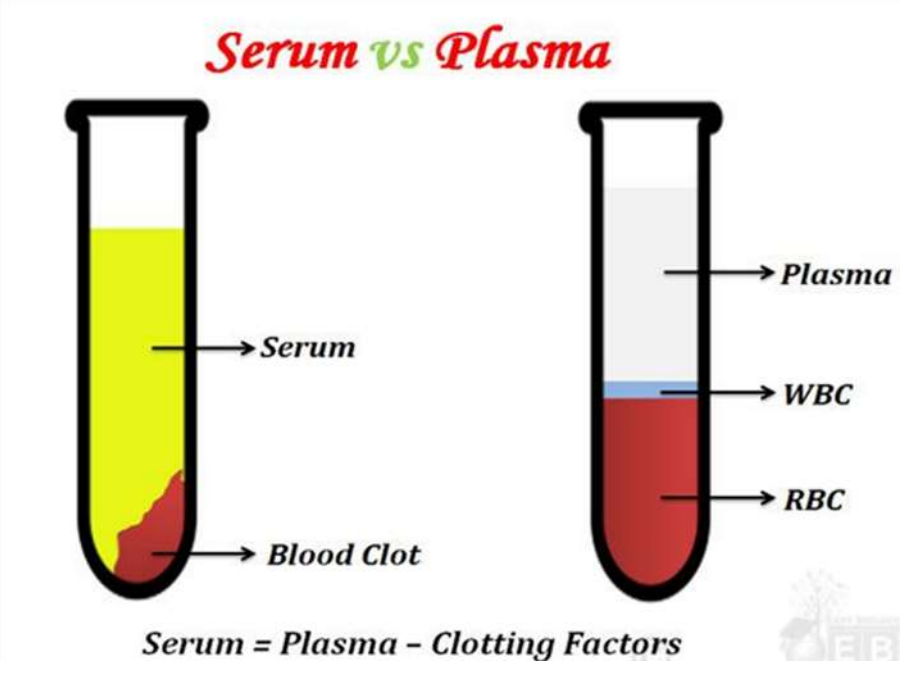
5. Keep the serum in labelled tubes at room temperature. If delay is anticipated, refrigerate the serum specimen. Protect the specimen from strong light (Sun) and heat which will lead to faster deterioration of the serum constituents sought in the analysis. Refrigerated specimens can be analysed within 24 h.

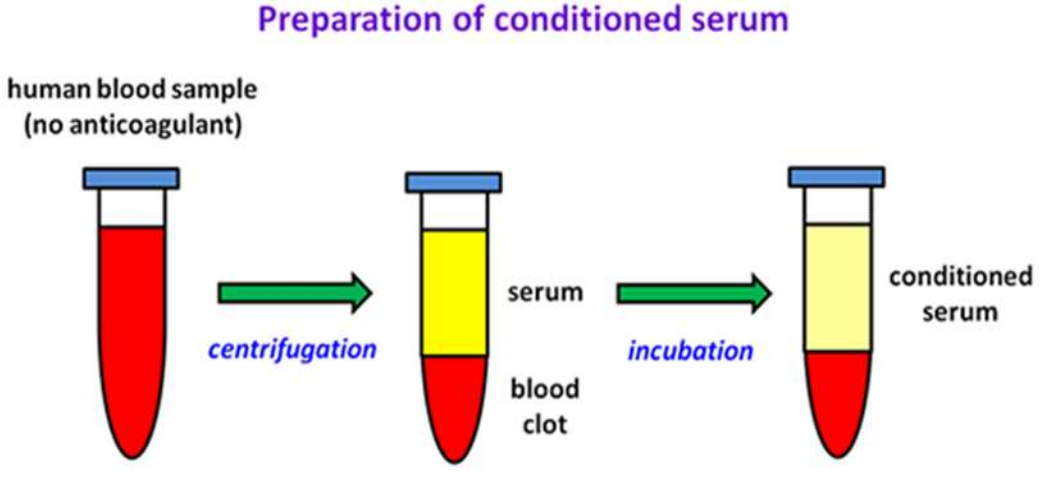
Precautions:

- Before taking the aliquot for analysis, the serum specimen must be brought to room temperature; otherwise, the volume of the aliquot will be inaccurate.
- Specimens for bilirubin analysis must be promptly processed and protected from direct light. Conjugated bilirubin is very sensitive towards light and leads to false low values if the specimens are not kept in the dark.
- Haemolysed serum is unsuitable for many biochemical analyses. As a result of haemolysis, constituents of red cells are released into the serum which leads to false elevated Values of serum lor those constituents which are found in higher concentration inside the red cells, e.g., potassium,

protein and cellular enzymes of red cells- acid phosphatases, Lactate dehydrogenase and transaminases. False low values may also result due to dilution of the serum and the cellular contents of red cells-cholesterol, sodium, chloride and others.

- Haemoglobin may directly interfere in some of the analysis such as lipase, bilirubin and enzymatic, glucose determinaton.
- Before the results of the biochemical analysis are finally accepted by the physician, possible interference in the biochemical analyses by the drugs that the patient is taking, must be considered.





2.Protein Free Filtrate Preparation by Folin Wu Method

Removing proteins from blood can be done via some techniques. The one method which is widely used is precipitation by large anions.

Why proteins are removed from blood?

Proteins interfere with some blood tests and may alter the results. Therefore, it is necessary to remove proteins or make a PFF before performing these tests.

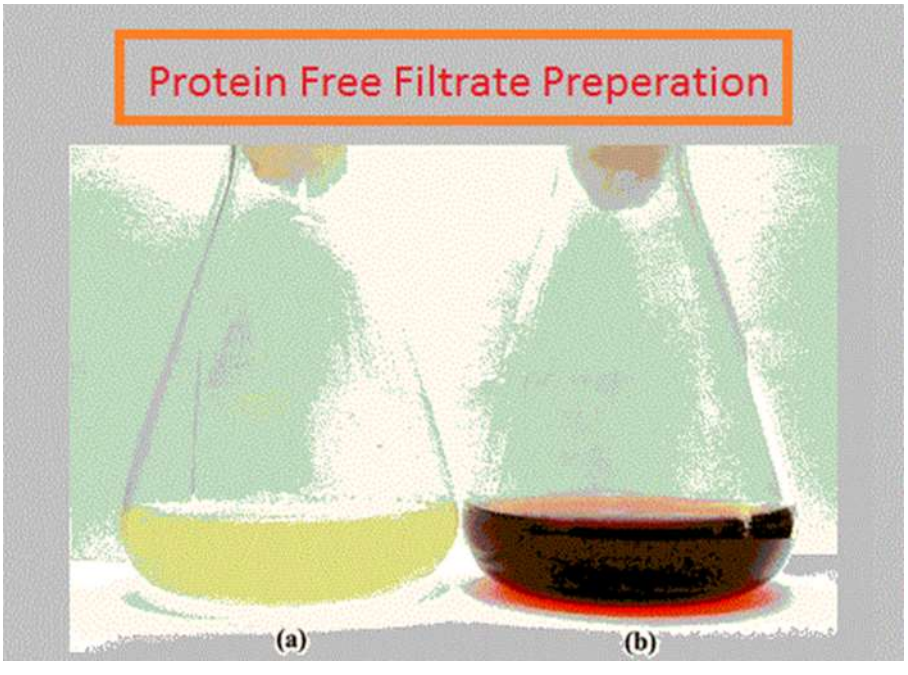
The list of some major tests in which you must prepare a protein less filtrate:

- Estimation of Blood Urea
- Estimation of Blood Creatinine
- Estimation of Blood Uric Acid

Precipitation By Large Anions:

Proteins can also be precipitated using large anions such as tungstic acid, tri chloroacetic acid, phosphor-tungstic acid, tannic acid etc.

This method is the basis of Folin Wu method and other similar methods for PFF preparation.



Protein Free Filtrate Preparation by Folin Wu Method

Folin Wu method is one of the most widely method used for the preparation of protein free filtrate. This method is done before doing laboratory estimation of creatinine, urine and uric acid in blood. This is method is based on precipitation of proteins in blood by large anions.

Reagents of Folin Wu method

There are a few reagents used for this. They are the chemicals that are mentioned in principle of this test and some other supporting chemicals. These are the following:

- Blood
- 10 percent sodium tungstate
- 2/3 Normal H2SO4.

- Potassium Oxalate crystals.

Procedure of Preparation of protein free filtrate

- First of all, take 2ml of blood.
- Then shift this blood to a container containing potassium oxalate.
- Then dilute the blood with distilled water. Add 14ml of distilled water.
- Then add 2ml of 10 percent sodium tungstate.
- Then add 2 ml 2/3 normal H2SO4.
- Color of solution will change from dark red to dark brown.
- Filter this solution using Whatman filter paper.
- After filtration, you will get colorless protein free filtrate.

There are two reasons for adding H2SO4. First is to convert sodium tungstate to tungstic acid which helps in protein precipitation (as sodium tungstate cannot precipitate proteins).

