MOLECULAR AND VECTOR DIAGNOSTIC TRAINING REPORT

Wolbachia Detection, Leptospirosis Surveillance,

Insecticide Resistance Testing and Rodent Biorepository Analysis

LABORATORY INTERNSHIP REPORT

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SEMARANG

2026

ACKNOWLEDGMENT

I would like to express my sincere gratitude to Dr. Martini for her guidance, supervision, and valuable support throughout my 14-day laboratory internship at Salatiga National Laboratory. Her mentorship greatly contributed to the successful completion of my practical training.

My deepest appreciation is also extended to the Faculty of Public Health, Diponegoro University, for designing this special academic internship program and for providing continuous support during my placement in Salatiga until I successfully completed my practical training.

I would also like to thank the General Administration of Salatiga National Laboratory for granting me the opportunity to conduct my internship and for facilitating a conducive learning environment.

My special thanks are extended to the Heads of Departments who supervised and guided me in their respective laboratories during the internship period:

1. Head of Molecular Laboratory

2. Head of Insecticide Resistance Laboratory

3. Head of Biorepository Laboratory

4. Head of Mosquito Colony Laboratory

Their professional guidance, technical instruction, and encouragement enriched my knowledge in molecular diagnostics, vector biology, and epidemiological surveillance.

Finally, I am grateful to all laboratory staff and colleagues who supported me during this training. This internship has significantly strengthened my practical competence and professional development in the field of molecular epidemiology and public health.

Semarang, 2026

Deng Manyang Anyieth Kuot

APPROVAL SHEET

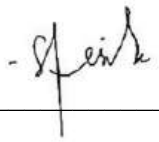
This laboratory internship report entitled:

"Molecular and Vector Diagnostic Training at Salatiga National Laboratory"

Has been examined and approved by:

Supervisor:

Dr. Martini

Signature: 

Date: 23 April 2026

Head of Laboratory:

_____

Signature: _____

Date: _____

LAB INTERNSHIP SUMMARY REPORT

Salatiga National Laboratory

Title: Molecular Diagnostics, Vector Surveillance, and Rodent Biorepository Activities

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Supervisor: Dr. Martini

Institution: Salatiga National Laboratory

Location: Salatiga

Duration: 2 – 16 February 2026

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A. Introduction

This 14-day internship was conducted from 2–16 February 2026 at Salatiga National Laboratory under the supervision of Dr. Martini. The program was designed to strengthen practical competencies in molecular diagnostics, vector surveillance, insecticide resistance monitoring, and zoonotic disease detection within a One Health framework.

The internship integrated laboratory-based molecular techniques with epidemiological surveillance principles. Activities included pathogen detection using PCR and Real-Time PCR, mosquito bioassays for insecticide resistance and repellent efficacy, and rodent biorepository surveillance for leptospirosis risk assessment.

Beyond the technical training, this internship was a deeply human experience. Each day involved collaboration with laboratory technicians, researchers, and fellow trainees. I observed how senior scientists mentored junior staff, discussed findings openly, and connected laboratory results to public health decision-making.

Handling mosquito specimens, extracting DNA from serum samples, and observing microscopic agglutination reactions were not merely procedural tasks—they represented real public health concerns affecting communities. Understanding that laboratory data contribute to disease prevention, outbreak response, and vector control strategies gave meaning to every experiment performed.

The integration of molecular diagnostics with vector surveillance and rodent monitoring highlighted the One Health approach in practice. It became clear that controlling infectious diseases requires cooperation between human health laboratories, environmental monitoring systems, and animal surveillance programs.

Professional and Academic Growth

This internship strengthened my practical laboratory competence while reinforcing epidemiological concepts learned during my academic training. It improved my technical skills in molecular diagnostics, vector identification, and resistance testing, while also developing soft skills such as discipline, communication, teamwork, and scientific responsibility.

Overall, the 14-day experience at Salatiga National Laboratory was not only a technical training period but also a transformative professional journey. It provided real-world exposure to public health laboratory systems and deepened my understanding of how laboratory science supports disease surveillance and prevention within the One Health framework.

B. Molecular Diagnostic Activities

1 Detection of Wolbachia in Aedes aegypti

1.1. Source of Mosquito Samples

The *Aedes aegypti* mosquitoes used in this experiment were obtained from a maintained laboratory colony at the Salatiga National Laboratory. The colony was reared under controlled environmental conditions (approximately 25–27°C, 70–80% relative humidity, and a 12:12 light-dark cycle). Larvae were fed with standard larval feed, and adult mosquitoes were maintained with a sugar solution.

For molecular analysis, adult female mosquitoes (3–5 days old) were selected. Female mosquitoes were chosen because they are the primary vectors responsible for dengue and other arboviral transmission. Prior to DNA extraction, the mosquitoes were immobilized by brief cold exposure to facilitate handling and prevent damage to body structures.

1.2 DNA Extraction Using Squash Buffer Method

The detection of *Wolbachia* began with DNA extraction using the squash buffer method, a simple and rapid technique suitable for mosquito tissue.

Step-by-Step Procedure:

1. **Sample Preparation**
   Individual mosquitoes were placed in sterile microcentrifuge tubes (1.5 mL). For this procedure, either the whole mosquito or the abdomen was used, depending on protocol requirements.
2. **Addition of Squash Buffer**
   Approximately 50–100 µL of squash buffer solution (containing Tris-HCl, EDTA, NaCl, and Proteinase K) was added to each tube.

   i. **Tris-HCl** maintains pH stability.
   ii. **EDTA** protects DNA by inhibiting nucleases.
   iii. **Proteinase K** digests proteins and facilitates cell lysis.

3. **Mechanical Homogenization**
   The mosquito tissue was crushed using a sterile micropestle until completely homogenized. This mechanical disruption helps release intracellular components, including bacterial DNA.

4. **Incubation (15 Minutes)**
   The homogenized samples were incubated at approximately 55°C for 15 minutes.

   i. This incubation allows Proteinase K to digest proteins effectively.
   ii. It promotes complete cell lysis of both mosquito cells and intracellular *Wolbachia* bacteria.

5. **Enzyme Inactivation**
   After incubation, the samples were heated at 95°C for about 5 minutes to inactivate Proteinase K.
6. **Centrifugation**
   The tubes were centrifuged briefly to pellet debris. The supernatant containing extracted DNA was carefully transferred to a new sterile tube and used as the DNA template for PCR.

This extraction method is rapid and cost-effective, suitable for screening large numbers of mosquito samples in vector control programs.

1.3 Preparation of PCR Master Mix

PCR (Polymerase Chain Reaction) was performed to amplify *Wolbachia*-specific gene targets (commonly the wsp gene or 16S rRNA gene).

The PCR master mix was prepared in a clean area to avoid contamination. The typical PCR mixture (per reaction) included:

a. PCR buffer
b. $MgCl_2$ (if not included in buffer)
c. dNTPs (deoxynucleotide triphosphates)
d. Forward primer (specific to *Wolbachia*)
e. Reverse primer
f. Taq DNA polymerase
g. Nuclease-free water
h. DNA template (added separately)

The master mix was prepared in bulk according to the number of samples plus additional volume to compensate for pipetting error.

1.4 PCR Templating

After preparing the master mix:

1. PCR tubes were labeled clearly.

2. A measured volume of master mix was aliquoted into each PCR tube.
3. Extracted DNA template (usually 1–2 µL) was added to each tube.
4. Positive control (known *Wolbachia*-infected DNA) and negative control (nuclease-free water) were included to validate the results.

The tubes were then briefly centrifuged to ensure all reagents collected at the bottom and placed in the PCR machine.

1.5 Real-Time PCR Procedure (Duration: ~1 Hour 30 Minutes)

Real-Time PCR (qPCR) was performed to detect and quantify *Wolbachia* DNA.

Thermal Cycling Conditions (Typical Example):

1. **Initial Denaturation** – 95°C for 3–5 minutes
2. **Amplification Cycles (40 cycles):**

    a. Denaturation – 95°C for 15 seconds
    b. Annealing – 55–60°C for 30 seconds
    c. Extension – 72°C for 30 seconds

Fluorescent dye (e.g., SYBR Green) binds to double-stranded DNA, and fluorescence increases proportionally as amplification progresses.

