INTERNSHIP REPORT

ICMR –NIMR (NATIONAL INSTITUTE OF MALARIA RESEARCH)

DURATION- 1ST DEC TO 30TH DEC

UNDER THE SUPERVISION OF-

Dr. Ajeet Mohanty

Mr.Abhishek Govekar

Mr.Debattum Muzumdar

Mr. Jagannath Nayak

Mrs. Sushma Bhingi

SUBMITTED BY

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Ref. No. NIMR/FU/GOA/ 53 /2023

Date: 6th March, 2023.

Experience Certificate

This is to Certify that Ms. Sanjana Sanjiv Gaikwad, student of MSc Marine Biotechnology, Goa University, Taleigao, underwent training in skill and research practices in the Laboratory of ICMR-National Institute of Malaria Research, Field Unit Goa from 1st to 30th December, 2022. This certificate is being issued to Ms. Sanjana S. Gaikwad on her request.

06/03/2023

(Officer in Charge)

Dr. Ajeet Kumar Mohanty
Scientist & Officer-In-Charge
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ACKNOWLEDGEMENT

First, I would like to express my sincere gratitude towards DBT-Department of Biotechnology, India for their support. I feel truly privileged and honoured to have the opportunity to be a beneficiary of dbt's sponsorship.

I would like to thank Prof. Savita ma'am (Dean of SBSB), Prof. Sanjeev Ghadi sir(Programme Director of marine biotechnology) , Dr. Meghnath sir, Dr. Samantha ma'am for providing this opportunity.

My special thanks to Dr. Ajeet Mohanty (scientist at NIMR) for giving me the chance to learn and work in the institute.

I would like to thank Mr. Abhishek Govekar for guide me and teaching all important molecular biological techniques and conduct hands on sessions.

I would like to thank Mr. Jagnnath Nayak for introducing Malarial microscopy and different stages of parasite infection in blood.

I would like to thank Mr. Debattum Muzumdar and Mrs. Sushma Bhingi for teaching and showing us identification of mosquito , dissection of mosquito and Rearing of mosquito in lab conditions.

It is indeed with a great sense of pleasure and gratitude that I acknowledge the help of these individuals.

I am extremely grateful to all staff members and friends who helped me in successful completion of this internship.

LEARNING OBJETIVES-

Sr.no	Duration	particulars	Learnings
1.	5th -6th December	Rearing and maintenance of different species of mosquito under laboratory condition.	1. Life cycle of Ades and Anopheles mosquito 2.Different stages of mosquito like larvae, pupa and adults.
2.	7th -14th December	Molecular techniques	1. DNA Isolation from salivary glands of mosquito by using Qiagen kit 2.Three types of PCR techniques- a] Nested PCR b] ITS (internal transcribed sequence) PCR c] Multiplex PCR 3. Gel electrophoresis and visualization by using gel documentation system.
3.	15th December	Malarial microscopy	1. Preparation of slides 2. Making thin film and thick film and staining 3. Observe under microscope.
4.	16th -21st December	Mosquito Identification, Dissection of mosquito and membrane feeding	1. Identification of mosquito under microscope by using manual. 2. Difference between ades , anopheles and culex mosquito. 3. Dissection of mosquito and observe salivary gland and midgut of mosquito.
5.	22nd – 27th December	Hands on molecular biology techniques.	1. DNA Isolation from salivary glands of mosquito by using Qiagen kit. 2. PCR and gel electrophoresis.

INSTITUTIONAL INFORMATION

ICMR- NATIONAL INSTITUTE OF MALARIA RESEARCH (NIMR).

The Field Unit (FU) of ICMR-National Institute of Malaria Research at Panaji was established on 6th Sept. 1989. The main objective of this FU was to control malaria in Goa by situational analysis, research evidence and demonstration of control of malaria in Panaji from 1989-92 by bio-environmental methods. The FU has assisted State National Vector Borne Diseases Control Programme (NVBDCP) in framing suitable bye-laws for vector control, preferential diagnosis and treatment of malaria in high risk groups and in the amendment of existing bye-laws in the Goa Public Health Act. The FU also provides training to Health Officers, Medical Officers and Senior Supervisory staff of the Directorate of Health Services, Goa.