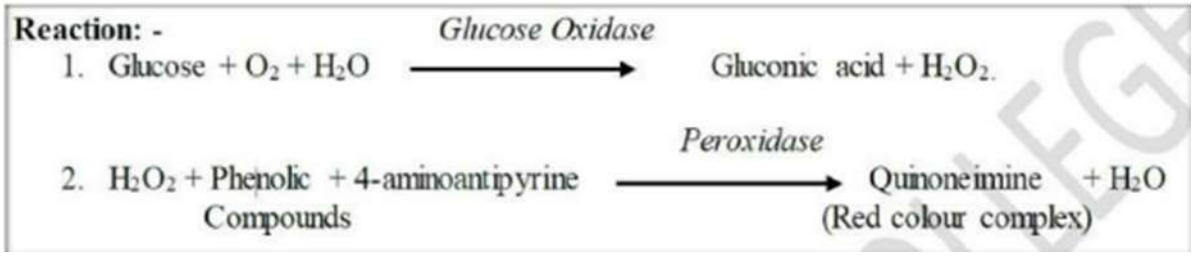
The second reason for adding sulfuric acid is to rupture the RBC's so that the color of solution changes from dark red to dark brown.

2.Estimation of plasma glucose by GOD-POD method

Principle:

Glucose oxidase (GOD) oxidizes the specific substrate β-D- glucose to gluconic acid and hydrogen peroxide (H2O2) is liberated. Peroxidase (POD) enzyme acts on hydrogen peroxide to liberate nascent oxygen (O2), then nascent oxygen couples with 4-amino antipyrine and phenol to form red quinoneimine dye. The intensity of the colour is directly proportional to the concentration of glucose present in plasma. The intensity of colour is measured by colorimeter at 530 nm or green filter and compared with that of a standard treated similarly. Final colour is stable for at least 2 hours if not exposed to direct sunlight.

Reaction:



Reagents:

1. Glucose colour reagent; it contains GOD, POD, 4- amino antipyrine, phenol & phosphate buffer (pH 7.5)

2. Glucose standard solution, Concentration = 100 mg/dl. 100 mg of anhydrous glucose is dissolved in 100 ml of distilled water

Procedure:

Pipette into clean, dry test tube labelled as Blank (B), Standard (S) and Test (T).

Then add the solution in each of test tubes separately as shown in table below:

	BLANK	STANDARD	TEST
Glucose colour Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	-------	--------
Standard	-----	10 µl	--------
Plasma	-----		10 µl

Mix thoroughly and keep the tubes at 37˚C for 15 minutes

OD at 530 nm	0.02	0.45	0.58

Data: Plasma Glucose standard concentration is 100 gm/dl

Calculation:

Concentration of Glucose = (OD of Test/OD of standard) × Concentration of standard

Result:

Plasma glucose concentration in given unknown blood sample = mg/dl

Normal Range

	Fasting	After eating	2-3 hrs after eating
Normal	80 - 100	170 - 200	120 - 140
Pre Diabetic	101 - 125	190 - 230	140 - 160
Diabetic	126+	220 - 300	200+

Interpretation:

• Hyperglycemia

• It is found in following conditions

I. Physiological:

• 1. Alimentary: After high carbohydrate diet

• 2. Emotional: Stress, anger, anxiety etc.

II. Pathological:

• 1. Diabetes mellitus

• 2. Hyperadrenalism

• 3. Hyperpituitarism

• Hypoglycemia:

• It is found in following conditions:

• I. Physiological:

– During starvation

– After Severe Exercise

II. Pathological:

– Prolonged fasting

– Due to excess of insulin e.g.

• Excessive dose of insulin

• No food intake after insulin administration

• Tumours of pancreas (insulinoma)

– Glycogen storage disease

– Hypoactivity of adrenal and pituitary gland

Glucose tolerance test (GTT)

GTT short for Glucose Tolerance Test is a test designed to assess the body response to glucose. In GTT, the patient is given a glucose solution and blood samples are drawn afterword at intervals to measure how well the body cells are able to absorb glucose. There are several variations to the glucose tolerance test used in different conditions but, the most common one of them is the Oral glucose tolerance test or OGTT.

The OGTT is mainly used in the diagnosis of gestational diabetes. For OGTT the patient is required to fast for 8 hours and then a fasting plasma glucose is tested, after that oral glucose solution is given. After that blood samples can be drawn up to 4 times at different intervals to measure the blood glucose. A OGTT is usually performed in the morning as glucose levels usually fall by afternoon.

OGTT used to be the gold standard in the diagnosis of diabetes type 2 but, is now being replaced with other GTT methodology. The GTT is primarily used for the diagnosis of diabetes, insulin resistance, impaired beta cell function, carbohydrate metabolism disorder and also reactive hypoglycaemia and acromegaly.

The GTT is usually given to pregnant women during 24th and 28th week of pregnancy. This test is also given to pregnant who has diabetes symptoms or have the risk of developing diabetes prior to the pregnancy. Besides that, the GTT is also given to other patients who are experiencing symptoms of varied diseases that can cause high glucose levels in the blood stream or restrict the proper absorption of glucose by the body cells.

Principle:

A glucose tolerance test is the administration of glucose in a controlled and defined environment to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycaemia. The glucose is most often given orally.

Preparation for GTT

TT is an elaborate blood test, that requires frequent testing and as the special requirements needfor GTT are as follows;

- Have a normal diet like any other day.
- Inform the doctor about the varied prescription drugs you are taking, as certain drugs likecorticosteroids, diuretics and anti- depressants can cause false results.
- Fasting is required for 8 to 10 hours prior to the test and only water is allowed during thisperiod.
- You might want to avoid using the washroom prior to testing as urine samples might beneeded.
- On the morning of the test do not smoke or have coffee or caffeine-based product.
- The GTT is not to be done on a sick person

GTT procedure

The GTT procedure is as follows:

- At first a zero -time or baseline blood sample is drawn.
- Then the patient is given a specific dose of glucose solution to drink
- After that the blood samples are drawn at regular intervals to measure the blood sugar levels and also insulin levels in certain cases. The blood sampling can be done as requested by the doctor and could involve up to 6 hours of testing.

GTT normal values:

- The GTT normal value is lower than 140 mg/dL and if the blood glucose level is between 140 and 199 mg/dL then it is a strong indication of prediabetes.
- The OGTT normal range for fasting results is between 100 – 125 mg/dL for prediabetes, 126 mg/dL or greater for diabetes and greater than 92 mg/dL for gestational diabetes.
- The OGTT normal range for after 2 hours test results is between 140 – 199 mg/dL for prediabetes, 200 mg/dL or greater for diabetes and greater than 153 mg/dL for gestational diabetes.

GTT result interpretation

For Gestational diabetes no further test is required and proper medication and treatment can start. In case of diabetes, further testing is advised to confirm the diagnosis. In case of pre diabetes doctors start the treatment with medication and dietary changes along with lifestyle changes.

Determination of hemoglobin A1c (HbA1c) in whole blood

BioMajesty

HbA1c net FS*

Diagnostic reagent for quantitative in vitro determination of hemoglobin A1c (HbA1c) in whole blood on BioMajesty JCA-BM6010/C

Order Information

Cat. No. 1 3348 99 10 962
R1: 6 x 300 tests
R2: 6 x 300 tests
Cat. No. 1 3348 99 10 964
R1: 2 x 160 tests
R2: 2 x 160 tests

Method

Hemoglobin: Photometric test
HbA1c: Colorimetric, enzymatic method

Principle

The concentrations of HbA1c and hemoglobin are determined separately and are used to calculate the HbA1c ratio from total hemoglobin exclusively.

Hemoglobin measurement
Whole blood samples are lysed with hemolyzing solution. Hemoglobin is released from the erythrocytes. The absorbance of hemoglobin is measured at 571 nm after addition of reagent R1 and is proportional to the total hemoglobin concentration in the sample.

HbA1c measurement [16]
After addition of R2, fructosylated dipeptides from the N-terminal part of the hemoglobin β-chain are released by a protease. Hydrogen peroxide (H_2O_2) is produced by oxidative cleavage of fructosylated dipeptides by FPOX (fructosyl peptide oxidase). The H_2O_2 generated is determined colorimetrically by reaction with a chromogen in presence of peroxidase at 658 nm. The absorbance increase is proportional to the HbA1c concentration.

Standardization

The assay is standardized according to IFCC [1] and DCCT/NGSP [4] reference methods. Calculation of patient and control values is possible according to IFCC [mmol/mol] as well as according to DCCT/NGSP [%].

NGSP and IFCC values show a linear relationship and, therefore, can be calculated from each other using the following equation:

HbA1c *(IFCC[b])* = (HbA1c *(NGSP[a])* – 2.15) / 0.0915
HbA1c *(NGSP[a])* = 0.0915 x HbA1c *(IFCC[b])* + 2.15

a: NGSP values in %
b: IFCC values in mmol/mol

IFCC: International Federation of Clinical Chemistry [1,2,7]
DCCT: Diabetes Control and Complications Trial [3]
NGSP: National Glycohemoglobin Standardization Program [4]

HbA1c and Average Glucose Concentrations [6]

Due to a linear correlation between hemoglobin A1c and average glucose concentrations HbA1c values can be converted into estimated average glucose values by means of the following equations:

Standardization according to IFCC (calculated referring to literature reference [8]):

Average glucose conc. [mg/dL] = 2.63 x HbA1c[b] + 15.01
Average glucose conc. [mmol/L] = 0.146 x HbA1c[b] + 0.829

b: HbA1c values in mmol/mol IFCC

Standardization according to NGSP:

Average glucose concentration [mg/dL] = 28.7 x HbA1c[a] – 46.7
Average glucose concentration [mmol/L] = 1.59 x HbA1c[a] – 2.59

a: HbA1c-values in % NGSP

No significant differences in the regression equation were observed for variations in individuals tested including sex, presence or absence of diabetes, type of diabetes, age, race, and ethnicity. Although this equation can be used for the majority of individuals each laboratory has to verify whether the regression equations mentioned are applicable for the patient group to be examined.

