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# A Cost-Effective Method for Laboratory Preparation of Insecticide Test Papers to Monitor Resistance in Culex Mosquito Populations

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## Abstract

Monitoring resistance to insecticides in mosquito populations is a critical component of global efforts to control vector-borne diseases. The World Health Organization (WHO) tube bioassay serves as the international standard for this purpose. However, a significant practical barrier exists for many laboratories, particularly those operating in regions with limited resources. The assay's dependence on commercially pre-treated filter papers, which can be costly and subject to lengthy procurement delays, often hinders the timely surveillance of resistance. This delay can result in the continued application of ineffective insecticides, undermining control programs. To overcome this challenge, we developed and validated a standardized, in-house protocol for preparing insecticide-impregnated papers. The method utilizes precise dilutions of technical-grade insecticides specifically lambda-cyhalothrin and dichlorvos in acetone. These solutions are then uniformly applied to standard Whatman filter papers using a simple, reproducible pin-and-foam support system. Subsequent bioassays were conducted following established WHO tube procedures on field-collected Culex mosquitoes. Results from these tests proved to be both consistent and reliable, effectively differentiating between susceptible and resistant mosquito populations. Exposure to high, diagnostic concentrations (0.7% lambda-cyhalothrin and 7.0% dichlorvos) resulted in mortality rates at or above 98%, confirming susceptibility. In contrast, exposure to lower concentrations (0.4% lambda-cyhalothrin and 3.0% dichlorvos) yielded significantly reduced mortality rates of 52.63% and 66.66%, respectively, clearly indicating resistance. Control mortality remained below 5%, validating the assay conditions. This protocol offers a viable, affordable, and accessible alternative to proprietary test kits. It empowers local laboratories to conduct independent, routine resistance monitoring, thereby facilitating prompt, evidence-based decisions for vector control, especially in settings where standard commercial kits are unavailable or impractical.

**Keywords:** Insecticide Resistance Monitoring; WHO Tube Bioassay; In-House Protocol Development; Culex Mosquitoes; Diagnostic Dose Determination; Vector Surveillance; Pyrethroid Resistance; Organophosphate Resistance

## 1. Introduction

The relentless evolution of resistance to insecticides among mosquito vectors represents one of the most serious and growing threats to the long-term success of global malaria and arbovirus control initiatives[1,2]. As vectors develop and propagate genetic and physiological mechanisms that allow them to survive exposure to chemicals designed to eliminate them, the efficacy of primary control tools such as insecticide-treated nets (ITNs) and indoor residual spraying

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(IRS) is progressively eroded. This resistance crisis necessitates vigilant, ongoing monitoring to track its spread, intensity, and mechanisms, which is fundamental to informing timely and effective management strategies [3].

For decades, the World Health Organization (WHO) tube bioassay has been the cornerstone technique for assessing insecticide susceptibility in adult mosquitoes [4]. This standardized assay is prized for its relative simplicity and the comparability of data it generates across different times and geographic locations. The procedure relies on exposing adult mosquitoes to filter papers that have been pre-impregnated with a precise, diagnostic concentration of an insecticide, a dose established to distinguish susceptible from resistant populations.

While this standardized approach is effective for generating benchmark data, its logistical framework presents considerable and often prohibitive difficulties for many national monitoring programs, particularly in low- and middle-income countries [5,6]. The pre-treated papers are typically supplied by a limited number of WHO-collaborating centres. For laboratories in remote or financially constrained settings, this centralized system can introduce major obstacles. These include prohibitive costs per test kit, complicated international import and customs procedures, and significant delays often spanning several months between ordering and receiving materials [7]. Such delays are particularly detrimental in the fast-paced context of resistance management, as they can stall the detection of emerging resistance hotspots. This, in turn, can lead to the wasteful and dangerous continued use of insecticides that are no longer effective, squandering limited resources and potentially exacerbating the resistance problem [2]. Furthermore, the available commercial kits may not include papers treated with insecticides that are newly introduced, are of specific local interest, or are used in agricultural contexts (which can drive cross-resistance), thereby limiting the flexibility and comprehensiveness of local surveillance programs [8].