The FU is currently involved in basic and applied research related to malaria. The ongoing research activities of FU include study of malaria vector bionomics in Goa to facilitate effective vector control strategies, studies on the changing behaviour of mosquito vectors such as feeding and resting behaviour and population dynamics in persistent transmission areas under different phases (categories) of malaria elimination, periodical assessment of malaria vectors susceptibility to commonly used insecticides and isolation, characterization and bio-assays of indigenous strains of mosquito-pathogenic Bacilli for vector control.

INTRODUCTION

- **Historical outline-**

It is assumed that the evolutionary history of mammalian plasmodia started with the adaptation of Coccidia of the intestinal epithelium to some tissue of internal organs and then to the invasion of free cells in the blood.

The next step was the possibility of transmission of parasites from one animal to another by blood sucking arthropod vector.

The main breakthrough in the long history of malaria is connected with the first therapeutic advances.

At the beginning of 17th century came discovery of the value of" Perruvian bark" for the treatment of fevers.

In 1880 the Laveran, a French army surgeon in Algeria, first saw and described malaria parasites in RBCs of man. Later after that Ramanawsky in Russia developed a new method of staining the malaria parasite in blood films and this, together with the improvement of the microscope, made further studies of plasmodia very much easier.

- The Malaria Parasite-

The microorganism causing malaria are commonly referred to as malaria parasite this term is usually restricted to –

Family- plasmodiidae

Order- coccidiida

Sub order- haemosparidiidea

Five species of Plasmodium (single-celled parasites) can infect humans and cause illness:

Plasmodium falciparum (or P. falciparum)

Plasmodium malariae (or P. malariae)

Plasmodium vivax (or P. vivax)

Plasmodium ovale (or P. ovale)

Plasmodium knowlesi (or P. knowlesi)

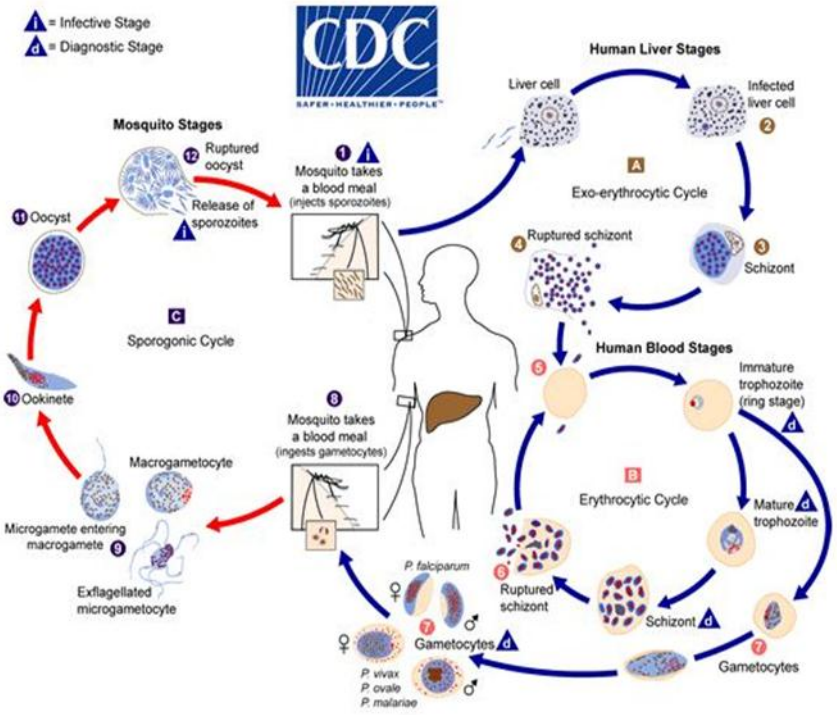
Falciparum malaria is potentially life-threatening. Patients with severe falciparum malaria may develop liver and kidney failure, convulsions, and coma. Although occasionally severe, infections with P. vivax and P. ovale generally cause less serious illness, but the parasites can remain dormant in the liver for many months, causing a reappearance of symptoms months or even years later.