Reagents

Components and Concentrations

R1:	Buffer	100 mmol/L
	FPOX	≥ 0.5 kU/L
	Ethylene glycol derivative	< 10%
R2:	Buffer	20 mmol/L
	Protease	≥ 500 kU/L
	Chromogen	≥ 0.05 mmol/L
	Ethylene glycol derivative	< 10%

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination and evaporation are avoided. Do not freeze the reagents! Protect reagents from light!

Warnings and Precautions

1. The reagents contain material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
2. Hemoglobin and HbA1c values in g/dL determined with DiaSys HbA1c net FS are used to calculate the HbA1c ratio from total hemoglobin exclusively. Individual results for hemoglobin and HbA1c must not be used for diagnostic purposes.
3. Falsely low values (low HbA1c despite high blood glucose) may occur in people with conditions such as shortened red blood cell survival (e.g. hemolytic diseases) or significant recent blood loss during the weeks before (higher fraction of young erythrocytes). Falsely high values (high HbA1c despite normal blood glucose) have been reported in iron deficiency anemia (high proportion of old erythrocytes). These circumstances have to be considered in clinical interpretation of HbA1c values. Care must also be taken in clinical interpretation of HbA1c results from patients with hemoglobin variants.
4. In very rare cases, samples of patients with gammopathy might give falsified results [15].
5. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
6. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, results should always be assessed with patient's medical history, clinical examinations and other findings.
7. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use. The bottles are placed directly into the reagent trays.
Homogenize HbA1c net Hemolyzing Solution by repeated inversion. Due to composition of the hemolyzing solution an opalescent and slightly turbid appearance remains. Avoid foaming! Do not shake!

Specimen

Whole blood collected with EDTA

Please collect whole blood by standard venipuncture and fill the blood collection tube according to manufacturer specifications!

Specimen Stability [5]:

Whole blood 1 week at 2 – 8°C

Discard contaminated specimens.

Sample Preparation:

For sample preparation the DiaSys HbA1c net Hemolyzing Solution is required.

	Cat. No.	Kit Size
HbA1c net Hemolyzing Solution	1 4590 99 10 967	4 x 450 tests
	1 4590 99 10 961	2 x 160 tests

The bottles of DiaSys HbA1c net Hemolyzing Solution are placed directly into the reagent trays.

Reagent tray position has to be defined as "Diluent position" in application for sample, control and calibrator. Position number must be between 1 and 45 respectively 1 and 50.

Hemolysis is performed on board of the instrument automatically. Whole blood collection tubes must not be higher than 75 mm; otherwise contamination may occur!
Processing in batch mode is recommended.

Calibrators, controls and samples have to be hemolyzed before use. Please refer to subsequent pipetting scheme for on-board hemolysis:

	Preparation			
	Calibrator Level 1	Calibrator Level 2	Control	Sample
TruCal HbA1c net Level 1	1.6 µL	-	-	-
TruCal HbA1c net Level 2	-	5 µL	-	-
TruLab HbA1c net Level 1 and Level 2 /Sample	-	-	5 µL	5 µL
Add:				
HbA1c net Hemolyzing solution	100 µL	100 µL	100 µL	100 µL

Calibration

The concentrations of HbA1c and hemoglobin in unknown samples are derived from linear calibration curves.
Each calibration curve is obtained with 2 calibrators at different levels without a zero value.

Calculation

After entering the calculation formula into the instrument, the calculation of HbA1c ratio from total hemoglobin is done by the instrument automatically. Please refer to the instrument manual.

Dependent on selected standardization enter the following formula:

IFCC

Values in mmol/mol according to IFCC:

$$\text{HbA1c [mmol/mol]} = \left(\frac{\text{HbA1c [g/dL]}}{\text{Hb [g/dL]}}\right) \times 1000$$

DCCT/NGSP

Values in percent according to DCCT/NGSP:

$$\text{HbA1c [\%]} = \left(91.5 \times \frac{\text{HbA1c [g/dL]}}{\text{Hb [g/dL]}}\right) + 2.15$$

Calibrators and Controls

For calibration the DiaSys TruCal HbA1c net calibrator is recommended. The assigned values of TruCal HbA1c net have been made traceable to the approved IFCC reference method [1]. For internal quality control, DiaSys TruLab HbA1c net controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal HbA1c net	1 3350 99 10 044	2 x 0.3 mL
TruLab HbA1c net Level 1	5 9930 99 10 076	6 x 1 mL
TruLab HbA1c net Level 2	5 9940 99 10 076	6 x 1 mL

Performance Characteristics

Measuring range from 20 – 150 mmol/mol according to IFCC (4 - 16% according to DCCT/NGSP). The assay is applicable for hemoglobin concentrations in blood from 6 - 30 g/dL (3.73 – 18.6 mmol/L).	
Limit of detection**: HbA1c Hemoglobin	 0.2 g/dL 1.5 g/dL
On-board stability	6 weeks
Calibration stability	6 weeks

** lowest measurable concentration which can be distinguished from zero
mean + 1.645 x SD (n = 60) of an analyte free specimen

Specificity/Interferences

According to CLSI protocol EP7-A2 a study on interferences was conducted.

IFCC

For each interfering substance three samples with different hemoglobin and HbA1c values have been tested; a low level sample within a hemoglobin range of 8 – 10 g/dL and a HbA1c range within 28 – 35 mmol/mol; a medium level sample within a hemoglobin range of 11 – 15 g/dL and a HbA1c range within 28 – 35 mmol/mol; a high level sample within a hemoglobin range of 11 – 15 g/dL and a HbA1c range > 60 mmol/mol.

Hemoglobin variants can lead to deviant HbA1c results: The tested Hemoglobin variants HbS, HbC, HbD, HbE, HbJ, HbG, HbSC, HbSE, HbEE and HbF showed no significant interference.

Hemoglobin Variant	Percentage of Hemoglobin Variant (≤)	Target Value Range HbA1c [% DCCT/NGSP]	Mean recovery HbA1c [%]
AS	40% S	5.2 – 8.8	94.7
AC	36% C	5.0 – 7.4	97.1
AD	41% D	5.6 – 7.0	93.9
AE	26% E	5.9 – 7.6	99.1
AJ	50% J	5.2 – 8.4	100
AG	20% G	6.1 – 6.6	97.4
SC	52% S, 44% C	4.5 – 7.0	91.6
SE	65% S, 27% E	7.4	95.4
EE	94% E	5.1 – 8.9	98.0
Elevated F	4.6% F	6.5 – 8.1	93.6

Impreolsion

Values according to IFCC

Within-run precision n = 20	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	32.7	0.309	0.947
Sample 2	33.2	0.207	0.623
Sample 3	63.7	0.308	0.483

Total precision CLSI n = 80	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	32.1	0.522	1.63
Sample 2	33.6	0.433	1.29
Sample 3	67.6	0.824	1.22

Method comparison (n=100)	
Test x	Competitor enzymatic HbA1c assay
Test y	DiaSys HbA1c net FS
Slope	0.983
Intercept	0.772 mmol/mol
Coefficient of correlation	0.9945

Method comparison (n=100)	
Test x	HPLC assay
Test y	DiaSys HbA1c net FS
Slope	0.996
Intercept	-0.0153 mmol/mol
Coefficient of correlation	0.9931

Reference Range

Suggested target values for HbA1c [6]:

	IFCC [mmol/mol]	NGSP [%]
Non-diabetics	20 – 42	4 – 6
Target of therapy	< 53	< 7
Change of therapy	> 64	> 8

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

RFT/KFT

Urea kit

(Mod. Berthelot method)

For the determination of urea in serum, plasma and urine (or Invitro Diagnostic Use Only)

Summary

Urea is the end product of protein metabolism. It is synthesized in the liver from the ammonia produced by the catabolism of amino acids. It is transported by the blood to the kidneys from where it is excreted. Increased levels are found in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy.

Principle

Urease hydrolyses urea to ammonia and CO2. The ammonia further reacts with a phenolic chromogen and Hypochlorite to form a green coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample.

Urease

Urea+ H2O = Ammonia+CO2

Ammonia + Phenolic chromogen+hypochlorite = Green coloured complex

Normal reference values

Serum/Plasma: 14- 40 mg/dl

Urine: Upto 20g/L

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	75 Assays	3x75Assays	2x150Assays
L1: Buffer Reagent	75 ml	3*75ml	2*150 ml
L2: Enzyme Reagent	7.5 ml	3*7.5ml	30 ml
L3: Chromogen Reagent	15 ml	2*22.5ml	2*30 ml
S: Urea Standard (40 mg/dl)	5 ml	5 ml	5 ml

Storage / stability

Contents are stable at 2-8°C till the expiry mentioned on Urea Nitrogen in mg/dl the tables.