The total run time was approximately 1 hour and 30 minutes.

The Real-Time PCR machine continuously monitored fluorescence signals, generating amplification curves for each sample.

1.6 Interpretation of Results

The interpretation of Real-Time PCR results was conducted using amplification plots and Ct (Cycle threshold) values.

1. Positive Result:

a. Clear exponential amplification curve.
b. Ct value typically below 35 cycles (depending on protocol).
c. Positive control must also show amplification.

2. Negative Result:

a. No amplification curve.

b. Undetermined Ct value or Ct above the cut-off.
c. Negative control must show no amplification (ensuring no contamination).

3. Quality Control:

a. If the negative control amplifies, contamination is suspected and the run is invalid.
b. If the positive control fails to amplify, reagents or thermal cycling conditions must be reviewed.

1.7 Honest Reflection on the Procedure

During the procedure, careful attention was required to prevent cross-contamination, especially during master mix preparation and DNA templating. Small errors in pipetting could significantly affect Ct values. Maintaining sterile technique and working systematically were essential.

The incubation step was critical for effective DNA extraction. Incomplete incubation could result in low DNA yield, affecting PCR sensitivity. Additionally, including proper controls ensured reliability of results.

Overall, the detection of *Wolbachia* in *Aedes aegypti* demonstrated how molecular tools support vector control strategies. Identifying *Wolbachia*-infected mosquitoes is important in programs aiming to reduce dengue transmission through biological control methods.

This procedure enhanced my practical skills in molecular diagnostics and strengthened my understanding of how laboratory findings contribute to public health interventions under the One Health framework.

DNA extraction was performed using the squash buffer method followed by Real-Time PCR targeting the wsp gene with RpS17 as internal control. Melt curve analysis and Ct value interpretation were conducted to determine infection density.

2.2 Detection of Leptospira spp. from Human Serum

The detection of *Leptospira* spp. from human serum was performed using conventional PCR targeting specific pathogenic genes (commonly **lipL32**, which is specific to pathogenic *Leptospira*). This method enables molecular confirmation of infection, particularly during the early phase of disease when antibodies may not yet be detectable.

1 Sample Collection and Preparation

Human serum samples were obtained from suspected leptospirosis cases. The blood had previously been centrifuged to separate serum from whole blood. Serum samples were stored at 2–8°C for short-term use or −20°C for longer storage prior to DNA extraction.

Strict biosafety procedures were followed because *Leptospira* is a zoonotic pathogen.

2. DNA Extraction from Human Serum

DNA extraction was performed to isolate bacterial DNA present in the serum.

Step-by-Step Procedure:

1. **Aliquoting Serum**
   Approximately 200 µL of human serum was transferred into a sterile microcentrifuge tube.
2. **Addition of Lysis Buffer and Proteinase K**
   Lysis buffer was added to break open bacterial cells. Proteinase K was included to digest proteins and release DNA.
3. **Incubation**
   The mixture was incubated at 56°C for 10–15 minutes to enhance cell lysis and protein digestion.
4. **Binding Step (Spin Column Method, if kit used)**
   Ethanol was added to promote DNA binding to the silica membrane in the spin column.
   The mixture was transferred into the column and centrifuged.
5. **Washing Steps**
   Wash buffers were applied to remove impurities and inhibitors that could interfere with PCR.
6. **Elution**
   DNA was eluted using 50–100 µL of elution buffer and stored at −20°C until PCR amplification.

This extraction ensured purification of bacterial DNA while minimizing PCR inhibitors commonly present in serum.

3. Preparation of Conventional PCR Master Mix

The conventional PCR mixture was prepared in a clean area to avoid contamination.

Components per Reaction:

a. PCR buffer (with $MgCl_2$)
b. dNTP mix
c. Forward primer (specific for *Leptospira* gene, e.g., lipL32)
d. Reverse primer
e. Taq DNA polymerase
f. Nuclease-free water
g. DNA template (added separately)

The master mix was prepared in bulk according to the total number of reactions, including positive and negative controls.

4. PCR Templating

1. PCR tubes were labeled clearly.
2. Master mix was aliquoted into each PCR tube.
3. 2–5 µL of extracted DNA template was added into each corresponding tube.
4. A **positive control** (known *Leptospira* DNA) was included.
5. A **negative control** (nuclease-free water) was included to check contamination.

The tubes were gently mixed and briefly centrifuged to collect contents at the bottom.

5. Running Conventional PCR

The PCR tubes were placed into the thermal cycler.

Typical Thermal Cycling Conditions:

1. **Initial Denaturation:** 95°C for 3–5 minutes
2. **35–40 Cycles of:**

    a. Denaturation: 95°C for 30 seconds
    b. Annealing: 55–60°C for 30 seconds (depending on primer Tm)
    c. Extension: 72°C for 45–60 seconds

3. **Final Extension:** 72°C for 5–7 minutes
4. **Hold:** 4°C

The total run time was approximately 1.5–2 hours.

After amplification, PCR products were stored temporarily at 4°C before gel electrophoresis.

6. Agarose Gel Preparation and Electrophoresis

To visualize PCR products, agarose gel electrophoresis was performed.

Step 1: Preparing Agarose Gel

1. **Weighing Agarose**
   1–2 grams of agarose powder was weighed depending on desired concentration (usually 1–2% gel).
2. **Adding Buffer**
   Agarose was mixed with 1× TAE or TBE buffer in a heat-resistant flask.
3. **Heating Using Microwave**
   The mixture was heated in a microwave until the agarose dissolved completely and the solution became clear.
   Care was taken to avoid boiling over.
4. **Cooling and Adding Stain**
   The solution was allowed to cool to about 50–60°C. DNA stain (e.g., ethidium bromide or safer alternative) was added and mixed gently.
5. **Casting the Gel**
   The agarose solution was poured into a casting tray with a comb inserted to form wells.
   The gel was left to solidify (about 20–30 minutes).

Step 2: Loading and Running the Gel

1. The gel was placed into the electrophoresis tank.
2. Running buffer (TAE/TBE) was added until the gel was submerged.
3. PCR products were mixed with loading dye.
4. DNA ladder (molecular weight marker) was loaded into one well.
5. PCR samples were loaded into separate wells.

Electrophoresis was run at 80–120 volts for approximately 30–45 minutes, allowing DNA fragments to migrate through the gel matrix.

7.Visualization

After electrophoresis:

a. The gel was placed on a UV transilluminator or gel documentation system.
b. DNA bands were visualized as fluorescent bands under UV light.
c. Images were captured for documentation.

8. Interpretation of Results

1. Positive Result:

a. Presence of a clear DNA band at the expected size (e.g., ~242 bp for lipL32 gene).
b. Positive control must show correct band.

2. Negative Result:

a. No visible band at expected size.
b. Negative control must show no band.

3. Invalid Result:

a. If the negative control shows amplification → contamination suspected.
b. If the positive control fails → PCR failure or reagent issue.

9. Honest Reflection

The detection of *Leptospira* spp. from human serum required strict attention to contamination control because PCR is highly sensitive. Proper separation of pre-PCR and post-PCR areas was essential.

Serum samples sometimes contain inhibitors; therefore, effective DNA extraction and washing steps were crucial. During gel preparation, careful microwave heating prevented uneven melting or bubble formation in the gel.

Seeing the expected DNA band under UV light was a significant confirmation of successful amplification. This procedure demonstrated the importance of molecular diagnostics in early detection of leptospirosis, supporting timely clinical management and epidemiological surveillance under the One Health framework.

2.3 Detection of Brugia spp. in Culex quinquefasciatus

Culex quinquefasciatus

The detection of *Brugia* spp. in *Culex quinquefasciatus* was performed using conventional PCR to identify filarial parasite DNA within mosquito vectors. This molecular detection supports lymphatic filariasis surveillance and helps evaluate transmission risk in endemic settings.