❖ **The life cycle of malaria-**

The natural history of malaria involves cyclical infection of humans and female Anopheles mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood. In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites ("merozoites") that continue the cycle by invading other red cells.

The blood stage parasites are those that cause the symptoms of malaria. When certain forms of blood stage parasites (gametocytes, which occur in male and female forms) are ingested during blood feeding by a female Anopheles mosquito, they mate in the gut of the mosquito and begin a cycle of growth and multiplication in the mosquito. After 10-18 days, a form of the parasite called a sporozoite migrates to the mosquito's salivary glands. When the Anopheles mosquito takes a blood meal on another human, anticoagulant saliva is injected together with the sporozoites, which migrate to the liver, thereby beginning a new cycle.

Thus the infected mosquito carries the disease from one human to another (acting as a "vector"), while infected humans transmit the parasite to the mosquito, In contrast to the human host, the mosquito vector does not suffer from the presence of the parasites.

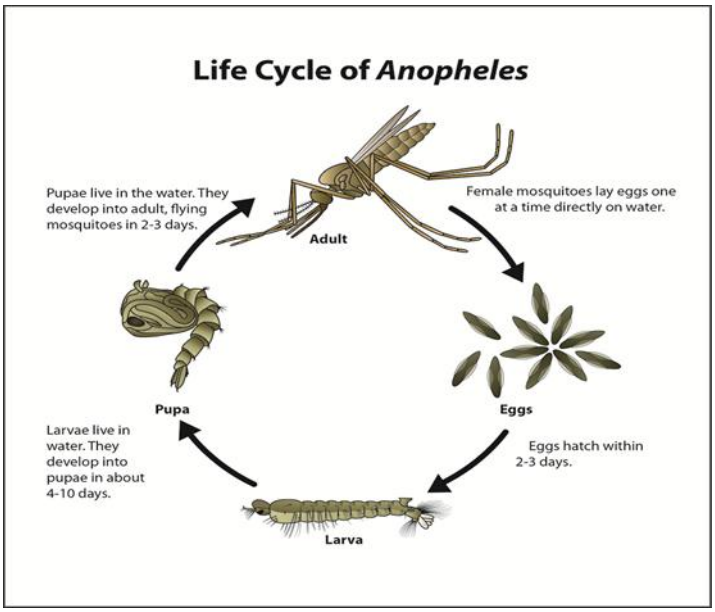


https://www.cdc.gov/malaria/about/biology/index.html#

Fig:1- Life cycle of malaria.

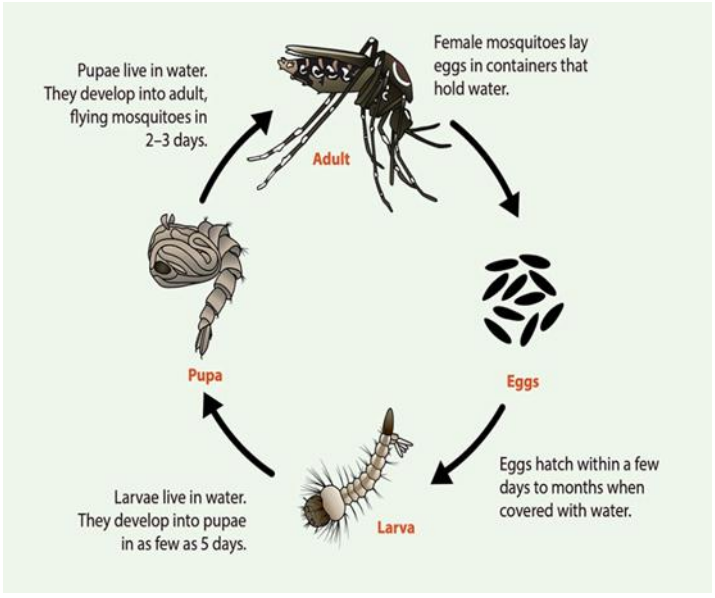
Rearing and maintenance of mosquito

- LIFE cycle of anopheles mosquito-



https://www.cdc.gov/mosquitoes/about/life-cycles/anopheles.html

- Life cycle of ades mosquito-



https://www.cdc.gov/mosquitoes/about/life-cycles/aedes.html

<u>Rearing of mosquito in laboratory is done in the different forms such as larvae, adults and eggs-</u>

1. **Larvae of mosquito-**

2. **Adults –**

The insectary rooms are maintained at 28°C and ~80% humidity, with a 12 hr. day/night cycle. For this procedure, you'll need mosquito cages, 10% sterile sucrose solution, paper towels, beaker, whatman filter paper, glass feeders, human blood and serum, water bath, parafilm, distilled water, clean plastic trays, mosquito food (described below), mosquito net to cover the trays, vacuum, and a collection chamber to collect adults.