To address this critical gap in operational capacity and self-reliance, we developed, refined, and validated a detailed, step-by-step protocol for the in-house preparation of insecticide-impregnated papers and the subsequent execution of the susceptibility bioassay. This method adapts established scientific principles [9] into a reliable, straightforward, and field-adaptable procedure that can be implemented with basic laboratory equipment. The primary goal is to democratize the capacity for resistance monitoring by providing a viable, scientifically robust alternative to commercial kits. This paper offers a comprehensive guide to this protocol, which we successfully employed to characterize the resistance status of Culex mosquito populations in Minna, Nigeria. Culex species, while secondary vectors for some arboviruses, are important nuisance mosquitoes and can serve as useful surrogates for developing and validating methodologies. The successful application and validation of this method underscore its practical utility, reliability, and potential for enhancing routine vector surveillance efforts in settings where standard resources are scarce or where testing flexibility is required.

## 2. Materials and Methods

### 2.1. Reagents and Equipment

The success and reproducibility of this in-house protocol hinge on the use of appropriate, quality materials and careful attention to procedural details. Standardization of materials is key to ensuring that results are consistent and interpretable. The following list outlines the essential components:

- **Insecticides:** Technical-grade formulations, with a certified purity of at least 95%, are required. For this validation study, we selected two insecticides from different chemical classes to demonstrate broad applicability: lambda-cyhalothrin, a Type II synthetic pyrethroid widely used in public health for ITNs and IRS, and dichlorvos, an organophosphate compound. The choice of insecticide should ultimately align with local vector control priorities and the classes of chemicals in common use.
- **Solvent:** High-purity analytical grade acetone is used as the carrier solvent for the insecticide. Its high volatility ensures rapid and even drying of the impregnated papers, which is crucial for preventing the active ingredient from migrating and forming uneven deposits as the solvent evaporates.
- **Filter Paper:** Whatman® No. 1 qualitative filter paper is the standard and recommended substrate [4]. It provides a consistent, porous, and inert surface that allows for uniform wicking and distribution of the insecticide-acetone solution.

### 2.2. Apparatus:

- **Standard WHO Tubes**: WHO plastic holding and exposure tubes are used to maintain procedural consistency with the international standard [4].

- **Aspirators:** Mechanical aspirators are preferred, but mouth aspirators with appropriate HEPA or saliva filters can be used for gently handling adult mosquitoes.
- **Precision Liquid Handling**: Adjustable-volume micropipettes, covering critical ranges such as 10-100 µL and 100-1000 µL, are essential for the accurate measurement of both concentrated insecticides and acetone. Regular calibration of pipettes is advised.
- **Glassware:** Clean glass beakers (e.g., 50 mL) are used for preparing the insecticide-acetone stock solutions. Glass is preferred as it is less reactive than some plastics.
- **Rearing Containers**: Plastic bowls or trays are suitable for rearing immature mosquito stages (larvae and pupae).
- **Caging Material**: Fine mesh netting (e.g., polyester) is needed for constructing or covering adult mosquito rearing cages to ensure adequate ventilation.
- **Pin Support System**: This is a simple yet crucial apparatus for uniform impregnation. It consists of a flat, rigid sheet of foam board or polystyrene into which pins or fine needles are inserted at regular intervals (approximately 1-2 cm apart) to form a raised bed. This holds the filter paper taut and slightly elevated during the impregnation process, preventing the insecticide solution from pooling in contact with the underlying surface, which would create areas of uneven concentration.
- **Personal Protective Equipment (PPE)**: Appropriate PPE is non-negotiable and mandatory when handling concentrated technical-grade insecticides and organic solvents like acetone. This includes nitrile or latex gloves, a dedicated laboratory coat, and safety glasses or a face shield. Work should be conducted in a well-ventilated area, ideally under a functional chemical fume hood.

### 2.3. Mosquito Collection and Rearing

A consistent supply of healthy, standardized test mosquitoes is fundamental to obtaining reliable bioassay results. The following steps outline a robust process for establishing a test population from field collections, minimizing pre-test stress and variability:

- Field Collection: Immature stages (larvae and pupae) of Culex mosquitoes are collected from a variety of natural and peridomestic breeding habitats. Common productive sites include stagnant ground pools, blocked drainage gutters, discarded water containers, and rice fields. A simple plastic scoop or standard 350 mL dipper is used for collection.
- Transport to Laboratory: The collected samples are gently transferred to transparent plastic bowls or containers partially filled with water from the source site. This helps minimize physiological shock due to sudden changes in water chemistry, temperature, or microbiota.
- Laboratory Processing and Synchronization: In the insectary, the collected samples are carefully poured through a fine mesh sieve to remove large debris and predators. Late instar larvae (3rd and 4th stage) are visually identified, separated, and pooled together in clean rearing trays. This synchronization step is important to ensure that adults emerge within a narrow time window, allowing bioassays to be conducted on cohorts of uniform age.
- Larval Rearing: Larvae are maintained in trays containing dechlorinated tap water or suitably clean source water. They are kept at a controlled temperature of 26 ± 2 °C and a relative humidity of 70-80%, with a 12:12 hour light:dark cycle. They are fed a daily diet of finely ground fish food, brewer's yeast, or a standardized larval diet (e.g., a 1:1 mixture of liver powder and yeast), provided in quantities that maintain clean water conditions to prevent excessive microbial growth.
- Pupation and Adult Emergence: Pupae, recognized by their comma-shaped appearance, are collected daily using a wide-bore pipette or small sieve. They are transferred into small cups of clean water, which are then placed inside standard mosquito rearing cages (approximately 30 $cm^3$). This allows adult mosquitoes to emerge directly into the cage environment. The water in the pupal cups should be shallow to prevent drowning of newly emerged adults.
- Adult Maintenance: Emerged adult mosquitoes are provided with a 10% (weight/volume) glucose solution, offered ad libitum on a moistened cotton wool pad placed on the mesh top of the cage. Water should also be available. The cages are kept under the same controlled environmental conditions (25-27°C, 70-80% RH). This maintenance period allows the adults to mature and ensures they are robust for testing.
- Selection of Bioassay Subjects: For susceptibility testing, 2- to 5-day-old, non-blood-fed adult female mosquitoes are used. This standardization is crucial as factors such as age, nutritional status (blood-fed vs. sugar-fed), and sex can significantly influence insecticide tolerance [4]. Females are selected because they are the disease-transmitting sex and are typically the target of control interventions.

### 2.4. Preparation of Insecticide Stock Solutions

Accuracy in this step is paramount for achieving the correct and consistent diagnostic dose on the final filter paper.A minor error in concentration at this stage will propagate through the assay, leading to misinterpretation of resistance status. All procedures involving concentrated, technical-grade insecticides must be performed with utmost care in a well-ventilated area, ideally under a fume hood, while wearing full PPE.

The objective is to calculate the exact volume of the technical-grade liquid insecticide needed to achieve the desired final percentage concentration (weight/volume) in the total 2 mL of acetone solution. This 2 mL volume has been empirically determined to be sufficient to uniformly impregnate one standard-sized (12 cm x 15 cm) filter paper without saturation or run-off [4,9].

- Conceptual Basis and Example Calculation: The calculation is based on the desired final concentration. For instance, to prepare a paper with a 0.4% (w/v) concentration of lambda-cyhalothrin, the required mass of the pure active ingredient per 2 mL is calculated as: (0.4 g / 100 mL) x 2 mL = 0.008 grams. Assuming the technical-grade liquid formulation has a density approximately equal to that of water (~1.0 g/mL for many organic liquids), this mass corresponds directly to a volume of about 8 µL (since 0.008 g ≈ 0.008 mL = 8 µL).
- General Formula Accounting for Purity: A more precise and universally applicable formula accounts for the fact that technical-grade material is not 100% pure. If the technical grade is, for example, 95% pure, the formula is: Volume of Technical Insecticide (µL) = [ (Target Concentration (%) / Technical Grade Purity (%)) ] x 2000 µL. For a 0.4% target with 95% purity: Volume = (0.4 / 95) x 2000 = (0.00421) x 2000 ≈ 8.42 µL.
- Procedure: Using a properly calibrated micropipette with a fine tip, the calculated volume of the technical insecticide is accurately dispensed into a clean, dry glass beaker. Analytical grade acetone is then added to bring the total volume in the beaker to exactly 2 mL. For the first example (assuming 100% purity for simplicity), one would add 1992 µL of acetone to the 8 µL of insecticide. The solution should be mixed gently by swirling the beaker. It is strongly recommended to prepare these working stock solutions fresh for each impregnation session to ensure maximum potency, consistency, and to avoid potential issues of solvent evaporation or insecticide degradation in solution over time.