Reagent Preparation: Reagents are ready to use for the given procedure.

Working Enzyme Reagent: For the flexibility and Convenience in performing large assay series, a working enzyme reagent may be made by pouring 1 bottle of L2.

(Enzyme Reagent) into 1 bottle of L1 (Buffer Reagent). For smaller series combine 10 parts ofL1 (Buffer Reagent) and 1 part of L2 (Enzyme Reagent). Use 1 ml of the working reagent per assay instead of 1 ml of L1 and 0.1 ml of L2 as given in the procedure. The working enzyme reagent is stable for at least 4 weeks when stored at 2-8°C.

Working Chromogen Reagent: For larger volume cuvettes, dilute 1 part of L3 (Chromogen Reagent) with 4 parts of fresh ammonia free distilled/ deionised water. Use 1 ml of working chrornogen instead of 0.2 ml in the assay. The working chromogen reagent is stable for at least 8 weeks when stored at 2-8°C in a tightly stoppered plastic bottle.

Sample material

Serum, plasma, Urine. Dilute urine 1+ 49 with distilled water before the assay (Results x 50). Urea is reported to be stable in the serum for 5 days when stored at 2-8°C.

Procedure

Wavelength/filter: 570 nm (Hg 578 nm)/ Yellow

Temperature: 37°C /R.T

Light path: 1 cm

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T)

Addition Sequence	B (ml)	S (ml)	T (ml)
Buffer Reagent (L1)	1.0	1.0	1.0
Enzyme Reagent (L2)	0.1	0.1	0.1
Distilled water	0.01	-	-
Urea Standard (S)	-	0.01	-
Sample	-	-	0.01

Mix well & incubate for 5 min. at 37°C or 10 min. at R.T. (25°C)

Chromogen Reagent (L3)	B (ml) 0.2	S (ml) 0.2	T (ml) 0.2

Mix well and incubate for 5 min. at 37°C or 10 min. at R.T (25°C). Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against the blank, within 60 Min.

Calculations

Urea in mg/dl = (Abs.T/Abs.S) x 40

Urea Nitrogen in mg/dl = Urea in mg/dl x 0.467

Linearity

This procedure is linear upto 250 mg/dl. Using the working chromogen reagent (1 ml) the linearity is increased to 4000 mg/dl. If values exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

Uric acid kit

(Uricase/PAP method)

For the determination of Uric Acid in serum or plasma

Summary

Uric acid is the end produced of purine metabolism. Uric acid is excreted to a large degree by the kidneys and to a smaller degree in the intestinal tract by microbial degradation. Increased levels are found in Gout, arthritis, impaired renal functions, and starvation, Decreased levels are found in Wilson's disease, Fanconi's syndrome and yellow atrophy of the liver.

Principle

Uricase converts uric acid to allantoin and hydrogen peroxide. The hydrogen peroxide formed further reacts with a phenolic compound and 4 aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of uric acid present in the sample.

Uric acid +H2O → (Uricase) → Allantoin +H2O2

O2+Aminoantieripynne+Phenolic compound = Red quinoneimine dye + H2O+ Phenolic compound

Normal reference values:

Serum/Plasma (Males): 3.4 -7.0 mg/dl

(Females): 2.5-6.0 mg/dl

Contents 25 ml 75 ml 2x 75 ml 2x 150 ml

L1: Buffer Reagent 20ml 60ml 2 x60 ml 2 x120ml

L2: Enzyme Reagent 5ml 15 ml 2 x15 ml 2 x30 ml

S: Uric Acid Standard (8mg/dl) 5ml 5ml5ml

Procedure:

Wavelength/filter: 520 nm (Hg 548 nm)/Yellow Green

Temperature: 37°C

Light path: 1 cm

Pipette into dean dry test tubes labelled as Blank (B), Standard (S), and Test (T):

|

Addition Sequence	B (ml)	S (ml)	T(ml)
Working reagent	1.0	1.0	1.0
Distillled water	0.02	-	-
Uric acid Standard (S)	-	0.02	-
Sample	-	-	0.02

Mix well and incubate at 37°C for 5 min. or at RT. (25°C) for 15 min. Measure the absorbance of the Standard (Abs.S) and test sample against the Blank, within 30 min.

Calculations

Uric acid in mg/dl = (ΔAT/ ΔAS) X 8.0

Creatinine kit

(Mod. Jaffe's Kinetic method)

For the determination of Creatinine in serum and urine)

(For Invitro Diagnostic Use Only)

Summary

Creatinine is the catabolic product of creatinine phosphate which is used by the skeletal muscle. The daily production depends on muscular mass and it is excreted out of the body entirely by the kidneys. Elevated levels are found in renal dysfunction, reduced renal blood flow (shock, dehydration, congestive heart failure) diabetes acromegaly. Decreased levels are found in muscular dystrophy.

Principle:

Picric acid in an alkaline medium reacts with creatinine to form orange coloured complex with the alkaline picrate. Intensity of the colour formed during the fixed time is directly proportional to the amount of creatinine present in the sample.

Creatinine+ Alkaline Picrate → Orange Coloured Complex

Reference values:

Serum Urine in 24hrs. collection

Males: 0.6-1.2 mg% 1.1-3.0 gm

Females: 0.5-1.1 mg% 1.0-1.8 gm

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	2x 35 ml	2x75 ml	2x150 ml	2x500 ml
L1: Picric acid reagent	35 ml	75 ml	150 ml	2x250 ml
L2: Buffer Reagent	35 ml	75 ml	150 ml	2 x150 ml
S: Creatinine Standard (2 mg/dl)	5ml	2 x5 ml	15 ml	50 ml population.

Storage/stability

All reagents are stable at R.T. till the expiry mentioned on the label.

Reagent Preparation

Reagents are ready to use. Do not pipette with mouth. Working reagent for larger assay series a working reagent may be prepared by mixing equal volumes of Picric Acid Reagent and Buffer Reagent. The Working reagent is stable at R.T. (25-30 °C) for at least one week.

Sample material

Serum or Urine. References Creatinine is stable in serum for 1 day at 2-8°C Urine of 24 hours collection is preferred. Dilute the specimen 1:50 with distilled/ deionised water before the assay.

Procedure:

Pipette into a clean dry test tube labelled Standard (S) or Test (T)

Addition Sequence	**(S)/(T) 30°C / 37°C**
Picric Acid Reagent (L1)	0.5 ml
Buffer reagent (L2)	0.5 ml

Bring reagents to the assay temperature and add	
Creatinine Standard (S)/ Sample / Diluted Urine	0.1 ml

Mix well and read the initial absorbance A, for the Standard and Test after exactly 30 seconds. Read another absorbance A, of the Standard and Test exactly 60 seconds later. Calculate the change in absorbance ΔA for both the Standard and Test.

For Standard ΔAS = A2S-A1S

For Test ΔAT = A2T – A1T

Calculations

Creatinine in mg/dl = (ΔAT/ ΔAS) X 2.0

Urine Creatinine in g/l = (ΔAT/ ΔAS) X 1.0

Urine Creatinine g/24Hrs = Urine Creatinine in g/L x Vol. of urine in 24 Hrs

Determination of Na, K in blood sample

Objectives

1. **To quantitatively determine the concentration of sodium (Na^+) and potassium (K^+) ions in a blood sample.**
   This helps assess the electrolyte balance and hydration status of the patient.
2. **To evaluate the physiological and clinical significance of Na^+ and K^+ levels.**
   Imbalances can indicate disorders such as dehydration, kidney disease, or cardiac conditions.
3. **To understand and apply appropriate techniques for electrolyte measurement (e.g., flame photometry or ion-selective electrodes).**
   Familiarizing with lab instrumentation and procedures used in clinical biochemistry.
4. **To correlate measured electrolyte levels with normal reference ranges.**
   Helps in diagnosing hypernatremia, hyponatremia, hyperkalemia, or hypokalemia.
5. **To develop proficiency in handling biological samples and ensuring accurate, contamination-free results.**

Principle for Sodium

The determination of sodium in blood samples is commonly performed using flame photometry or ion-selective electrode (ISE) techniques. Sodium ions (Na+) are selectively measured due to their specific emission wavelength (589 nm) in flame photometry or by selective binding to membrane electrodes in ISE. These techniques allow for rapid, accurate quantification of sodium levels in biological fluids.

Principle for Potassium

Potassium can be determined by a number of different methods. It can be directly estimated by flame photometry, colorimetry. It can also be measured by the use of ion selective electrode. The method

is based on the measurement of turbidity of the reaction mixture containing Sodium Tetraphenyl Boron, Alkaline EDTA,Formaldehyde and sample containing potassium or standard potassium salt.

Normal Reference Values:

- Serum sodium (Na+): 135 - 145 mmol/L

-Potassium : 3.5 to 5.5 mmol/L

Storage / Stability:

- Blood samples should be collected in plain or lithium heparin tubes.
- Serum or plasma should be separated from cells within 1 hour of collection.
- Store samples at 2°C - 8°C if analysis is delayed.
- Sodium in serum is stable for up to 7 days at 2°C - 8°C and up to 6 months when frozen at -20°C.