Mosquito food:

- Food A: grounded fish food (Aquaricare). A small pinch needs to be added.
- Food B: grounded CAT food (Purina). A small pinch needs to be added.
- Food C: cat food (Purina). Two tablets needs to be added.

Blood: Animal or human blood can be used to rear mosquitoes.

MALARIAL MICROSCOPY

Malaria parasites can be identified by examining under the microscope a drop of the patient's blood, spread out as a “blood smear” on a microscope slide.

- **Washing and preparing slides-**

1. Separate new slides one from the other and soak in detergent solution for 4–8 h,

Conveniently overnight.

2. After soaking, clean each slide on both sides by rubbing the two surfaces in the

Washing cloth .

3. Rinse the slides individually in clean water to wash off the detergent. dry it thoroughly with a clean, lint-free cotton cloth. Always handle slides by the edges. The slide is ready for use.

- **Preparing blood films-**

Preparation of a thin and a thick blood film on the same slide-

After recording the patient’s details on the form or register, wearing protective latex gloves, hold the patient’s left hand, palm facing upwards, and select the third finger from the thumb, called the ring finger.

1. Clean the finger with cotton wool dampened with alcohol. Use firm strokes to remove dirt and oils from the ball of the finger.

2. Dry the finger with a clean cotton cloth, using firm strokes to stimulate blood circulation.

3. using a sterile lancet and a quick rolling action, puncture the ball of the finger or toe.

4. Apply gentle pressure to the finger or toe and express the first drop of blood; wipe it away with dry cotton wool, making sure that no cotton strands remain that might later be mixed with the blood.

5. Apply gentle pressure to the finger and collect a single small drop of blood about this size □ on the middle of the slide. This is for the thin film.

6. Apply further gentle pressure to express more blood, and collect two or three larger drops on the slide, about 1 cm away from the drop intended for the thin film. Wipe the remaining blood off the finger with cotton wool.

7. The thin film: Using another clean slide as a spreader and with the slide with the blood resting on a flat, firm surface, touch the small drop of blood with the edge of the spreader, allowing the blood to run right along the edge. blood to run right along the edge. Firmly push the spreader along the slide, keeping it at an angle of 45o. The edge of the spreader must remain in even contact with the surface of the other slide while the blood is being spread.

8. The thick film: Handling the slides by the edges or a corner, make the blood film by using the corner of the spreader to join the drops of blood, and spread them to make an even, thick film. Do not stir the blood. A circular or rectangular film can be made by three to six quick strokes with the corner of the spreader. The circular thick film should be about 1 cm in diameter.

9. . The thick film should be dried level and be protected from dust, flies, sunlight and extreme heat. from dust, flies, sunlight and extreme heat.

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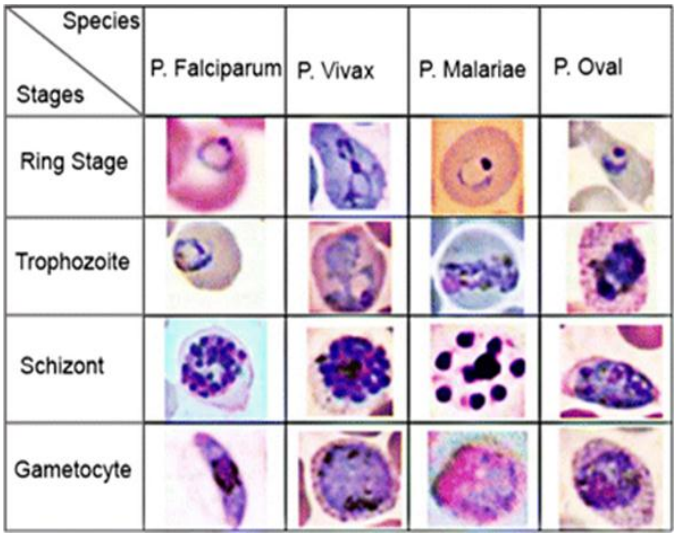
10. Fix the thin film by dabbing it with a pad of cotton wool dampened with methanol or by briefly dipping the film into methanol. Avoid contact between the thick film and methanol, as methanol and its vapours quickly fix the thick film, and it does not stain well