1 Mosquito Collection and Species Background

Mosquitoes were obtained from a maintained laboratory colony. Although the focus of this experiment was *Culex quinquefasciatus*, the laboratory colony contained six *Culex* species for training and comparative identification purposes:

1. *Culex quinquefasciatus*
2. *Culex pipiens*
3. *Culex tritaeniorhynchus*
4. *Culex vishnui*
5. *Culex gelidus*
6. *Culex sitiens*

Among these, *Culex quinquefasciatus* is an important vector of *Brugia malayi* in certain endemic regions.

Adult female mosquitoes (3–7 days old) were selected for molecular analysis. Female mosquitoes were chosen because they are responsible for parasite transmission. Prior to processing, mosquitoes were immobilized using cold treatment and morphologically confirmed under a stereomicroscope to ensure correct species identification.

2. DNA Extraction from Mosquito Samples

DNA extraction was performed to detect parasite DNA within mosquito tissues.

Step-by-Step Procedure:

1. **Sample Preparation**
   Individual mosquitoes were placed into sterile 1.5 mL microcentrifuge tubes.
2. **Addition of Lysis Buffer**
   Approximately 100 µL of lysis buffer containing Tris-HCl, EDTA, NaCl, and Proteinase K was added.
3. **Mechanical Homogenization**
   Mosquitoes were crushed using sterile micropestles until fully homogenized.
4. **Incubation**
   Samples were incubated at 55–56°C for 15–30 minutes to promote digestion of proteins and release of DNA from both mosquito tissues and any *Brugia* larvae present.
5. **Enzyme Inactivation**
   Tubes were heated at 95°C for 5 minutes to inactivate Proteinase K.
6. **Centrifugation**
   Debris was pelleted, and the supernatant containing DNA was transferred into a new sterile tube.

Extracted DNA was stored at −20°C until PCR analysis.

3. Preparation of Conventional PCR Master Mix (Contextual Explanation)

Conventional PCR was used to amplify *Brugia*-specific gene targets (commonly ITS2 or HhaI repeat region).

The preparation of PCR master mix is a critical step because it ensures uniformity across all samples and reduces pipetting errors.

Components and Their Functions:

1. **PCR Buffer**: Maintains optimal pH and ionic strength for enzyme activity.
2. **$MgCl_2$**: Essential cofactor for Taq polymerase; influences amplification efficiency.
3. **dNTPs (A, T, G, C)**: Building blocks for new DNA strand synthesis.
4. **Forward and Reverse Primers**: Short DNA sequences designed to bind specifically to *Brugia* DNA regions.
5. **Taq DNA Polymerase**: Heat-stable enzyme that synthesizes new DNA strands.
6. **Nuclease-Free Water**: Adjusts final reaction volume.

The master mix was prepared in a clean PCR preparation area. The volume was calculated based on the number of samples plus positive and negative controls, including extra volume to compensate for pipetting loss.

DNA template was added separately to avoid cross-contamination.

4. PCR Templating

1. PCR tubes were labeled clearly.
2. Equal volumes of master mix were aliquoted into each tube.
3. 2–5 µL of extracted mosquito DNA was added to corresponding tubes.
4. A **positive control** (known *Brugia* DNA) was included.
5. A **negative control** (nuclease-free water) was included.

Tubes were gently mixed and briefly centrifuged before placing into the thermal cycler.

5. Running Conventional PCR

The PCR amplification was performed under the following typical cycling conditions:

1. **Initial Denaturation**: 95°C for 3–5 minutes
2. **35 Cycles of:**
    i. Denaturation: 95°C for 30 seconds
    ii. Annealing: 55–60°C for 30 seconds
    iii. Extension: 72°C for 45 seconds
3. **Final Extension**: 72°C for 5–7 minutes
4. **Hold**: 4°C

The total run time was approximately 1.5–2 hours.

6.Agarose Gel Electrophoresis

To confirm amplification:

Gel Preparation

1. 1.5–2% agarose was prepared by dissolving agarose powder in 1× TAE buffer.
2. The mixture was heated using a microwave until completely dissolved.
3. After cooling to ~50°C, DNA stain was added.
4. The gel was poured into a casting tray with comb inserted and allowed to solidify.

Running the Gel

1. The gel was placed into the electrophoresis tank.
2. Running buffer was added to submerge the gel.
3. PCR products were mixed with loading dye and loaded into wells.
4. DNA ladder was loaded in one lane.
5. Electrophoresis was conducted at 100 volts for 30–45 minutes.

7. Visualization and Interpretation

The gel was examined under UV transillumination.

Positive Result:

a. Clear band at expected size (e.g., ~320 bp depending on target gene).
b. Positive control shows correct band.

Negative Result:

a. No band at expected size.
b. Negative control shows no amplification.

Invalid Result:

a. Band present in negative control → contamination suspected.
b. No band in positive control → PCR failure.

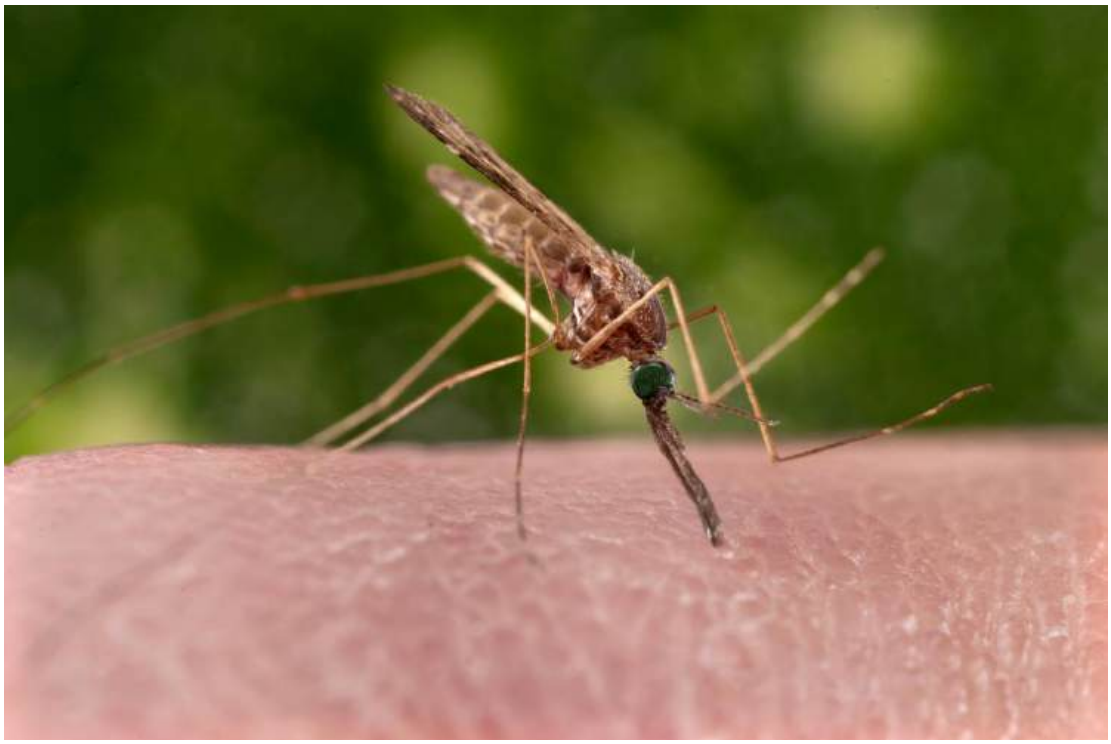
8. Honest Reflection

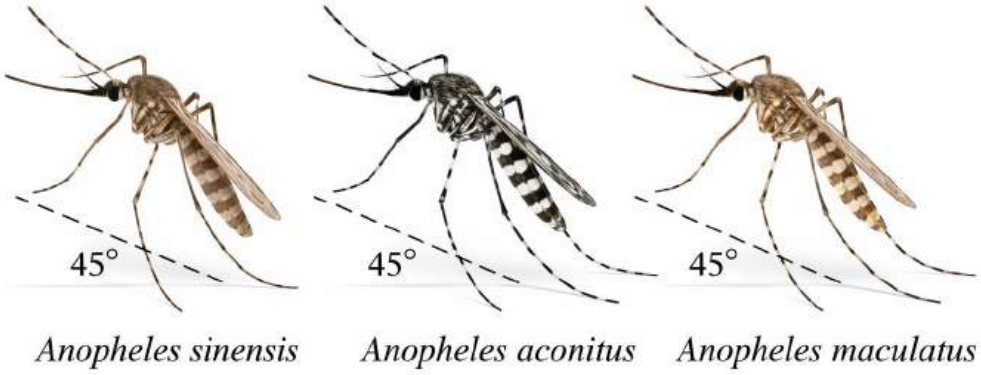
Detecting *Brugia* spp. in *Culex quinquefasciatus* demonstrated the importance of molecular xenomonitoring in lymphatic filariasis surveillance. Careful mosquito identification was essential to avoid species misclassification.