Procedure:

1. Collect venous blood using standard phlebotomy techniques.
2. Allow blood to clot and centrifuge to separate serum (if not using plasma).
3. Calibrate the flame photometer or ISE system with sodium standards.
4. Aspirate standard solutions and samples into the instrument.
5. Record the readings from the instrument.
6. Compare sample readings to standard curve to determine sodium concentration.

Determination of Calcium in Blood Sample (OCPC Method)

1. Principle

The **o-Cresolphthalein Complexone (OCPC)** method is based on the reaction between calcium ions and o-cresolphthalein complexone in an alkaline medium, forming a purple-colored complex. The intensity of the color is directly proportional to the calcium concentration and is measured spectrophotometrically at **570–580 nm**.

2. Requirements

- **Reagents:**
  - o-Cresolphthalein Complexone (OCPC)
  - Alkaline buffer (e.g., 8-hydroxyquinoline to prevent magnesium interference)
  - Standard calcium solution
  - Distilled water
- **Equipment:**
  - Spectrophotometer or colorimeter
  - Test tubes, pipettes, cuvettes
  - Centrifuge (if using whole blood)
  - Water bath (optional)

3. Specimen Collection and Storage

- **Sample Type**: Serum (preferred) or plasma (heparinized).
- **Collection**:
  - Use a plain or heparinized vacutainer.

  - Avoid using EDTA, citrate, or oxalate anticoagulants as they chelate calcium.
- **Storage**:
  - Store serum/plasma at **2–8°C** if analysis is delayed.
  - Analyze within **24 hours** to avoid significant changes.
  - Bring to room temperature before testing.

4. Procedure

a. Preparation of Standard Curve

1. Prepare a series of calcium standards (e.g., 0, 5, 10, 15 mg/dL).
2. Add reagents as per the kit/manual or standard protocol.
3. Measure absorbance at 570–580 nm.
4. Plot absorbance vs. calcium concentration to get the standard curve.

b. Sample Analysis

1. Pipette:
   - 1.0 mL reagent blank (distilled water)
   - 1.0 mL calcium standard
   - 1.0 mL serum sample
2. Add 1.0 mL of OCPC reagent to each.
3. Mix and incubate at room temperature for **5–10 minutes**.
4. Read absorbance at **570–580 nm** using the reagent blank to zero the instrument.

5. Calculation

$$\text{Calcium (mg/dL)} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \right) \times \text{Concentration of standard (mg/dL)}$$

4. i) LFT (Liver function tests)

Bilirubin kit

(Mod. Jendrassik& Grof's method)

For the determination of Direct & Total Bilirubin in serum.

(For Invitro Diagnostic Use Only)

Summary

Bilirubin is mainly formed from the heme portion of aged or damaged RBCs. It then combines with albumin to form a complex which is not water soluble. This is referred to as indirect or unconjugated Bilirubin. In the liver this Bilirubin Complex is combined with glucuronic acid into a water-soluble conjugate. This is referred to as conjugated or direct Blirubin. Elevated levels of bilirubin are found in liver diseases (Hepatitis, cirrhosis), excessive hemolysis/destruction of RBC (hemolytic jaundice) obstruction of the bilary tract (obstructive jaundice) and in drug induced reactions. The differentiation between the direct and indirect bilirubin is important in diagnosing the cause of hyperbilirubinemia.

Principle

Bilirubin reacts with diazotised sulphanilic acid to form a coloured azobilirubin compound. The unconjugated bilirubin couples with the sulphanilic acid in the presence of a caffeine-benzoate accelerator. The intensity of the colour formed is directly proportional to the amount of bilirubin present in the sample.

Bilirubin + Diazotized sulphanilic acid = Azobilirubin compound

Normal reference values

Serum (Direct): upto 0.2 mg/dl

(Total): upto 1.0 mg/dl

Procedure

Wavelength / filter: 546 nm/Yellow- Green

Temperature: R.T.

Light path: 1cm

ii)Direct Bilirubin Assay

Pipette into clean dry test tubes labelled as Blank (B) and Test (T)

Addition Sequence	B(ml)	T (ml)
Direct Bilirubin Reagent (L1)	1.0	1.0
Direct Nitrite Reagent (L2)	-	0.05
Sample	0.1	0.1

Mix well and incubate at R.T. for exactly 5 min. Measure the absorbance of the Test Samples (Abs.T) immediately against their respective Blanks.

Total Bilirubin Assay

iii) **Pipette into clean dry test tubes labelled as Blank (B), and Test (T):**

Addition Sequence	B(ml)	T (ml)
Total Bilirubin Reagent (L1)	1.0	1.0
Total Nitrite Reagent (L2)	-	0.05
Sample	0.1	0.1

Mix well and incubate at R.T. for 10 min. Measure the absorbance of the Test Samples (Abs.T) immediately against their respective Blanks.

Calculations

Total or Direct Bilirubin in mg/dl = Abs.T X 13

Indirect bilirubin = Total bilirubin- Direct bilirubin

Linearity

This procedure is linear upto 20 mg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

iv)

SGPT (ALAT) kit

(Modified IFCC Method)

Summary:

SGPT is found in a variety of tissues but it is mainly found in liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction.

Principle:

SGPT (ALAT) catalyzes the transfer of amino group between L Alanine and α-Ketoglutarate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT (ALT) activity in the sample.

SGPT

L-Alanine + α-Ketoglutarate → Pyruvate + L-Glutamate

LDH

Pyruvate + NADH + H+ → Lactate + NAD+

Expected values:

Serum (Males): upto 40 U/L at 37°C

(Females): upto 31U/L at 37°c

Procedure:

Wavelength/filter: 340 nm

Temperature: 37°C/30°C/25°C R.T.

Light path: 1 cm

Substrate Start Assay:

Pipette into a clean dry test tube labelled as Test (T):

Addition Sequence	**(T) 25°C/30°C**	**(T) 37°C**
Enzyme Reagent (L 1)	0.8 ml	0.8 ml
Sample	0.2ml	0.2ml

Incubate at the assay temperature for 1 min. and add

Starter Reagent (L2)	0.2 ml	0.2 ml

Mix well and read the initial absorbance A, after 1 min. and repeat the absorbance reading after every 1, 2, & 3 mins. Calculale the mean absorbance change per min. (ΔA /min).

Calculations

Substrate /Sample start assay

SGPT (ALAT) Activity in U/L 25°C/30°C = ΔA/min x 952

SGPT (ALAT) Activity in U/L 37°C = ΔA/min. x 1746

Serum Glutamic-Oxaloacetic Transaminase (SGOT)/ Aspartate Aminotransferase (ASAT) kit

(Modified IFCC Method)

Summary:

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood. Elevated levels are found in myocardial infarction, Cardiac operations, Hepatitis, Cirrhosis, acute pancreatitis, acute renal diseases, primary muscle diseases. Decreased levels may be found in Pregnancy, Beri Beri and Diabetic ketoacidosis.

Principle:

SGOT (ASAT) catalyses the transfer of amino group between L-Aspartate and α-Ketoglutarate to form Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with NADH in the presence of Malate dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT (ASAT) activity in the sample.

SGOT

L-Aspartate + α-ketoglutarate → Oxaloacetate + L-glutamate

MDH

Oxaloacetate + NADH + H+ → Malate + NAD+

Expected values:

Serum (males): upto 37 U/L at 37°C

(Females): upto 31 U/L at 37°C

Procedure:

Wavelength/filter: 340 nm

Temperature: 37°C/30°C/25°C R.T.

Light path:1 cm

Substrate Start Assay

Serum Pipette into a clean dry test tube labelled as Test (T)

Mix well and read the initial absorbance A, after 1min. & repeat the absorbance reading after every 1,2, & 3 mins. Calculate the mean absorbance change per min. (ΔA/min.).

Addition Sequence	(T) 25°C/30°C	(T) 37°C
Enzyme Reagent (L1)	0.8 ml	0.8 ml
Sample	0.2 ml	0.1ml
Incubate at the assay temperature for 1 min. and add		
Starter Reagent (L2)	0.2 ml	0.2 ml

Mix well and read the initial absorbance A, after 1min. & repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (ΔA /min.).

Sample Start Assay:

Pipette into a clean dry test tube labelled as Test (T)

Addition Sequence	**(T) 25°C/30°C**	**(T) 37°C**
Working Reagent	1.0 ml	1.0 ml

Incubate at the assay temperature for 1 min and add

Sample	0.2 ml	0.1 ml

Mix well and read the initial absorbance A, after 1 min. & repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (ΔA/min.)

Calculations:

Substrate/Sample start assay

SGOT (ASAT) Activity in U/L 25C/30°C = ΔA /min. x 952

37C °C = ΔA /min. x 1746

vi) **γ-Glutamyl transferase (GGT)**

Colorimetric Assay Kit

Principle:

The GGT Activity Colorimetric Assay kit provides asimple and direct procedure for measuring GGT activityin a variety of samples. GGT activity is determined by acoupled enzyme assay, in which the GGT transfers theγ-glutamyl group from the substrate L-γ-Glutamylp-nitroanilide, liberating the chromogen p-nitroanilide(pNA, 418 nm) proportional to the GGT present. Oneunit of GGT is the amount of enzyme that will generate1.0mole of pNA per minute at 37°C.