11. Using a test tube or a small container to hold the prepared stain, make up a 10% solution of Giemsa in the buffered water by mixing three drops of Giemsa from the stock solution, using the Pasteur pipette, with 1 ml of buffered water. Each slide needs approximately 3 ml of stain to cover it.

12. pour the stain gently under the staining 4. tray until each slide is covered with stain, or gently pour the stain onto the slides lying face upwards on the plate or rack. Stain the films for 8–10 min. Experience with the stain you are using will help you to decide the exact time needed for good staining.

13. Gently wash the stain from the slide by adding drops from the slide by adding drops of clean water. Do not pour the stain directly off the slides, or the metallic-green surface scum will stick to the film, spoiling it for microscopy. When the stain has been washed away, place the slides in the drying rack, film side downwards, to drain and dry. Ensure that thick films do not scrape the edge of the rack. Wait for 15-20 minutes.

- **Different stages of parasite infection-**



Jan, Z., Khan, A., Sajjad, M. et al. A review on automated diagnosis of malaria parasite in microscopic blood smears images. Multimed Tools Appl 77, 9801–9826 (2018). https://doi.org/10.1007/s11042-017-4495-

MOLECULAR BIOLOGY TECHNIQUES

- **Isolation of plasmid from salivary gland of mosquito by using Qiagen kit-**

Principle- In this type the lysate conditions are adjusted to allow optimal binding of DNA. DNA adsorbed onto silica membrane as silica membrane is positively charged and DNA have negative charge. Salt and pH conditions in the lysate ensure that protein and other

contaminants, which can inhibit PCR and other downstream enzymatic reactions, are

not retained on the silica membrane

Procedure-

1. Firstly, we need mosquito thorax, which we get after dissection of mosquito
2. Take this mosquito thorax in centrifuge tube and add 50ul of ATL (all tissue lysis buffer) in the tube.
3. Now, crush mosquito part with the sterile pestle , after that wash pestle with 130ul of ATL in tube .
4. Take this tube and centrifuge it at 3000 rpm for 30 sec
5. Add 20 µl proteinase K, mix by vortexing and short spin and incubate at 56°C in dry bath until the tissue is completely lysed. To ensure efficient lysis, vortex and spin after every 15 min.
6. Now, take AL buffer of 200 ul and ethanol of 200 ul in the silica column and place this silica column in 1ml collection tube provided in kit.
7. Now, centrifuge at 8000rpm for 1min and after spinning discard the tube which contain filtrate.
8. Carefully open the silica column and add 500 µl Buffer AW1 Close the cap, and centrifuge at (8000 rpm) for 1 min. Place the silica column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.
9. Carefully open the silica column and add 500 µl Buffer AW2 Close the cap and centrifuge at full speed 14,000 rpm for 3 min.
10. Place the silica column in a new 2 ml collection tube not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. This step helps to eliminate the chance of possible Buffer AW2 carryover
11. Place the silica column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the silica column and add 200 µl Buffer AE or distilled water. Incubate at room temperature for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.
12. DNA successfully isolated.

- **DNA Isolation from dried blood spots by Thermofisher method of extraction-**

Principle-

Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard procedure takes less than 20 minutes following cell lysis and yields purified DNA greater than 30 kb in size. Isolated DNA can be used directly in PCR, Southern blotting, and enzymatic reactions.

Procedure-

1. Add 20 µL of Proteinase K Solution to 200 µL of whole blood, then mix by vortexing.

2. Add 400 µL of Lysis Solution, then mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

Note: If using less than 200 µL of blood, adjust sample volume to 200 µL with 1X PBS or TE buffer (not provided). If using larger volumes.