The preparation of PCR master mix required precision, especially in measuring $MgCl_2$ and primers, as small variations could affect amplification specificity.

Gel electrophoresis provided clear visual confirmation of results, and the inclusion of controls ensured reliability. This procedure highlighted how entomological surveillance combined with molecular tools supports disease elimination programs within the One Health framework.

2.4 Detection of VGSC Gene in *Anopheles* Mosquitoes (Insecticide Resistance Study)





The detection of the **Voltage-Gated Sodium Channel (VGSC) gene** in *Anopheles* mosquitoes was performed to investigate potential knockdown resistance (kdr) mutations associated with pyrethroid and DDT resistance. The VGSC gene is an important molecular marker used in vector control surveillance programs.

1. Mosquito Species Used

Three *Anopheles* species obtained from the laboratory colony were analyzed:

1. ***Anopheles sinensis*** – Common in Asia and associated with malaria transmission.
2. ***Anopheles aconitus*** – Found in Southeast Asia, often linked to rural malaria transmission.
3. ***Anopheles maculatus*** – A known malaria vector in forested and hilly areas.

Adult female mosquitoes (3–5 days old) were selected because they are responsible for malaria transmission and insecticide exposure in the field.

Each mosquito was morphologically identified under a stereomicroscope prior to molecular analysis to ensure species accuracy.

2. DNA Extraction from Mosquito Samples

DNA extraction was performed to isolate genomic DNA containing the VGSC gene.

Step-by-Step Procedure:

1. **Sample Preparation**
   Individual mosquitoes were placed into sterile 1.5 mL microcentrifuge tubes.
2. **Addition of Lysis Buffer**
   Approximately 100 µL of lysis buffer (containing Tris-HCl, EDTA, NaCl, and Proteinase K) was added.
3. **Homogenization**
   The mosquito was crushed using a sterile micropestle until completely homogenized.
4. **Incubation**
   The homogenate was incubated at 55–56°C for 15–30 minutes.
   - This step allows Proteinase K to digest proteins and release genomic DNA.
5. **Enzyme Inactivation**
   Samples were heated at 95°C for 5 minutes to inactivate Proteinase K.
6. **Centrifugation**
   Tubes were centrifuged briefly to pellet debris.
   The supernatant containing DNA was transferred into a new sterile tube.

Extracted DNA was stored at −20°C until PCR amplification.

3. Preparation of Conventional PCR Master Mix (VGSC Gene Amplification)

Conventional PCR was used to amplify a fragment of the VGSC gene, typically targeting regions where kdr mutations occur (e.g., codon 1014).

PCR Master Mix Components and Explanation:

- **PCR Buffer** – Provides optimal pH and salt conditions.
- **$MgCl_2$** – Required cofactor for Taq polymerase activity.
- **dNTPs** – Nucleotide building blocks for DNA synthesis.
- **Forward Primer** – Specifically binds upstream of the VGSC target region.
- **Reverse Primer** – Binds downstream of the target region.
- **Taq DNA Polymerase** – Heat-stable enzyme for DNA amplification.

- **Nuclease-Free Water** – Adjusts final reaction volume.

The master mix was prepared in a PCR clean area to prevent contamination. Volumes were calculated based on the number of samples, including positive and negative controls.

DNA template was added separately after distributing master mix into PCR tubes.

4. PCR Templating

1. PCR tubes were labeled according to species and sample number.
2. Master mix was aliquoted equally into each tube.
3. 2–5 µL of extracted DNA was added to each tube.
4. Positive control (known VGSC-positive DNA) was included.
5. Negative control (nuclease-free water) was included.

Tubes were gently mixed and briefly centrifuged before placing into the thermal cycler.

5. Running Conventional PCR

The PCR tubes were loaded into the thermal cycler with the following typical cycling conditions:

Thermal Cycling Profile:

1. **Initial** **Denaturation:**
   95°C for 3–5 minutes
2. **35 Cycles of:**

   I. Denaturation: 95°C for 30 seconds
   II. Annealing: 55–60°C for 30 seconds (depending on primer melting temperature)
   III. Extension: 72°C for 45–60 seconds

3. **Final** **Extension:**
   72°C for 5–7 minutes
4. **Hold:**
   4°C

The total PCR run time was approximately 1.5–2 hours.

6. Agarose Gel Electrophoresis

To confirm amplification of the VGSC gene fragment, agarose gel electrophoresis was performed.

Gel Preparation:

1. 1.5–2% agarose powder was weighed.
2. Mixed with 1× TAE buffer.
3. Heated using a microwave until fully dissolved.
4. Cooled to ~50°C before adding DNA stain.
5. Poured into casting tray with comb inserted.
6. Allowed to solidify for 20–30 minutes.

Running the Gel:

1. Gel placed into electrophoresis tank.
2. Running buffer added.
3. PCR products mixed with loading dye.
4. DNA ladder loaded in one well.
5. Samples loaded into separate wells.
6. Electrophoresis run at 100 volts for 30–45 minutes.

7. Visualization and Interpretation

The gel was visualized under UV transillumination.

Expected Result:

- A clear DNA band at the expected fragment size (commonly ~200–300 bp depending on primer design).

Interpretation:

Positive Amplification:

a. Clear band at correct size.
b. Positive control shows band.
c. Negative control shows no band.

No Amplification:

a. No visible band.

b. Could indicate absence of target gene fragment or PCR failure.

Contamination:

- Band present in negative control → results invalid.

Further sequencing or allele-specific PCR may be required to confirm specific kdr mutations (e.g., L1014F or L1014S).