Procedure

All samples and standards should be run in duplicate. pNA Standards for Colorimetric detection Add 0, 4, 8, 12, 16, and 20µL of the 2 mM standard solution into a 96 well plate, generating 0 (blank), 8, 16, 24, 32, and 40 nmol/well standards. Add GGT Assay Buffer to each well to bring the volume to 100 µL.

Sample Preparation

Tissue (10 mg) or cells (1x 10^6) can be homogenized in 200µL of ice-cold GGT Assay Buffer. Centrifuge the samples at 13,000xg for 10 minutes to remove insoluble material. Serum samples can be directly added to the wells. For the positive control, add 10µL of the GGT positive control solution to wells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve. Bring samples to a final volume of 10 µL with GGT Assay Buffer.

Assay Reaction:

1. Add 90 L of GGT Substrate Solution to each well containing test samples. Do not add to pNA Standards.
2. Incubate the plate at 37°C. After 3 minutes, take the initial measurement (Tinitial). Measure the absorbance at 418 nm at the initial time (A418) initial. Of the standard curve.
3. Continue to incubate the plate at 37°C taking measurements (A418) every 5 minutes. Protect the plate from light during the incubation.
4. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (40 nmol/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.
5. The final measurement [(A418) final] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds theend of the linear range of the standard curve, see step 5. The time of the penultimate reading is Tfinal.

Results:

Calculations

Plot the pNA standard curve from the initial measurement (Tinitial).

A418 = (A418)final – (A418)initial

Compare the ΔA418 of each sample to the standardcurve to determine the amount of pNA generatedbetween Tinitial and Tfinal (B).

GGT Activity = B xSample Dilution Factor/ (Reaction Time) xV

The GGT activity of a sample may be determined by the following equation:

B = Amount (nmol) of NADH generated between Tinitial and Tfinal.

Reaction Time = Tfinal – Tinitial (minutes)

V = sample volume (mL) added to well

GGT activity is reported as nmol/min/mL = milliunit/mL

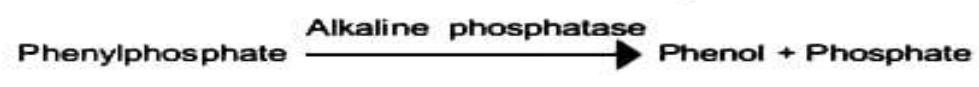
One unit of GGT is the amount of enzyme that will generate 1.0 mole of pNA per minute at 37°C.

vii) **Alkaline phosphatase**

Colorimetric method

Principle:

Free phenol liberated by hydrolysis of the substrate reacts then with 4-amino-antipyrine in the presence of alkaline potassium ferricyanide to form a red-coloured complex which absorbance measured at 510 nm is directly proportional to the ALP activity in the specimen.



Procedure:

Prepare tubes as follows :	Reagent blank	Specimen blank	Standard	Assay
Reagent R1	2 mL	2 mL	2 mL	2 mL
Incubate 5 minutes at 37°C.				
Specimen				50 µL
Reagent R2 (Standard)			50 µL	
Let stand **exactly 15 minutes at 37°C.**				
Reagent R3	0,5 mL	0,5 mL	0,5 mL	0,5 mL
Mix well.				
Reagent R4	0,5 mL	0,5 mL	0,5 mL	0,5 mL
Specimen		50 µL		
Demineralised water	50 µL			

Mix. Incubate 10 minutes at room temperature and away from light. Read absorbances of the blank specimen, standard and assay at 510 nm against reagent blank.

Coloration is stable for 45 minutes away from light.

Normal range:

The normal range for alkaline phosphatase is 20 to 140 IU/L, but ranges of normal must be adjusted for factors such as age, sex, and the laboratory's own normal values.

Calculation:

Quantity of enzyme which on reactions conditions liberates 1 mg of phenol in 15 minutes at 37°C.

ALP activity

(Kind and king units/100 ml)= (Abs Assay-Abs specimen blank)/Abs standard x20

High ALP levels can be a sign of many different conditions, including:

- Bacterial infections
- Cholangiocarcinoma (bile duct cancer)
- Cholelithiasis (gallstones)
- Biliary obstruction or dyskinesia (lack of motility)
- Bone cancers, including osteosarcoma and chondrosarcoma
- Bone conditions, including osteomalacia and osteoporosis
- Cholecystitis (gallbladder inflammation)
- Cirrhosis (scarring of the liver)
- Congestive heart failure
- Drug-induced liver toxicity

- Gallstones
- Hepatitis, infectious and non-infectious
- Hepatocarcinoma (liver cancer)
- Hyperthyroidism (overactive thyroid gland)
- Hyperparathyroidism (overactive parathyroid gland)
- Kidney cancers, including renal cell carcinoma
- Lymphoma (cancer of the lymphatic system)
- Metastatic cancer to the liver or bone

Low ALP is less common than high levels. Abnormally low values may be the result of:

- Achondroplasia (a form of dwarfism)
- Aplastic anaemia (anaemia caused by bone marrow failure)
- Celiac disease (an autoimmune disease triggered by gluten)
- Congenital iodine deficiency
- Hypophosphatasia (a congenital disorder affecting bone growth)
- Hypothyroidism (low thyroid function)
- Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease

viii) **Total protein kit**

(Biuret method)

For the determination of Total Proteins in serum and plasma.

(For Invitro Diagnostic Use Only)

Summary

Proteins are constituents of muscle. enzymes, hormones and several other key functional and structural entities in the body. They are involved in the maintenance of the normal distribution of water between blood and the tissues. Consisting mainly of albumin and globulin the fractions vary 1ndependently and widely in diseases. Increased levels are found mainly in dehydration. Decreased levels are found mainly in malnutrition, impaired synthesis, protein losses as in haemorrhage or excessive protein catabolism.

Principle

Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet coloured complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample.

Proteins+cu2+ = Blue-Violet coloured complex

Normal reference values

Serum & Plasma: 6.0-8.0g/dl

It is recommended that each laboratory establish its own normal range representing its patient population.

Procedure

Wavelength/filter: 550nm (Hg546nm)/Yellow-Green

Temperature: R.T./37°C

Light path: 1 cm

Pipette into dean dry test tubes labelled as Blank (B), Standard (S). and Test (T):

Addition Sequence	B (ml)	S (ml)	T (ml)
Biuret reagent (L1)	1.0	1.0	1.0
D1stilled water	0.02	-	-
Protein Standard (S)	-	0.02	-
Sample	-	-	0.02

Mix well and read the initial absorbance A, after 1 min. and repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (ΔA /min).

Calculations

Total Proteins in g/dl = (Abs.T/Abs.S) X 8

Linearity

This procedure is linear upto 15 g/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Serum Globulin = (Serum total protein – Serum albumin) g/dL

ix) Albumin-Globulin ratio

The albumin globulin (A/G) ratio is determined through a blood test that measures the levels of albumin and globulins in the blood. Here's a step-by-step overview of the process:

1**. Blood collection:** A healthcare professional collects a blood sample from a vein in your arm.

2. **Serum separation:** The blood is centrifuged to separate the serum (liquid portion) from the blood cells.

3. **Protein analysis:** The serum is then analysed for protein content using techniques like electrophoresis or chromatography.

4. **Albumin measurement:** The level of albumin is measured in grams per deciliter (g/dL).

5. **Globulin measurement:** The level of globulins is measured in g/dL.

6. **A/G ratio calculation:** The A/G ratio is calculated by dividing the albumin level by the globulin level.

7. **Result interpretation:** The resulting A/G ratio is compared to the normal range (1.5-2.5) to determine if it's within normal limits or indicates an imbalance.

Some common methods for determining the A/G ratio include:

- **Electrophoresis:** Separates proteins based on their electrical charge.
- **Chromatography:** Separates proteins based on their size and binding properties.
- **Nephelometry:** Measures protein levels by detecting changes in light scattering.
- **Immunoassays:** Measures specific proteins using antibodies

The Albumin/Globulin (A/G) ratio has significant clinical implications in various medical conditions. Here are some key aspects:

Clinical Significance:

1. **Liver Disease:** A low A/G ratio (<1.5) may indicate liver damage, cirrhosis, or liver failure.

2. **Kidney Disease**: A low A/G ratio may indicate nephrotic syndrome, kidney failure, or glomerulonephritis.

3. **Malnutrition:** Alow A/G ratio may indicate protein malnutrition, kwashiorkor, or marasmus.

4. **Inflammation:** A high A/G ratio (>2.5) may indicate acute or chronic inflammation.

5. **Infection:** A high A/G ratio may indicate bacterial or viral infections.

6. **Cancer:** Abnormal A/G ratios may be seen in various types of cancer, such as multiple myeloma or liver cancer.

7. **Autoimmune Disorders:** Abnormal A/G ratios may be seen in conditions like rheumatoid arthritis or systemic lupus erythematosus.