3. Incubate the sample at 56°C for 10 minutes while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed.

4. Add 200 µL of ethanol (96–100%), then mix by pipetting.

5. Transfer the prepared mixture to the spin column, then centrifuge at 6,000 × g for 1 minute. Discard the collection tube containing

the flow-through solution, then place the column into a new 2 mL collection tube (included).

6. Add 500 µL of Wash Buffer WB I (with ethanol added), then centrifuge 8,000 × g for 1 minute. Discard the flow-through and place the

column back into the collection tube.

7. Add 500 µL of Wash Buffer II (with ethanol added) to the column, then centrifuge at 20,000 rpm for 3 minutes.

8. Empty the collection tube, then place the purification column back into the tube. Centrifuge at 20,000 rpm× g for 1 minute.

9. Discard the collection tube containing the flow-through solution, then transfer the column to a sterile 1.5 mL microcentrifuge tube.

10. Add 200 µL of Elution Buffer to the center of the column membrane to elute genomic DNA. Incubate for 2 minutes at room temperature, then centrifuge at 8,000 × g for 1 minute

11. Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20°C.

- **PCR and its different types**-

General Principle of PCR-

The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer mediated enzymes. DNA Polymerase synthesises new strands of DNA complementary to the template DNA. The DNA polymerase can add a nucleotide to the pre-existing 3'-OH group only. Therefore, a primer is required. Thus, more nucleotides are added to the 3' prime end of the DNA polymerase.

Components of PCR-

DNA Template– The DNA of interest from the sample.

DNA Polymerase– Taq Polymerase is used. It is thermostable and does not denature at very high temperatures.

Oligonucleotide Primers- These are the short stretches of single-stranded DNA complementary to the 3' ends of sense and anti-sense strands.

Deoxyribonucleotide triphosphate– These provide energy for polymerization and are the building blocks for the synthesis of DNA. These are single units of bases.

Buffer System– Magnesium and Potassium provide optimum conditions for DNA denaturation and renaturation. It is also important for fidelity, polymerase activity, and stability.

PCR Steps-

he PCR involves three major cyclic reactions:

Denaturation

Denaturation occurs when the reaction mixture is heated to 94℃ for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into a single-stranded DNA.

The single strands now act as a template for the production of new strands of DNA. The temperature should be provided for a longer time to ensure the separation of the two strands.

Annealing

The reaction temperature is lowered to 54-60℃ for around 20-40 seconds. Here, the primers bind to their complementary sequences on the template DNA.

Primers are single-strand sequences of DNA or RNA around 20 to 30 bases in length.

They serve as the starting point for the synthesis of DNA. The two separated strands run in the opposite direction and consequently there are two primers- a forward primer and a reverse primer.

Elongation

At this step, the temperature is raised to 72-80℃. The bases are added to the 3’ end of the primer by the Taq polymerase enzyme

This elongates the DNA in the 5’ to 3’ direction. The DNA polymerase adds about 1000bp/minute under optimum conditions.

Taq Polymerase can tolerate very high temperatures. It attaches to the primer and adds DNA bases to the single strand. As a result, a double-stranded DNA molecule is obtained.

These three steps are repeated 20-40 times in order to obtain a number of sequences of DNA of interest in a very short time period.

General procedure-

A standard polymerase chain reaction (PCR) setup consists of four steps:

1. Add required reagents or mastermix and template to PCR tubes.

2. Mix and centrifuge

3. Amplify per thermo cycler and primer parameters and Evaluate amplified DNA by agarose gel electrophoresis.

Types of PCR-

1. Nested PCR-

Nested polymerase chain reaction involves two sets of primers, used in two successive runs of polymerase chain reaction, the second set intended to amplify a secondary target within the first run product. This allows amplification for a low number of runs in the first round, limiting non-specific products. The second nested primer set should only amplify the intended product from the first round of amplification and not non-specific product. This allows running more total cycles while minimizing non-specific products. This is useful for rare templates or PCR with high background.