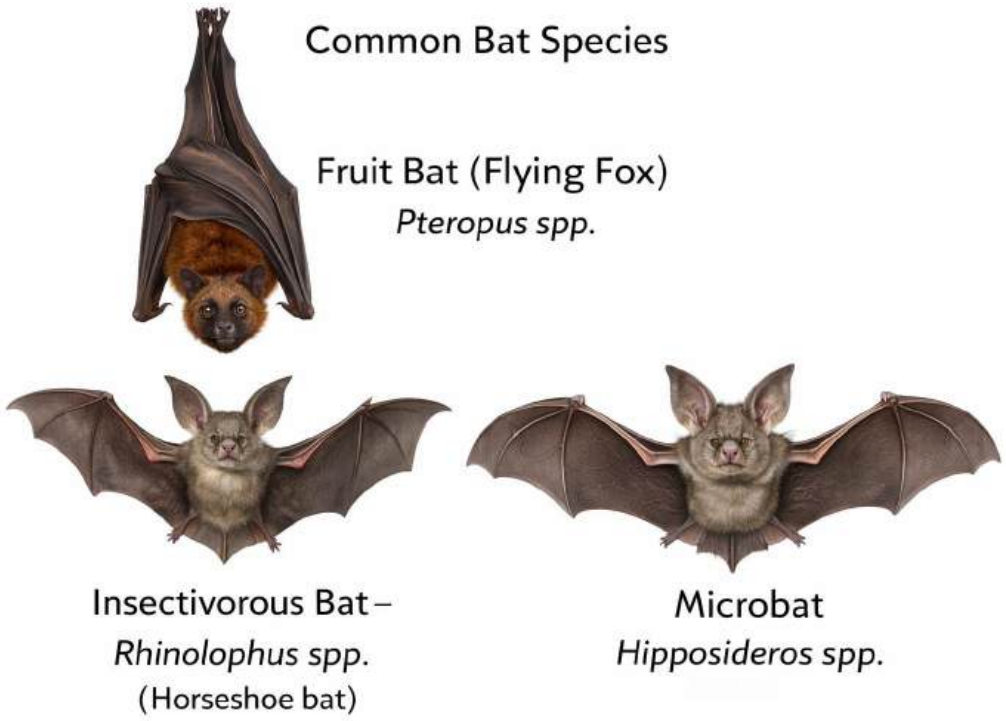
8. Honest Reflection

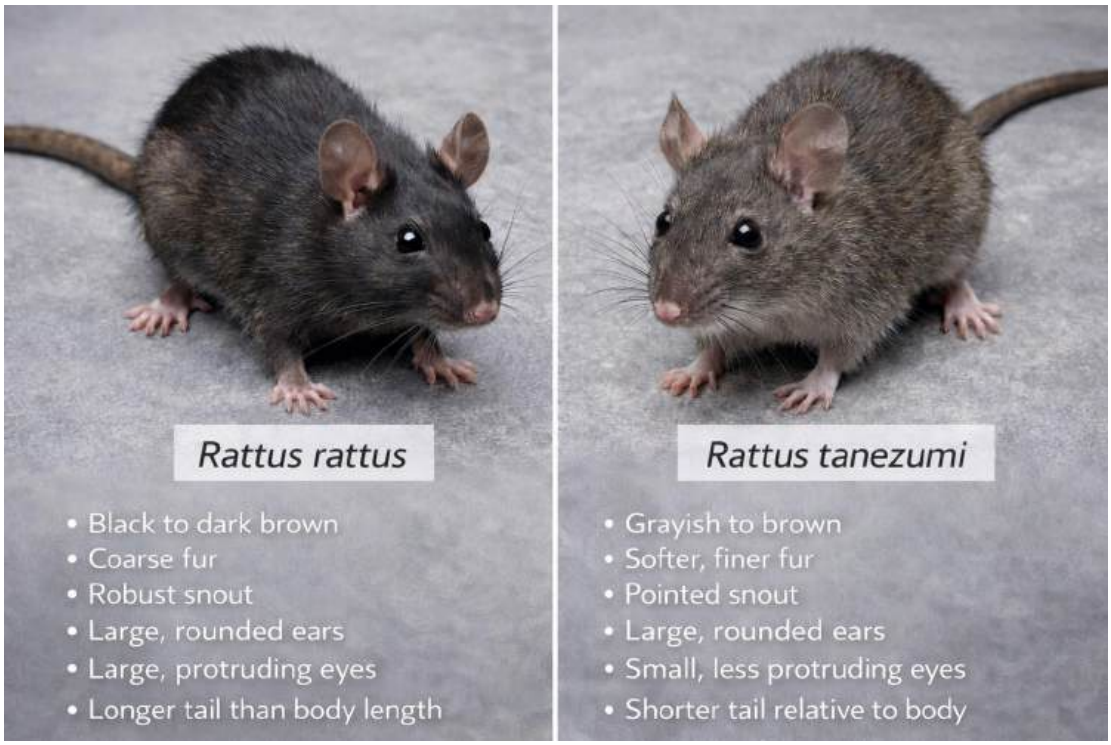
Detection of the VGSC gene in *Anopheles sinensis*, *Anopheles aconitus*, and *Anopheles maculatus* provided practical understanding of molecular resistance monitoring.

Precise pipetting and contamination control were critical throughout the process. Minor errors in primer concentration or annealing temperature could affect amplification specificity.

This procedure demonstrated how molecular diagnostics contribute to insecticide resistance surveillance. Identifying VGSC mutations is essential for guiding vector control strategies, ensuring effective insecticide selection, and supporting malaria control programs within the One Health and integrated vector management framework.

C.Vector Control and Biorepository Surveillance of Rodents and Bats





Vector control activities in the biorepository laboratory focused on **rodents and bats**, which together represent nearly **60% of all mammalian species globally**. Rodents alone account for about 40–45% of mammal species, while bats contribute approximately 20%. Their high diversity, adaptability, and close association with human settlements make them important reservoirs of zoonotic pathogens.

The biorepository laboratory plays a key role in collecting, identifying, preserving, and testing these animals for pathogens that may threaten public health.

1. Importance of Rodents and Bats in Public Health

Rodents and bats are significant because:

a. They live close to human environments.
b. They reproduce rapidly.
c. They can carry multiple zoonotic pathogens.
d. They may serve as reservoirs without showing clinical signs.

Common Rodent-Associated Diseases:

a. Leptospirosis (*Leptospira* spp.)
b. Hantavirus infection
c. Plague (*Yersinia pestis*)
d. Salmonellosis
e. Rat-bite fever

Common Bat-Associated Diseases:

a. Rabies virus
b. Nipah virus
c. Coronaviruses
d. Histoplasmosis (fungal exposure from guano)

The biorepository laboratory supports surveillance by collecting specimens and preserving tissues for molecular and serological testing.

2. Identification of Rats in the Laboratory

Accurate species identification is essential because disease risk differs by species.

Rodent identification is based on:

a. Body size and weight
b. Tail length relative to body
c. Ear size
d. Fur color
e. Skull morphology
f. Mammary gland position
g. Geographic location

3. Comparison: *Rattus rattus* vs *Rattus tanezumi*

Two commonly encountered species in Southeast Asia include:

1 *Rattus rattus* (Black Rat)

a. Slender body
b. Tail longer than body
c. Large ears
d. Pointed snout

e. Usually found in roofs, ceilings, trees
f. More arboreal behavior

2 *Rattus tanezumi* (Asian House Rat)

a. Similar body shape to *R. rattus*
b. Slightly shorter tail relative to body
c. Adapted to urban and peri-urban environments
d. Often found inside houses and markets
e. Highly associated with human settlements

Key Difference:
While morphologically similar, *R. tanezumi* is more strongly associated with dense urban environments and may have higher exposure to food contamination sources.

Because of their similarity, molecular confirmation may sometimes be required for accurate differentiation.

4. Measuring Disease Risk in Rodents

Risk assessment in rodents involves multiple steps:

a. Population Surveillance

1. Live trapping using baited traps
2. Recording trap success rate (number caught / traps set)
3. Estimating rodent density
4. Identifying dominant species

Higher density often correlates with higher disease transmission risk.

b. Sample Collection

After humane handling:

1. Blood (serum)
2. Kidney tissue
3. Liver tissue
4. Spleen
5. Ectoparasites (fleas, mites)

Samples are labeled and stored properly in the biorepository.

c. Laboratory Testing

Disease detection methods include:

1. Molecular Methods

i. PCR for *Leptospira*, hantavirus, etc.
ii. Sequencing for pathogen typing

2. Serological Methods

i. Microscopic Agglutination Test (MAT) for leptospirosis
ii. ELISA for antibody detection

3. Microscopic Examination

- Direct visualization in certain cases

d. Calculating Risk Indicators

1. Prevalence Rate

(Number of infected rodents / total rodents tested) × 100

2. Infection Intensity

Pathogen load measured via PCR Ct value or bacterial count

3. Species-Specific Risk

Comparing infection rates between species (e.g., *R. rattus* vs *R. tanezumi*)

4. Environmental Risk Factors

i. Proximity to markets
ii. Waste accumulation
iii. Flood-prone areas
iv. Human population density

5. Diseases Commonly Found in Rodents

Leptospirosis

Bacteria colonize kidneys and are shed in urine. Transmission occurs through contaminated water or soil.

Hantavirus

Transmitted via inhalation of aerosolized rodent excreta.

Plague

Transmitted through infected fleas.

Salmonella

Contaminates food and surfaces.

Many rodents are asymptomatic carriers, meaning they appear healthy while shedding pathogens.

6. Role of the Biorepository Laboratory

The biorepository laboratory:

a. Maintains specimen archives
b. Preserves tissues at −20°C or −80°C
c. Stores serum samples
d. Supports long-term epidemiological studies
e. Enables retrospective outbreak investigation

It connects field ecology with molecular diagnostics, forming part of the One Health surveillance system.