5.Lipid profile

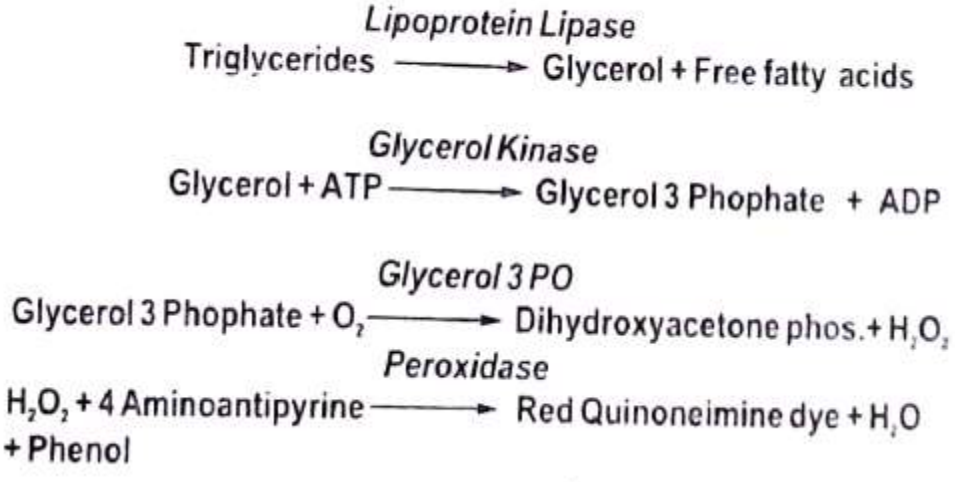
i) Triglycerides kit (Serum TG)

Summary

Triglycerides are a form of fatty acid esters. They are produced in the liver by binding glycerol and other fatty acids. They are transported by VLDL and LDL and act as a storage source for energy. Increased levels are found in hyperlipidemias, diabetes, nephrotic syndrome, hypothyroidism. Increased levels are risk factor for arteriosclerotic coronary disease and peripheral vascular disease. Decreased levels are found in malnutrition andhyperthyroidism.

Principle

Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphate which is oxidised by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinonimine dye complex. Intensity of the colour formed is directly proportional to the amount of triglycerides present in the sample.



Normal reference values:

Serum/Plasma (Suspicious): 150 mg/dl and above

(Elevated): 200 mg/dl and above

Sample material

Serum, plasma. Triglycerides is reported to be stable in the sample for 5 days when stored at 2-8°C.

Procedure:

Wavelength/filter: 505nm (Hg 546 nm)/Green

Temperature: 37C/R.T.

Light path:1 cm

Pipette into clean dry test tubes labelled as Blank (B), Standard (S) and Test (T):

Additiion Sequence	B (ml)	S (ml)	T (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Triglycerides Standard (S)	-	0.01	-
Sample	-	-	0.01

Mix well and incubate at 37'C for 5 min. or at R.T. (25'C) for 15 mins. Measure the absorbance of the Standard. Normal reference values (Abs.S), and Test Sample (Abs.T) against the Blank, within 60 Min.

Calculations:

Triglycerides in mg/dl = Abs.T/Abs.SX 200

Linearity

This procedure is linear upto 1000 mg/dl. If values exceed this limit, dilute the serum with normal saline (Nacl 0.9%) and repeat the assay.

ii)

Cholesterol kit

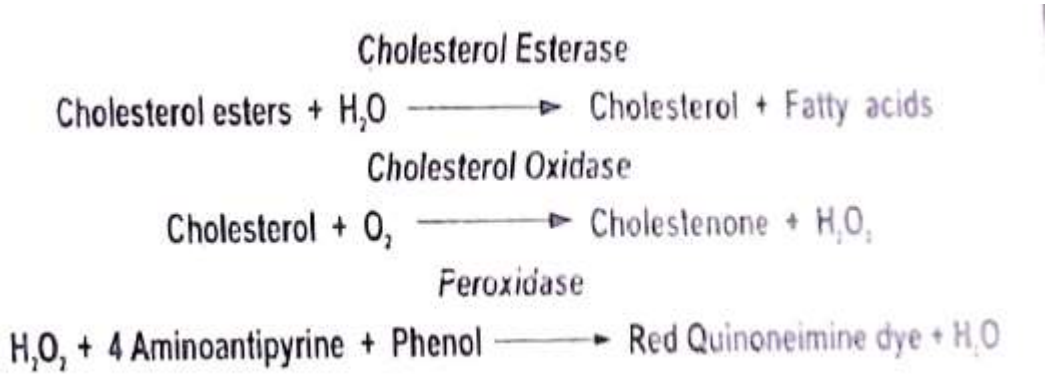
(CHOD/ PAP METHOD)

Summary

Cholesterol is the main lipid found in blood, bile and brain tissues. It is the main lipid associated with arteriosclerotic vascular diseases. It is required for the formation of steroids and cellular membranes. The liver metabolizes the cholesterol and it is transported in the blood stream by lipoproteins. Increased levels are found in hypercholesterolaemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephrotic syndrome, and cirrhosis. Decreased levels are found in malabsorption, malnutrition, hyperthyroidism, anaemias and liver diseases.

Principle:

Cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol is oxidised to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinonimine dye complex. Intensity of colour formed is directly proportional to the amount of cholesterol present in the sample.



Normal reference values:

Serum/Plasma (Suspicious): 220 mg/dl and above

Elevated:260 mg/dl and above

Procedure:

Wavelength/filter: 505 nm (Hg 546 nm)/ Green

Temperature: 37°C/R.T.

Light path: 1 cm

Pipette into clean dry test tubes labelled as Blank (B), Standard (S) and Test (T):

Addition Sequence	B (ml)	S (ml)	T (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Cholesterol Standard (S)	-	0.01	-
Sample	-	-	0.01

Mix well and incubate al 37"C for 5 min. or at R.T. (25°C) for 15 min. Measure the absorbance of the Standard (Abs.S). and Test Sample (Abs.T) against the Blank, within 60 Min.

Calculations

Cholesterol in mg/dl = (Abs.T/Abs. S) X 200

Linearity

This procedure is linear upto 750 mg/dl. If the value exceeds this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

HDL (High-Density Lipoprotein) in blood using the Direct Method, specifically using a **Euro HDL-Cholesterol kit**

Objective

To **determine the concentration of HDL cholesterol** in human serum or plasma using a **direct enzymatic method** without requiring prior separation of lipoprotein fractions.

Principle

The **direct HDL-C assay** by the **Euro kit** works based on a **two-reagent enzymatic colorimetric method**:

1. **Reagent 1** contains detergents and enzymes that **selectively block or remove non-HDL lipoproteins** (VLDL, LDL, chylomicrons), preventing them from participating in the reaction.
2. **Reagent 2** contains cholesterol esterase and cholesterol oxidase that react **only with HDL-bound cholesterol**, generating **hydrogen peroxide (H_2O_2)**.
3. The H_2O_2 formed reacts with a chromogen (e.g., 4-aminoantipyrine and phenol) in the presence of peroxidase to form a **colored quinoneimine dye**, which is measured **photometrically**.

Absorbance is directly proportional to the concentration of HDL-C in the sample.

 Reagents Required (Euro Kit Components)

- **Reagent 1**: Selective detergent and masking agents
- **Reagent 2**: Cholesterol esterase, cholesterol oxidase, peroxidase, chromogen

 Sample

- Serum or plasma (fasting sample recommended)
- Avoid hemolysis or lipemic samples

 Procedure

1. **Label test tubes** as: Blank (B), Standard (S), and Test (T)
2. Pipette as follows:

Component	Blank	Standard	Test
Sample	—	—	0.02 mL (20 µL)
HDL Standard	—	0.02 mL (20 µL)	—
Reagent 1	1.0 mL	1.0 mL	1.0 mL
Mix and incubate at 37°C for 5 minutes			
Reagent 2	0.25 mL	0.25 mL	0.25 mL
Mix and incubate at 37°C for 5–10 minutes			
Measure absorbance at λ = 600 nm or 500–550 nm depending on the kit			

📊 Calculation

$$\text{HDL-C (mg/dL)} = \left(\frac{A_T - A_B}{A_S - A_B}\right) \times \text{Concentration of Standard}$$

Where:

- A_T = Absorbance of Test
- A_S = Absorbance of Standard
- A_B = Absorbance of Blank
- Standard concentration is usually **50 mg/dL** (check Euro kit label)

LDL (Low-Density Lipoprotein) in blood using the Direct Method

Direct Enzymatic Colorimetric Method
For In-Vitro Diagnostic
Store at 2-8°C

INTENDED USE

For the measurement of cholesterol concentration in human serum.

Principle

Direct determination of serum LDLc (low-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation steps.

The assay takes place in two steps.

1. Elimination of lipoprotein no –LDL:

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{cholesterol+ fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

2. Measurement of LDLc

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{cholesterol+ fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{TOOS} + \text{4-AA} \xrightarrow{\text{POD}} 4H_2O + \text{Quinonimine}$$

The intensity of the color formed is proportional to the LDLc concentration in the sample.