The target DNA undergoes the first run of polymerase chain reaction with the first set of primers. The selection of alternative and similar primer binding sites gives a selection of products, only one containing the intended sequence.

The product from the first reaction undergoes a second run with the second set of primers, shown in red. It is very unlikely that any of the unwanted PCR products contain binding sites for both the new primers, ensuring the product from the second PCR has little contamination from unwanted products.

Reagent	Concentration
P mix or master mix	15ul
Forward primer	3ul
Reverse primer	3ul
Nuclease free water	3ul
Template dna	1.5ul
Positive control	1.5ul
Negative control	1.5ul

2. ITS PCR-

It is also know n as internal transcribed sequence PCR. In which the 18s rRNA , conserved part of internal transcribed sequence of mosquito that is amplified by PCR .

Reagent	Concentration
P mix or master mix	15ul
Forward primer(ITS primer)	3ul
Reverse primer(ITS primer)	3ul
Nuclease free water	3ul
Template dna	1.5ul
Positive control	1.5ul
Negative control	1.5ul

3. Multiplex PCR-

Multiplex polymerase chain reaction (Multiplex PCR) refers to the use of polymerase chain reaction to amplify several different DNA sequences simultaneously (as if performing many separate PCR reactions all together in one reaction). This process amplifies DNA in samples using multiple primers and a temperature-mediated DNA polymerase in a thermal cycler. The primer design for all primers pairs has to be optimized so that all primer pairs can work at the same annealing temperature during PCR.

It is used for sub species identification.

Procedure of different types of pcr-

NESTED PCR	ITS PCR	MULTIPLEX PCR
Add 5ul mastermix ,1st set of primer (1ul each).	Add 5ul mastermix, ITS primers(1ul each)	Add mastermix and more than two primers
Add template	Add template	Add template
Mix and centrifuge	Mix and centrifuge	Mix and centrifuge
Amplify by using thermal cycler.	Amplify by using thermal cycler.	Amplify by using thermal cycler.
Amplified DNA used as template now add mastermix and 2nd set of primer	Evaluate amplified DNA by agarose gel electrophoresis	Evaluate amplified DNA by agarose gel electrophoresis
Mix and centrifuge		
Evaluate amplified DNA by agarose gel electrophoresis.		

❖ Gel electrophoresis-

Electrophoresis is a scientific laboratory technique that is used to separate DNA, RNA, or protein molecules based on their size and electrical charge. An electric current is passed through the molecules to move them so that they can be separated via a gel. The pores present in the gel work like a sieve, allowing smaller molecules to pass through more quickly and easily than the larger molecules. According to the way conditions are adjusted during electrophoresis, the molecules can be separated in the desired size range.

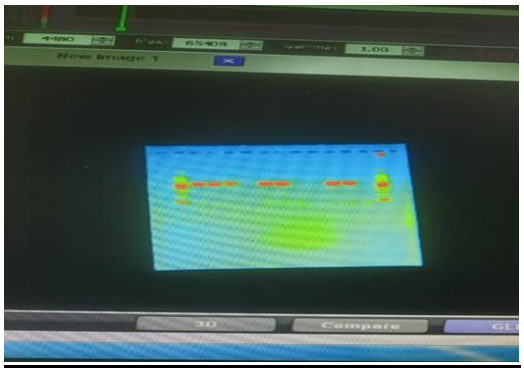
Principle-

The fundamental principle of electrophoresis is the existence of charge separation between the surface of a particle and the fluid immediately surrounding it. An applied electric field acts on the resulting charge density, causing the particle to migrate and the fluid around the particle to flow. The charged molecules are placed at one end of the field according to their charge, and an electric field is applied.

On passing electric current, depending upon the kind of charge the molecules carry, they move towards the opposite electrodes – either cathode (negative electrode) or anode (positive electrode).

Procedure-

1. Preparing of gel- add 3% of agarose powder in 1x of TBE buffer mix it well and heat in microwave for 10-15 min
2. Allow it to cool for few minutes and add 7ul of Syber to it.
3. Pour this solution into the gel cast and allow it to solidify.
4. Remove end blocks and comb and submerged gel under buffer in the electrophoresis chamber
5. Load each sample into the wells and connect leads to power source and initiate electrophoresis.
6. Wait for an hour.
7. After sample ran toward anode take a gel and put it into gel documentation system and observe results . Gel documentation system contain an ultraviolet (UV) or visible (blue or white) transilluminator, a hood to block the samples from external light sources and a high-resolution camera for image capture.