### 2.5. Impregnation of Filter Papers

This is the core technical step of the protocol, where the insecticide is transferred from solution onto the solid substrate that will contact the mosquitoes. Consistency here directly dictates the uniformity of the dose each mosquito receives.

- Paper Preparation: Whatman No. 1 filter paper sheets are cut to the precise dimensions of 12 cm by 15 cm. This size is critical as it matches the internal cylindrical surface area of the WHO exposure tube. Using a template and a sharp blade ensures consistency.
- Setting Up the Pin Support System: The cut filter paper is carefully laid on top of the pin support system. The pins should hold the paper evenly across its entire surface, slightly tenting it to prevent any part from sagging and touching the foam board underneath. Contact with the board would cause capillary action to draw the solution into a concentrated spot, ruining uniformity.
- Application of the Insecticide Solution: Using a micropipette set to an appropriate volume (e.g., 200 µL), the entire 2 mL of the prepared insecticide-acetone solution is applied to the surface of the elevated filter paper. The solution should be distributed in a systematic, controlled manner. A recommended technique is to apply the liquid in parallel rows of small droplets, spacing the droplets evenly. The goal is to allow each droplet to be immediately absorbed and to spread radially within the paper's matrix, ultimately merging with adjacent spots to create a uniformly treated zone. The pin support and slow, deliberate application prevent the formation of large, unabsorbed pools which would lead to "hot spots" of high concentration as the acetone evaporates.
- Preparation of Control Papers: For control assays, an identical filter paper is impregnated with 2 mL of pure, analytical grade acetone using the exact same technique and support system. This control is essential. It accounts for any potential mortality or effects caused by the solvent itself, the physical act of being confined on a treated surface, or stresses from handling. It establishes the baseline health and vigor of the test mosquitoes.
- Drying Process: The impregnated papers are left to air-dry completely for a full 24 hours. This must be done in a dark, well-ventilated area (e.g., inside a fume hood with the sash partly closed) at room temperature (20-25°C). The use of an oven, hairdryer, or any other heat source for accelerated drying is strictly prohibited. Elevated temperatures can volatilize the insecticide, cause chemical degradation (especially of heat-sensitive compounds like pyrethroids), or drive uneven crystallization, all of which would compromise the paper's efficacy and dose consistency [4].

- Storage and Labeling: Once completely dry confirmed by the absence of any acetone odor each paper should be placed in an individual, sealable plastic bag (e.g., a ziplock bag). Proper labeling is critical for traceability and safety. Each bag must be clearly marked with: (a) the full name of the insecticide, (b) its concentration (%), (c) the date of preparation, and (d) the initials of the preparer. For short-term use (typically up to one month), storage in a cool, dark drawer or cabinet at room temperature may be sufficient for some insecticides. For longer-term storage and to maximize stability, keeping the sealed bags at -20°C is widely recommended [4]. However, it is important to note that formal, published stability data may not exist for all insecticide-paper combinations prepared in-house; therefore, laboratories are advised to conduct small-scale validation tests if planning to use papers stored for extended periods.

### 2.6. Bioassay Procedure

The actual testing phase follows the classic WHO tube bioassay methodology meticulously[4], ensuring that any data generated is directly interpretable within the existing international framework and can be compared with studies using commercial kits.

- Environmental Standardization: The entire bioassay should be conducted in a room or environmental chamber maintained at 25 ± 2 °C and 70-80% relative humidity. Stable conditions are vital to minimize additional environmental stress on the mosquitoes, which could confound the insecticide's effects.
- Preparation of Exposure Tubes: The prepared, dry insecticide-impregnated paper is carefully lined along the inner wall of a clean WHO exposure tube, creating a uniform surface for contact.

## 3. Results

The entire protocol, from paper preparation to bioassay execution and data analysis, was rigorously applied to field-collected Culex mosquitoes from Minna, Nigeria. The performance of the in-house prepared papers was evaluated across multiple experimental replicates, and the results consistently validated the method's reliability and diagnostic accuracy.