Reagents

R1 Enzymes	• PIPES Ph 7.0 (20°C)	50mmol/L
	• Cholesterol esterase (CHE)	≥600U/L
	• Cholesterol oxidase (CHOD)	≥500 U/L
	• Catalase	≥600 KU/L
	• N-Ethyl-N-(2-hydroxy-3-sulfopropyl) -3-methylaniline(TOOS)	2mmol/L
R2 Enzymes	• PIPER Ph 7.0	50mmol/L
	• 4-Aminoantipyrine (4-AA)	4mmol/L
	• Peroxidase (POD)	≥4KU/L
LDL CAL.	standard	

Preparation

- R1 and R2 : are ready to use.
- Calibrator:
  1. Reconstitute with 1.0 ml of distilled water mix well , making sure there is no material left on the walls.
  2. Let stand the vial for at least 2 hours before using.
  3. Mix well before using.

or 3 months -20 °C.

Samples

- Serum, After sampling, the test should be performed without delay. Repeated freezing and thawing should be avoided.
- Stability of the samples :7 days at 2-8°C

Procedure

1. Assay conditions
   Wavelength.....600 ±10 nm
   cuvette.....1cm. light path
   Temperature.....37°C
2. Adjust the instrument to zero with distelled water.
3. Pipette into a cuvette

	Blank	Standard	Sample
R1(µL)	300	300	300
Standard(µL)	--	4	--
Sample(µL)	--	--	4

4. Mix and incubate for 5 minutes at 37°C.
5. Add:

R2(µL)	100	100	100

Cholesterol esters $\xrightarrow{CHE}$ cholesterol+ fatty acids

Cholesterol $+O_2$ $\xrightarrow{CHOD}$ 4-Cholestenone $+ H_2O_2$

$2H_2O_2$ $\xrightarrow{catalase}$ $2H_2O + O_2$

2. Measurement of LDLc

Cholesterol esters $\xrightarrow{CHE}$ cholesterol+ fatty acids

Cholesterol$+O_2$ $\xrightarrow{CHOD}$ 4-Cholestenone $+H_2O_2$

$2H_2O_2$ +TOOS+4-AA $\xrightarrow{POD}$ $4H_2O$ + Quinonimine

The intensity of the color formed is proportional to the LDLc concentration in the sample.

Clinical significance

The LDLc particle is lipoproteins that transport cholesterol to the cells.

Often called "bad cholesterol" because high levels are risk factor for coronary heart disease and are associated with obesity ,diabetes and nephrosis.

Clinical diagnosis should not be made on a single test result, it should integrate clinical and other laboratory data.

	• Peroxidase (POD) ≥4KU/L
LDL CAL.	standard

Preparation

- R1 and R2 : are ready to use.
- Calibrator:
  1. Reconstitute with 1.0 ml of distilled water mix well , making sure there is no material left on the walls.
  2. Let stand the vial for at least 2 hours before using.
  3. Mix well before using.

Additional equipment

- spectrophotometer or colorimeter measuring at 600±10nm
- matched cuvettes 1.0 cm light path.
- General laboratory equipment.

Storage and stability

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use.

	Blank	Standard	Sample
R1(µL)	300	300	300
Standard(µL)	–	4	–
Sample(µL)	–	–	4

4. Mix and incubate for 5 minutes at 37°C.
5. Add:

R2(µL)	100	100	100

6. Mix and incubate for 5 minutes at 37°C.
7. Read the absorbance (A) , against the blank.

Calculations

$$\frac{\text{(A) Sample}}{\text{(A) standard}} \times \text{standard concentration}$$

=mg/dL of LDLc in the sample

Conversion factor: mg/dL x 0.02586= mmol/L

Determination of Troponin T and Troponin I in Blood

1. Objective:

To determine the levels of cardiac-specific **Troponin T (cTnT)** and **Troponin I (cTnI)** in human blood samples for diagnostic evaluation of myocardial injury.

2. Principle:

Troponins are regulatory proteins found in skeletal and cardiac muscle. **Troponin T and I are released into the bloodstream** when the heart muscle is damaged.
Their detection is based on **immunoassay techniques**, such as:

- **Enzyme-Linked Immunosorbent Assay (ELISA)**
- **Chemiluminescent Immunoassay (CLIA)**
- **Lateral flow immunoassay (Point-of-care test kits)**

These methods use antibodies specific to cTnT or cTnI to detect and quantify the proteins in serum or plasma samples.

3. Materials and Reagents:

- Patient blood sample (serum or plasma)
- ELISA/CLIA/Lateral flow assay kit for Troponin T/I
- Standard controls and calibrators
- Microplate reader (for ELISA)
- Pipettes and tips
- Centrifuge
- Sample tubes
- Washing buffer
- Substrate solution (e.g., TMB for ELISA)
- Stop solution (usually H_2SO_4 for ELISA)

4. Sample Collection and Storage:

- **Sample type:** Serum or plasma (EDTA or heparinized)
- **Collection:** Venous blood collected via venipuncture
- **Processing:** Centrifuge at 3000 rpm for 10 minutes to separate serum/plasma
- **Storage:**
  - **Short-term:** 2–8°C for up to 48 hours
  - **Long-term:** −20°C or below for up to 6 months
- Avoid repeated freeze-thaw cycles.

5. Procedure (ELISA Example):

1. Bring all reagents and samples to room temperature.
2. Pipette standards, controls, and patient samples into wells coated with anti-cTnT or anti-cTnI antibody.
3. Incubate at 37°C for 30–60 minutes.
4. Wash wells to remove unbound substances.
5. Add enzyme-linked detection antibody specific to Troponin T or I.
6. Incubate again, followed by washing.
7. Add substrate solution (TMB).
8. Incubate until color develops (10–15 minutes).
9. Add stop solution.
10. Read absorbance at 450 nm using a microplate reader.

6. Calculation:

- Plot a **standard curve** using known concentrations of troponin standards.
- Use the sample absorbance values to interpolate from the curve.

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6 .Lipase

(Methyl resorufin)

Introduction: Lipases are glycoproteins with a molecular weight of 47000 Daltons. Lipases hydrolyzes the ester linkages. Specifically, lipase catalyzes the partial hydrolysis of dietary triglycerides in the intestine to the 2-monoglyceride intermediate, with the production of long chain fatty acids.

Principle:

Enzymatic color test.

Colorimetric substrate 1, 2-o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin)-ester is cleaved by pancreatic lipase and the resulting dicarboxylic acid ester is hydrolysed under the alkaline test conditions to yield the chromophore methylresorufin. The kinetic of colour formation at 580 nm is monitored and it is proportional to lipase activity in sample.

Assay Procedure:

	Calibrator	Sample
Reagent 1	1000 µL	1000 µL
Calibrator	10 µL	-
Sample		10 µL

Mix and after 60 seconds incubation, measure the change in absorbance for 60 seconds at 37°C.

Determine the ΔAbs,

Calculation:

Lipase activity (U/I) = (ΔAbs. of sample/ΔAbs. of calibrator) x Concentration of calibrator

Reference normal value:

Serum lipase activity: 13-60 U/l

Clinical Significance:

Determination of lipase is used for investigation of pancreatic disorders. In acute pancreatitis the lipase concentrations rise to 2-50 fold to upper reference limit within 4-8 hours after begin of abdominal pain peaking at 24 hours and decreasing within 8 to 14 days. Elevated lipase values can also be observed in chronic pancreatitis and obstruction of the pancreatic duct.

Alpha amylase (single reagent)

Introduction

α-Amylase is derived mainly from the salivary glands and the exocrine pancreas. α-Amylase catalyses the hydrolysis of α-1-4 glycosidic linkages of starch and other related polysaccharides to produce maltose and other oligosaccharides. The enzyme is a relatively small molecule which is rapidly cleared by the kidneys and excreted in the urine.

Principle:

2-Chloro-4-nitrophenol-β -1- 4 galactopyranosylmaltotrioside (CNP-G) is a direct substrate for determination of α-amylase activity, which does not require the presence of ancillary enzymes. The rate of 2-chloro-4-nitrophenol formation can be monitored at (400-420) nm and is proportional to the α-amylase activity.

Gal – G2 - α-CNP → Gal – G2 + CNP

Expected values at 37°C

Serum: up to 80 U/l

Urine: up to 500 U/l

Assay procedure

Wavelength: 405 (400 – 420) nm

Cuvette: 1cm

Working solution	1000 µl
Sample	20 µl

Mix, incubate 1 min. at 37°C and then measure the initial absorbance of calibrator and sample against reagent blank. Measure the absorbance change exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

Calculation:

1. Amylase activity (U/l) = $\frac{\Delta A sam/min}{\Delta A cal/min} \times$ Concentration of Cal

2. Using factor: Amylase activity (U/l) = f x ΔA/min

f = factor, f = 3128 (at 405 nm)

Clinical significance:

α-Amylase is derived mainly from the salivary glands and the exocrine pancreas. α-Amylase catalyses the hydrolysis of α-1-4 glycosidic linkages of starch and other related polysaccharides to produce maltose and other oligosaccharides. The enzyme is a relatively small molecule which is rapidly cleared by the kidneys and excreted in the urine.

α-Amylase is most frequently measured in the diagnosis of acute pancreatitis when serum levels may be grossly elevated. In acute pancreatitis α-amylase starts to rise approximately 4 hours after the onset of pain, reaches a peak at 24 hours and remains elevated for 3-7 days. Hyperamylasaemia is also associated with other acute abdominal disorders, biliary dysfunction, salivary gland disorders, ruptured ectopic pregnancy and macroamylasemia.

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