Identification of mosquito

AEDES	ANOPHELES	CULEX
Aedes aegypti, the yellow fever mosquito, is a mosquito that can spread dengue fever, chikungunya, Zika fever, Mayaro and yellow fever viruses, and other disease agents. The mosquito can be recognized by black and white markings on its legs and a marking in the form of a lyre on the upper surface of its thorax. This mosquito originated in Africa, but is now found in tropical, subtropical and temperate regions throughout the world.	Anopheles is a genus of mosquito first described and named by J. W. Meigen in 1818.[2] About 460 species are recognised; while over 100 can transmit human malaria, only 30–40 commonly transmit parasites of the genus Plasmodium, which cause malaria in humans in endemic areas. Anopheles gambiae is one of the best known, because of its predominant role in the transmission of the most dangerous malaria parasite species (to humans) – Plasmodium falciparum.	Culex is a genus of mosquitoes, several species of which serve as vectors of one or more important diseases of birds, humans, and other animals. The diseases they vector include arbovirus infections such as West Nile virus, Japanese encephalitis, or St. Louis encephalitis, but also filariasis and avian malaria.

Dissection of mosquito

The mosquito midgut and salivary glands are key entry and exit points for pathogens such as Plasmodium parasites. Therefore dissection of mosquito is impotant to observe different infected stages of plasmodium such as sporozites in salivary glands and oocytes in midgut. It is observe under the Phase contrast microscope.

Procedure-

Dissection of midgut and salivary gland tissues from mosquitoes requires prior preparation of 1X Phosphate Buffered Saline (1X PBS) solution and anesthetization of mosquitoes by subjecting to a temperature of 4°C, until immobilized. The mosquitoes remain anesthetized by placing in a Petri dish that is kept cold on ice. Other materials required include: light microscope fitted with 10x objective, pipettor, fine-tipped forceps, glass slide, needle-tip probes.

Salivary gland dissection

1.Place a drop of 1X PBS onto a glass slide mounted under a light microscope.

2Pick up a mosquito by stabbling the thorax with a needle-tip probe.

Pull off mosquito legs using your fingers.

Transfer the mosquito onto the slide.

Remove the head of the mosquito using forceps.

While holding down the mosquito thorax with the probe, use another probe to gently push down on the thorax.

The salivary glands are located at the anterior portion of the thorax and can be isolated by using a needle-tip probe and severing the attachments that connect the gland to the thorax. Intact salivary glands are comprised of three lobes: two lateral lobes and a medial lobe.

Midgut dissection

Place a drop of 1X PBS onto a glass slide mounted under the light microscope.

Transfer a mosquito onto the prepared slide by stabbing the mosquito thorax with a needle-tip probe.

While holding down the mosquito with the probe, use the forceps to grasp the second to the last abdominal segment and gently pull off the mosquito abdomen in a single motion. The midgut should remain attached to the immobilized thorax.

Discard the abdomen. Use the forceps to detach the midgut from the thorax.

Add mercurochrome stain and observe under phase contrast microscope.

Bibliography

1. 1.Protocol for Mosquito Rearing (A. gambiae) Suchismita Das, Lindsey Garver, and George Dimopoulos
2. https://www.cdc.gov/malaria/about/biology/index.html
3. Malaria Microscopy Quality Assurance Manual – Version 2https://www.who.int › docs › publications › gmp
4. file:///C:/Users/HOME/Downloads/HB-0329-004-1102728-HB-QIAamp-DNA-Mini-Blood-Mini-0516-WW.pdf
5. https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2FMAN0012667_GeneJET_Whole_Blood_Genomic_DNA_Purification_Mini_Kit_UG.pdf
6. Dissection of Midgut and Salivary Glands from Ae. aegypti Mosquitoes Judy Coleman, 1 Jennifer Juhn, 1 and Anthony A. James 2.