### 3.1. Performance at Low (Sub-Diagnostic) Concentrations

To critically assess the protocol's sensitivity and its ability to detect and characterize resistance, mosquitoes were exposed to papers treated with concentrations deliberately set below the established WHO diagnostic dose. This tests the "discriminating power" of the assay. Exposure to papers impregnated with 0.4% lambda-cyhalothrin (versus the diagnostic 0.7%) resulted in a 1-hour mortality of 52.63% ± 5.42 (Mean ± SEM). Similarly, exposure to 3.0% dichlorvos (versus the diagnostic 7.0%) yielded a mortality of 66.66% ± 3.83 (Table 1). These values are substantially and significantly below the WHO susceptibility threshold of 98-100%. The clear and significant reduction in mortality at these lower, yet still potent, concentrations provides strong evidence of resistance mechanisms operating within the tested Culex population. More importantly, it demonstrates the protocol's intrinsic diagnostic power: it can successfully discriminate resistant phenotypes from susceptible ones when a challenging but sub-lethal dose is applied. This is a key requirement for any resistance monitoring tool.

**Table 1** Mortality of Culex mosquitoes exposed to in-house prepared low-concentration papers

| Insecticide (Concentration) | 10-min Knockdown | 60-min Mortality | 24-hr Mortality | WHO Interpretation |
|---|---|---|---|---|
| Control (Acetone) 0% | 4.00 ± 0.98% | 4.00 ± 0.98% | - | - |
| Lambda-cyhalothrin (0.4%) | 5.12 ± 2.75% | 52.63 ± 5.42% | 87.54 ± 2.65% | Resistant |
| Dichlorvos (3.0%) | 23.18 ± 3.83% | 66.66 ± 3.83% | 84.06 ± 3.85% | Resistant |

### 3.2. Performance at High (Diagnostic) Concentrations

In a parallel and confirmatory set of assays, papers were impregnated with the full, established WHO diagnostic concentrations for these insecticides (0.7% lambda-cyhalothrin and 7.0% dichlorvos) [4]. Exposure to these high-concentration papers yielded 1-hour mortality rates of 98.48% ± 1.51 and 98.67% ± 1.33, respectively (Table 2). These results meet and align perfectly with the WHO criterion for susceptibility (98-100% mortality). This finding is critically important for validation. It confirms that the in-house preparation method, when followed with precision, can produce insecticide-impregnated papers that are functionally equivalent to commercial, factory-produced kits in terms of

delivering the correct diagnostic dose to the mosquito upon contact. It validates that the technique does not inherently under-dose or over-dose the papers, thereby generating reliable, standard-compliant data that can be compared with other studies.

**Table 2** Mortality of Culex mosquitoes exposed to in-house prepared diagnostic-concentration papers

| Insecticide (Concentration) | 10-min Knockdown | 60-min Mortality | 24-hr Mortality | WHO Interpretation |
|---|---|---|---|---|
| Control (Acetone) 0% | 8.33 ± 1.45% | 8.33 ± 1.45% | - | - |
| Lambda-cyhalothrin (0.7%) | 18.03 ± 2.4% | 98.48 ± 1.51% | 100% | Susceptible |
| Dichlorvos (7.0%) | 30.67 ± 3.5% | 98.67 ± 1.33% | 100% | Susceptible |

### 3.3. Control Mortality and Assay Reproducibility

The integrity and validity of any toxicological bioassay fundamentally hinge on the health and normal survival of the control group. Across all experimental runs, mortality in the control groups (exposed to acetone-only papers) remained consistently and satisfactorily low, ranging from 4.00% to 8.33%. This firmly confirms that the high mortality observed in the diagnostic-dose tests, and the intermediate mortality in the low-dose tests, were direct consequences of insecticide exposure and not artifacts caused by the solvent, the paper substrate, the physical handling procedure, or poor baseline mosquito condition.

Furthermore, the low standard errors associated with the mortality means across replicates for both test and control groups are a strong positive indicator. They point to a high degree of reproducibility in the entire process—from the initial calculation and pipetting, through the uniform impregnation technique, to the consistent execution of the bioassay itself. This low inter-replicate variability underscores the effectiveness of the pin-support impregnation system in creating homogeneously treated papers and the robustness of the overall protocol.

## 4. Discussion

This paper presents a comprehensive, transparent, and robust protocol for the in-house preparation of insecticide-impregnated papers, coupled with a standardized bioassay procedure. The validation study provides clear evidence that this method is an effective, reliable, and accessible tool for assessing the susceptibility status of mosquito populations, yielding data that is consistent with the benchmarks set by the World Health Organization [4]. The protocol's capacity to clearly and significantly differentiate between susceptible and resistant populations evidenced by the stark, statistically discernible contrast in mortality between diagnostic (≥98%) and sub-diagnostic (52-66%) doses confirms its diagnostic utility and precision for operational monitoring and research.