7. Honest Reflection

Working in the biorepository laboratory highlighted the close relationship between wildlife and human health. Handling rodents requires strict biosafety precautions, including PPE and controlled processing environments.

Identifying subtle differences between *Rattus rattus* and *Rattus tanezumi* required careful observation and sometimes molecular confirmation. The experience reinforced that vector control is not limited to mosquitoes—rodents and bats are equally important in zoonotic disease transmission.

Understanding rodent population density, infection prevalence, and environmental factors allows prediction of disease outbreaks and supports targeted public health interventions.

This integrated approach reflects the practical application of the One Health concept, where animal surveillance directly contributes to protecting human communities.

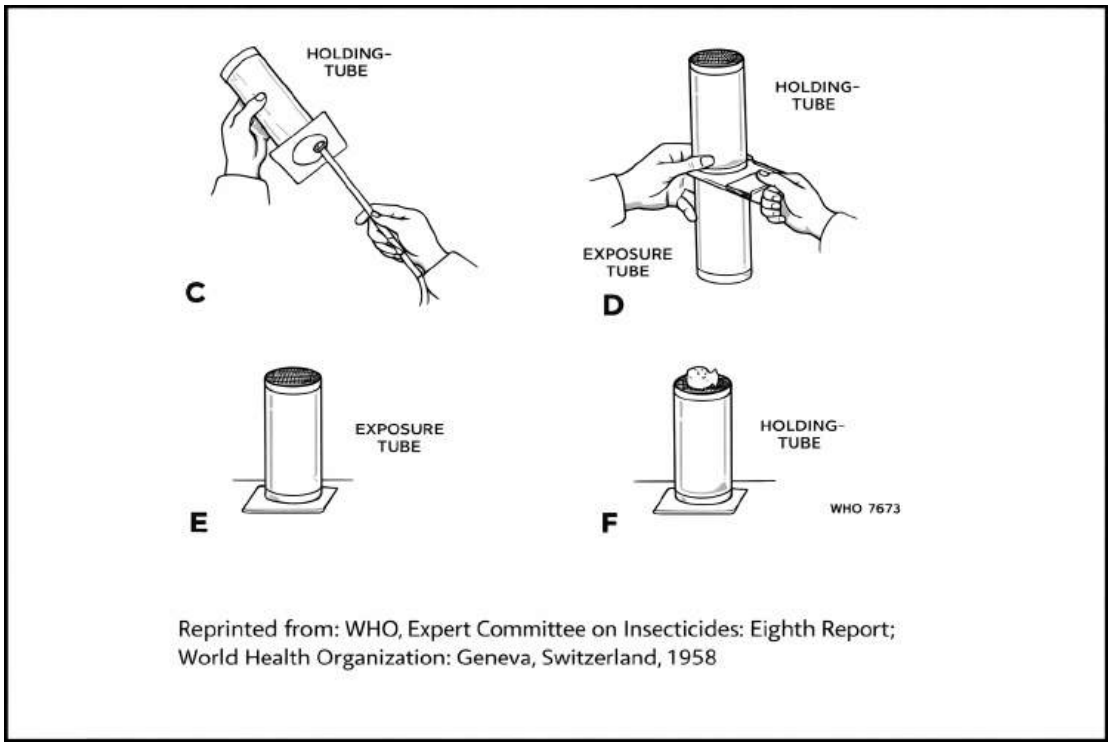
3.1 Mosquito Repellent Efficacy Testing

The arm-in-cage method was used to evaluate protection percentage calculated as: Protection (%) = (C − T) / C × 100. Results were interpreted according to effectiveness levels.

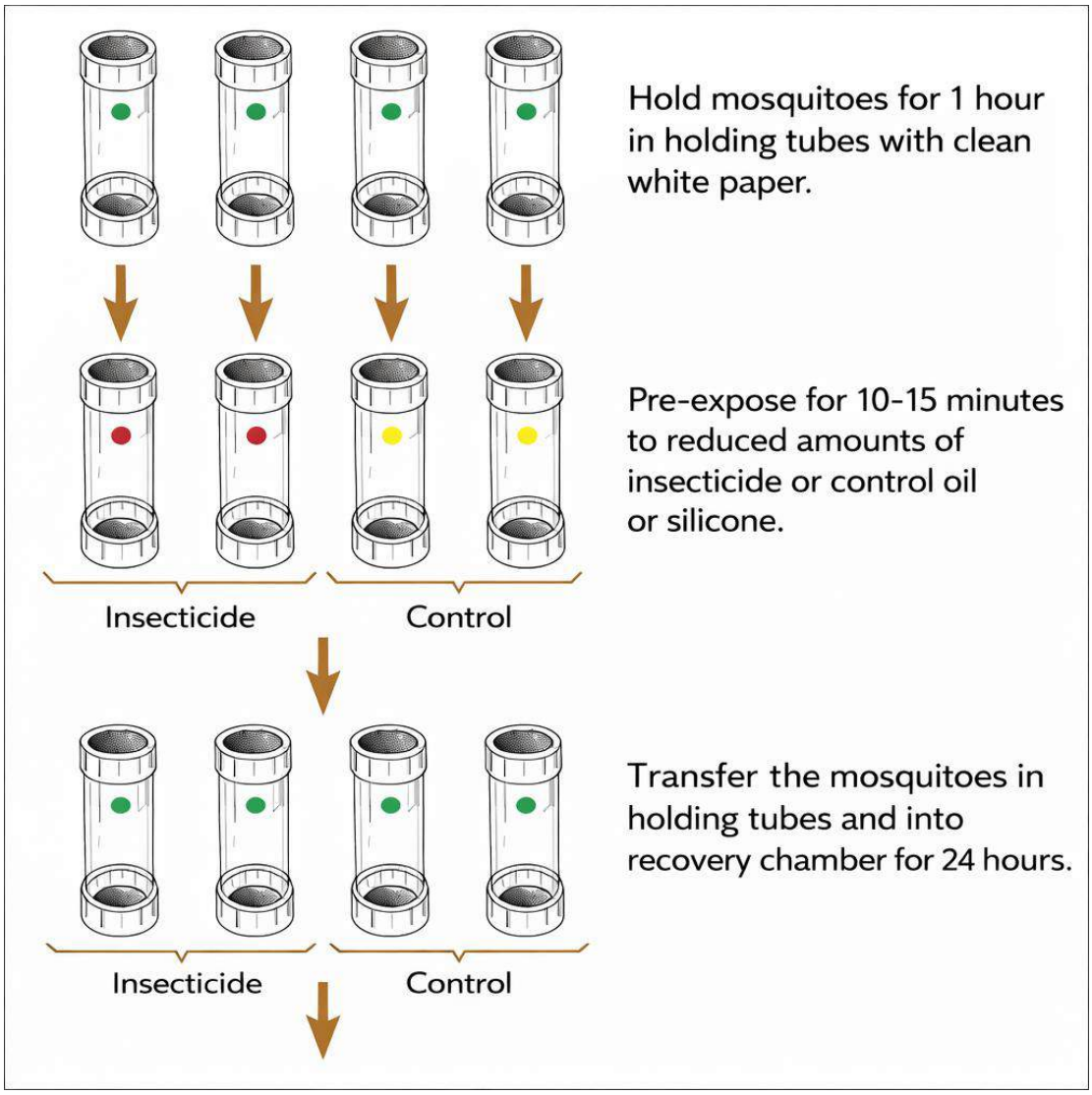
3.2 Insecticide Susceptibility Testing (WHO Tube Test)

Twenty to twenty-five mosquitoes per tube were exposed for one hour. Mortality was recorded after 24 hours. WHO criteria were used to classify susceptibility or resistance.

D.Insecticide Susceptibility Testing (WHO Tube Test), Repellent Test, and Larvicide Testing



Reprinted from: WHO, Expert Committee on Insecticides: Eighth Report; World Health Organization: Geneva, Switzerland, 1958


Hold mosquitoes for 1 hour in holding tubes with clean white paper.
Pre-expose for 10-15 minutes to reduced amounts of insecticide or control oil or silicone.
Insecticide
Control
Transfer the mosquitoes in holding tubes and into recovery chamber for 24 hours.
Insecticide
Control

4

These procedures were conducted to evaluate vector control tools used against mosquito populations. Testing insecticide effectiveness, repellents, and larvicides is essential for monitoring resistance and guiding evidence-based vector control strategies.

1. Insecticide Susceptibility Testing – WHO Tube Test

These procedures were conducted to evaluate vector control tools used against mosquito populations. Testing insecticide effectiveness, repellents, and larvicides is essential for monitoring resistance and guiding evidence-based vector control strategies.

The WHO Tube Test is a standardized method used to determine whether adult mosquitoes are susceptible, possibly resistant, or resistant to a specific insecticide.

a. Purpose

1. To measure knockdown effect and mortality rate
2. To detect insecticide resistance
3. To support vector control decision-making

b. Materials

1. WHO test kit tubes (holding tube + exposure tube)
2. Insecticide-impregnated papers (e.g., permethrin, deltamethrin, malathion)
3. Control papers (untreated)
4. Adult female mosquitoes (2–5 days old, non-blood-fed)
5. Cotton pads soaked in 10% sugar solution

c. Procedure

Step 1: Preparation

a. Select 20–25 healthy female mosquitoes per tube.
b. Use 4 replicates per insecticide (total ~100 mosquitoes).
c. Include 1 control tube with untreated paper.

Step 2: Transfer to Holding Tube

a. Mosquitoes are gently introduced into the holding tube.
b. Allow 1-hour acclimatization.

Step 3: Exposure

a. Mosquitoes are transferred into exposure tubes lined with insecticide-treated paper.

b. Exposure time: 60 minutes.

Step 4: Record Knockdown

- Knockdown mosquitoes are counted at intervals (e.g., every 10–15 minutes).

Step 5: Recovery Period

a. After 1 hour exposure, mosquitoes are transferred back to holding tubes.
b. Provided with 10% sugar solution.
c. Observed for 24 hours.

d. Interpretation (After 24 Hours Mortality)

a. **98–100% mortality** → Susceptible
b. **90–97% mortality** → Possible resistance (needs confirmation)
c. **<90% mortality** → Resistant

If control mortality is between 5–20%, Abbott's formula is used to correct mortality.

2 Repellent Effectiveness Test

Repellent testing measures the ability of a substance to prevent mosquito landing and biting.

a. Purpose

a. To evaluate personal protection efficacy
b. To compare different repellent formulations

b. Method (Arm-in-Cage Test – Laboratory Standard)

Step 1: Mosquito Preparation

- Use 50–100 female mosquitoes (5–7 days old, starved for 12 hours).

Step 2: Control Test

a. Volunteer inserts untreated arm into cage for fixed time (e.g., 3 minutes).
b. Count number of landings/bites.

Step 3: Repellent Application

a. Apply measured amount of repellent to exposed skin area.
b. Allow drying.

Step 4: Exposure

a. Insert treated arm into cage for 3 minutes.
b. Record number of landings or bites.

Step 5: Time Interval Testing

- Repeat every 30–60 minutes until protection fails.

C. Calculating Protection Percentage

Protection (%) =

(C−T)C×100\frac{(C - T)}{C} \times 100C(C−T)×100

Where:
C = Number of landings in control
T = Number of landings in treated arm

d. Interpretation

a. 100% protection → Complete repellency
b. Protection decreases over time → Duration of effectiveness
c. Compare products by protection time and percentage

3 Larvicide Testing (WHO Larval Bioassay)

Larvicide testing determines effectiveness of chemicals against mosquito larvae.

a. Purpose

1. Evaluate larval control agents
2. Determine lethal concentration (LC50, LC90)
3. Monitor larvicide resistance

b. Materials

1. Late 3rd or early 4th instar larvae
2. Test solutions at different concentrations
3. Control solution (distilled water)
4. Plastic cups or trays
5. Pipettes

c. Procedure

Step 1: Preparation of Test Solutions

a. Prepare serial dilutions of larvicide (e.g., temephos).
b. At least 4–5 concentrations tested.

1. Step 2: Larvae Introduction

a. 20–25 larvae per cup.
b. 4 replicates per concentration.
c. Include control group.

Step 3: Exposure

a. Larvae exposed for 24 hours.
b. No feeding during test.

Step 4: Mortality Recording

a. Dead larvae counted after 24 hours.
b. Larvae considered dead if they do not move when probed.

d. Interpretation

1. Mortality ≥98% → Susceptible
2. Mortality 80–97% → Possible resistance
3. Mortality <80% → Resistant

For quantitative analysis, LC50 and LC90 values are calculated using probit analysis.

Honest Reflection

Conducting WHO tube tests required careful mosquito handling to avoid injury that could bias mortality results. Environmental conditions (temperature and humidity) had to be controlled because they influence mosquito survival.

Repellent testing highlighted the importance of standardizing exposure time and mosquito hunger levels. Small variations could influence landing rates.

Larvicide testing required precise preparation of concentrations. Accurate dilution is critical for calculating reliable lethal concentration values.

These vector control evaluations demonstrated how laboratory-based evidence supports malaria and dengue prevention programs. Monitoring susceptibility ensures that insecticides remain effective and helps guide public health policy within integrated vector management strategies

E. Insect Colony Maintenance in the Laboratory


Musca domestica (housefly) colony in Salatiga National Lab
Housefly Colony


Cockroaches in Salatiga National Lab
Cockroach Colony

During the internship, I witnessed and participated in the maintenance of insect colonies, particularly **mosquitoes (*Anopheles*, *Culex*, and *Aedes*)**, as well as **cockroaches and flies**. Maintaining a healthy insect colony requires strict environmental control, regular feeding, and continuous monitoring to ensure stable growth for experimental and surveillance activities.

Colony maintenance involved monitoring survival rate, adult emergence, feeding behaviour, temperature (26–28°C), and humidity (70–80%). Healthy colony conditions ensured reliability for experimental and surveillance activities.

1 Mosquito Colony Maintenance

Mosquito rearing is a multi-stage process involving egg, larva, pupa, and adult stages. Each stage requires specific environmental and nutritional conditions.

a. Environmental Conditions

1. **Temperature:** 26–28°C
2. **Relative Humidity:** 70–80%
3. **Photoperiod:** 12:12 light-dark cycle
4. **Clean water supply for larvae**
5. **Well-ventilated insectary room**

These conditions simulate tropical environments and optimize mosquito survival and reproduction.

b. Egg Collection and Hatching

Aedes aegypti

1. Lay eggs on moist filter paper.
2. Eggs can survive dry conditions for weeks.
3. Eggs are submerged in water to stimulate hatching.

Anopheles spp.

1. Lay eggs individually on water surface.
2. Eggs hatch within 1–2 days.

Culex spp.

1. Lay eggs in rafts on water surface.
2. Egg rafts hatch within 24–48 hours.

Egg trays were monitored daily to ensure proper hatching rates.

c. Larval Rearing

1. Larvae were transferred into plastic trays containing clean dechlorinated water.
2. Fed with finely ground fish food or yeast powder.
3. Feeding was done carefully to avoid overfeeding, which can cause fungal contamination.

Larval density was controlled to prevent overcrowding, which may reduce survival rate and adult size.

Larval development was monitored daily until pupation (5–7 days depending on species).

d. Pupal Collection

1. Pupae were collected using droppers.
2. Transferred into small cups placed inside adult cages.
3. Adult emergence was recorded to assess colony productivity.

E. Adult Mosquito Maintenance

Adults were maintained in mesh cages.

Feeding:

1. 10% sugar solution provided continuously for energy.
2. Blood feeding (if required for egg production) provided using artificial membrane feeder or animal host (according to ethical guidelines).

Monitoring:

1. Adult survival rate
2. Feeding behavior
3. Egg production
4. Mating activity

Healthy adults are active, responsive, and have consistent egg production cycles.