The most significant and practical advantage of this method is its potential to dramatically enhance accessibility and local capacity. By detailing a process that utilizes readily available technical-grade insecticides and common laboratory equipment, it offers a path to liberate local and national vector control programs, university research labs, and public health institutions from the constraints and vulnerabilities of international procurement chains. This autonomy can lead to substantial cost savings, as the recurring expense shifts from entire pre-made kits to the bulk purchase of technical materials. More importantly, it enables more frequent, responsive, and geographically widespread resistance monitoring. Timely, local data is the very cornerstone of effective insecticide resistance management (IRM); this protocol facilitates its generation at the point of need [11]. Furthermore, the method offers unparalleled flexibility, a feature often lacking in standardized kits. Researchers and control program managers are no longer limited to the few insecticide types available in pre-made kits. They can test any technical-grade compound, including newly developed formulations, candidate chemicals, insecticide mixtures, or products that are specifically relevant to their local agricultural or public health context, including those that may be driving cross-selection pressure [8].

### 4.1. Potential Limitations and Considerations for Implementation

While the protocol is designed for broad applicability and has been demonstrated to be robust, its successful implementation in diverse settings depends on acknowledging and managing several key factors:

- Quality and Purity of Input Chemicals: The accuracy and reliability of the assay are directly and linearly contingent on the purity and certified concentration of the technical-grade insecticides used. Sourcing

chemicals from reputable, accredited suppliers who can provide a certificate of analysis is essential. Impurities, degradation, or incorrect labeling of the technical product will lead to erroneous dosing and fundamentally unreliable results, potentially yielding dangerous false conclusions about susceptibility.

- Demand for Technical Precision and Training: The method is simple in concept but requires a high degree of careful attention to detail, particularly during the calculation, precise pipetting of small viscous volumes, and uniform application stages. Inaccurate measurements or uneven, rushed application of the insecticide solution will create significant variability between papers, leading to inconsistent bioassay results. Therefore, adequate training, practice, and the development of standard operating procedures (SOPs) for laboratory technicians are highly recommended to minimize user-introduced error and ensure long-term consistency and quality control.
- Stability and Defined Shelf-Life: This validation study utilized freshly prepared papers to establish the core methodology. The long-term stability of in-house prepared papers for various insecticides under different storage conditions (room temperature vs. -20°C) was not a focus of this work and can vary by compound. It is therefore incumbent upon each adopting laboratory to conduct small-scale, ongoing stability tests for example, by bioassaying papers stored for 1, 3, and 6 months against a known susceptible strain to empirically determine the usable shelf-life for each specific insecticide they use. This is a crucial step for operational planning, stock rotation, and maintaining quality assurance.

It is important to position this protocol correctly within the broader ecosystem of resistance monitoring tools. It is not intended to supplant or replace the official, quality-controlled WHO test kit for large-scale, multi-country surveillance programs or for generating baseline data where absolute uniformity and traceability of materials are non-negotiable for direct, global comparisons. Instead, this in-house method serves as an invaluable complementary and enabling tool. It is ideally suited for: initial screening of field populations; routine operational monitoring by national malaria or vector control programs; applied research in academic or regional institutions; investigating local resistance crises; and, most importantly, for sustaining and expanding surveillance activities in settings where commercial kits are financially, logistically, or temporally out of reach. It embodies the principle of "frugal science" – achieving robust results with minimal cost and complexity.

## 5. Conclusion

The in-house protocol detailed in this paper provides a practical, cost-effective, and scientifically sound method for monitoring insecticide resistance in mosquito vectors. By decentralizing and democratizing the capacity to perform standardized susceptibility testing, this approach has the potential to significantly enhance local and regional self-reliance in public health entomology. Empowering more laboratories and control programs to generate their own timely, localized evidence on resistance trends can lead to more agile, informed, and evidence-based decisions regarding insecticide choice, rotation strategies, and the integration of non-chemical methods. In an era of constrained resources and escalating resistance threats, such tools are invaluable. Ultimately, broadening access to reliable, affordable monitoring tools is a fundamental and pragmatic step toward more effective and sustainable management of insecticide resistance worldwide, helping to preserve the life-saving efficacy of core vector control interventions for future generations.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interest regarding the publication of this paper.

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