2. Cockroach Colony Maintenance

Cockroach colonies (e.g., *Periplaneta* or *Blattella* species) were maintained in plastic containers with ventilation.

Environmental Conditions:

1. Temperature: 26–30°C
2. Humidity: Moderate (avoid excessive moisture)

Feeding:

1. Dry food (dog pellets, bread)
2. Fresh vegetables occasionally
3. Water provided using soaked cotton to prevent drowning

Egg cases (oothecae) were monitored for hatching success. Colony cleanliness was important to prevent mold growth and door.

3. fly Colony Maintenance

Fly colonies (e.g., houseflies) were maintained in screened cages.

Life Cycle Maintenance:

Eggs:
Laid on protein-rich medium (e.g., fish meal, liver).

Larvae (Maggots):
Reared in moist organic substrate.
Maintained proper moisture to prevent desiccation or rot.

Pupae:
Collected and transferred to adult cages.

Adults:
Fed with sugar solution and protein source for egg production.

Proper sanitation was crucial because flies breed rapidly and contamination spreads easily.

4 Monitoring Colony Health

Daily monitoring included:

1. Survival rate
2. Larval mortality
3. Adult emergence percentage
4. Feeding activity
5. Abnormal behavior
6. Fungal or bacterial contamination

Any decline in survival or unusual mortality triggered investigation (food quality, water condition, temperature variation).

5 Importance of Healthy Colonies

A well-maintained colony ensures:

a. Reliable insecticide susceptibility testing
b. Accurate repellent evaluation
c. Stable vector competence studies
d. Consistent experimental results

Poor colony conditions can lead to stress, altered behavior, or biased susceptibility results.

Honest Reflection

Maintaining insect colonies required discipline and consistency. Small changes in temperature or feeding schedules could significantly affect survival and development rates.

I observed that colony work demands patience and daily attention. The insects depend entirely on controlled environmental conditions, and any neglect may disrupt the entire experimental process.

Through this experience, I understood that strong vector research begins not only with molecular tools or bioassays but with maintaining a stable and healthy insect colony. Proper colony management forms the foundation of reliable vector surveillance and control research.

Colony maintenance involved monitoring survival rate, adult emergence, feeding behavior, temperature (26–28°C), and humidity (70–80%). Healthy colony conditions ensured reliability for experimental and surveillance activities.

F. Competencies Developed During the Internship

The 14-day internship provided both **technical (hard skills)** and **professional (soft skills)** competencies essential for laboratory science, epidemiology, and vector surveillance under the One Health framework. The experience strengthened my capacity to work in multidisciplinary laboratory environments and enhanced my scientific confidence.

1 Technical Competencies (Laboratory Skills)

1. Molecular Diagnostic Skills

a. DNA extraction from mosquitoes, rodents, and human serum
b. Preparation of PCR master mix (conventional and real-time PCR)
c. PCR templating and contamination control
d. Agarose gel preparation and electrophoresis
e. Interpretation of amplification curves and gel bands

I developed confidence in detecting:

a. *Wolbachia* in *Aedes aegypti*
b. *Leptospira* spp. from human serum
c. *Brugia* spp. in *Culex quinquefasciatus*
d. VGSC gene mutations in *Anopheles* species

2. Entomological and Vector Identification Skills

a. Morphological identification of mosquito species (*Anopheles*, *Culex*, *Aedes*)
b. Differentiating rodent species such as *Rattus rattus* and *Rattus tanezumi*
c. Understanding vector behavior and ecology
d. Colony maintenance and life cycle monitoring

3. Insecticide and Vector Control Testing

a. WHO tube test for insecticide susceptibility
b. Repellent efficacy testing (arm-in-cage method)
c. Larvicide bioassay and mortality calculation
d. Interpretation of resistance status using WHO criteria

This enhanced my understanding of insecticide resistance monitoring and integrated vector management.

4. Zoonotic Surveillance and Biorepository Handling

a. Rodent sample processing
b. Safe handling of potentially infected specimens
c. Risk assessment of rodent-borne diseases
d. Understanding disease prevalence calculation and epidemiological interpretation

2 Epidemiological Competencies

a. Understanding disease transmission dynamics
b. Linking laboratory findings to public health risk
c. Calculating prevalence and mortality rates
d. Applying the One Health concept in surveillance
e. Appreciating the importance of environmental, animal, and human health interaction

I learned how laboratory data support outbreak prediction and prevention strategies.

3 Biosafety and Quality Control Competencies

a. Proper use of PPE
b. Prevention of cross-contamination
c. Waste disposal management
d. Documentation and sample labeling accuracy
e. Maintaining laboratory discipline

This strengthened my awareness of laboratory ethics and safety standards.

4 Analytical and Critical Thinking Skills

a. Interpreting PCR and gel electrophoresis results
b. Identifying experimental errors
c. Understanding control validation
d. Evaluating insecticide resistance patterns
e. Problem-solving during technical challenges

I developed the ability to critically analyze laboratory outcomes rather than simply observing results.

5 Professional and Soft Skills

Communication

a. Reporting laboratory findings clearly
b. Working collaboratively with supervisors and technicians
c. Discussing scientific procedures confidently

Teamwork

a. Participating in shared laboratory tasks
b. Supporting colony maintenance and surveillance work

Time Management

a. Managing multiple experiments simultaneously
b. Meeting procedural timelines

Discipline and Responsibility

a. Maintaining consistency in daily colony monitoring
b. Handling samples carefully and ethically

6 Personal Growth and Professional Identity

This internship strengthened my identity as a future epidemiologist and public health professional. I gained:

a. Confidence in laboratory-based research
b. Appreciation for precision and accuracy
c. Awareness of real-world public health challenges
d. Commitment to evidence-based disease control

The integration of molecular diagnostics, vector surveillance, and zoonotic monitoring demonstrated how laboratory science directly supports community health protection.

Overall Reflection

The internship provided comprehensive training that bridged theory and practice. It enhanced my technical expertise, improved my analytical capacity, and strengthened my professional discipline.

Most importantly, it deepened my understanding that effective disease prevention relies on collaboration between laboratory science, epidemiology, and environmental health — a true application of the One Health framework.

G. Conclusion

The 14-day internship at the Salatiga National Laboratory provided comprehensive exposure to molecular diagnostics, vector surveillance, insecticide resistance monitoring, insect colony maintenance, and zoonotic disease risk assessment. This experience successfully integrated theoretical epidemiological knowledge with practical laboratory application under the One Health framework.

Throughout the internship, I gained hands-on experience in detecting *Wolbachia* in *Aedes aegypti*, identifying *Leptospira* spp. from human serum, detecting *Brugia* spp. in *Culex quinquefasciatus*, and amplifying the VGSC gene in *Anopheles* species to assess insecticide resistance. These procedures strengthened my understanding of molecular techniques such as DNA extraction, PCR preparation, gel electrophoresis, and result interpretation. I also developed competence in applying WHO-standard insecticide susceptibility tests, repellent evaluations, and larvicide bioassays, which are essential for evidence-based vector control.

In addition, participation in rodent and bat biorepository surveillance expanded my knowledge of zoonotic disease reservoirs and risk assessment. Learning to differentiate rodent species, monitor infection prevalence, and link laboratory findings to epidemiological implications enhanced my ability to view disease control from a multidisciplinary perspective.

The internship also emphasized the importance of maintaining healthy insect colonies under controlled environmental conditions (26–28°C and 70–80% humidity). Proper colony management ensured reliable experimental outcomes and reinforced the value of consistency, precision, and discipline in laboratory research.

Beyond technical competencies, this internship strengthened my professional skills, including teamwork, time management, biosafety compliance, and scientific

communication. I learned that laboratory science requires not only technical expertise but also responsibility, accuracy, and ethical commitment.

Overall, the internship was a transformative academic and professional experience. It bridged classroom learning with real-world laboratory practice and deepened my commitment to contributing to infectious disease surveillance and control. The knowledge and competencies acquired during this period will serve as a strong foundation for my future career in epidemiology and public health research.

This training significantly strengthened practical laboratory skills and prepared the trainee for advanced research in molecular epidemiology and public health.