A survey of users on the WHO insecticide susceptibility tube test kit

1. Introduction

The major vector control interventions use insecticides to control vectors of disease. Repeated or regular use of these insecticide-based interventions may result in the development of insecticide resistance, which may potentially reduce their impact. To assess vector populations for the presence of insecticide resistance, the World Health Organization (WHO) developed an insecticide susceptibility test kit, currently produced and supplied by Universiti Sains Malaysia under a memorandum of understanding with WHO. This test kit has been widely used since its development.

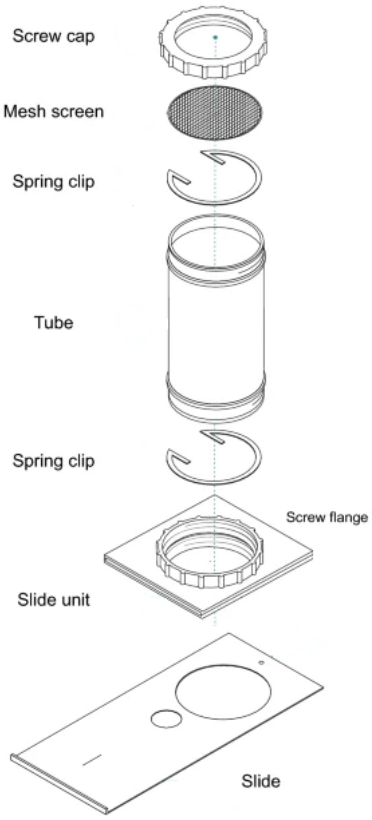
The test kit includes two plastic tubes held together by a slide unit. One tube functions as a mosquito holding chamber to be lined with untreated paper; the other acts as an exposure chamber, where an insecticide-treated paper or control paper (with or without carrier oil) can be inserted to line the interior walls of the tube. A fixed number of the insects (e.g. mosquitoes, sand flies) to be tested are first held for one hour in the holding tube to acclimatize them. Then, the surviving insects are transferred to the exposure tube for one hour. The insects are then returned to the holding tube for observation and recording of mortality at 24 hours after the one hour of exposure. See the *Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions (1)* for more information.

Over the years, there have been reports of quality issues associated with this equipment, such as damage to kits with use, breakage, loss of parts and long shipping times *(2)*. WHO and the Vector Control Research Unit (VCRU) at Universiti Sains Malaysia have worked on addressing a number of issues relating to quality and timeliness, including by conducting a review in 2018 of the impediments to timely production and supply of quality-assured kits. However, it is recognized that there may be additional suggestions to improve on these aspects and on the overall design of the kit to enhance the user experience. To this end, WHO sought feedback on the quality and ease of use of the WHO susceptibility kits (at this time limited to the tube test kit, not the WHO bottle bioassay) from individuals and/or institutions that regularly use this equipment for insecticide resistance testing. An online survey was posted from 30 June to 15 October 2023.

World Health Organization

The terminology used in this document has been standardized to that used in Fig. 1 (adapted from *(2)*).

Fig. 1. Parts of the WHO susceptibility tube test kit.



Taken from *(2)*.

2. Results

2.1 Respondents

There was a total of 36 responses. The respondents were from: India (9), Rwanda (5), Ghana (3), United Republic of Tanzania (3), Uganda (2), United Kingdom of Great Britain and Northern Ireland (2), United States of America (2), Brazil (1), Cameroon (1), Côte d'Ivoire (1), Djibouti (1), Germany (1), Islamic Republic of Iran (1), Kenya (1), Liberia (1), Nigeria (1), and Zambia (1).

The respondents had used the WHO susceptibility test kits for different lengths of time. Of those that responded, 37% had used the kits for 11 or more years, 41% had used them for 6–10 years, 19% had used them for 1–5 years, and 4% had used them for less than one year.

The mosquito species the respondents had used in the tests varied. Many users did not provide the names of species they had tested (44%). Of those that reported the species used, *Anopheles funestus* was the most common (35%), followed by *An. gambiae* (30%), *An. stephensi* (15%), *An. culicifacies* (15%), *An. arabiensis* (5%), *Aedes aegypti* (5%), *An. subpictus* (5%), *An. vagus* (5%), *An. maculatus* (5%), *An. aconitus* (5%), *Mansonia uniformis* (5%), *Culex gelidus* (5%), and *Cx. quinquefasciatus* (5%).

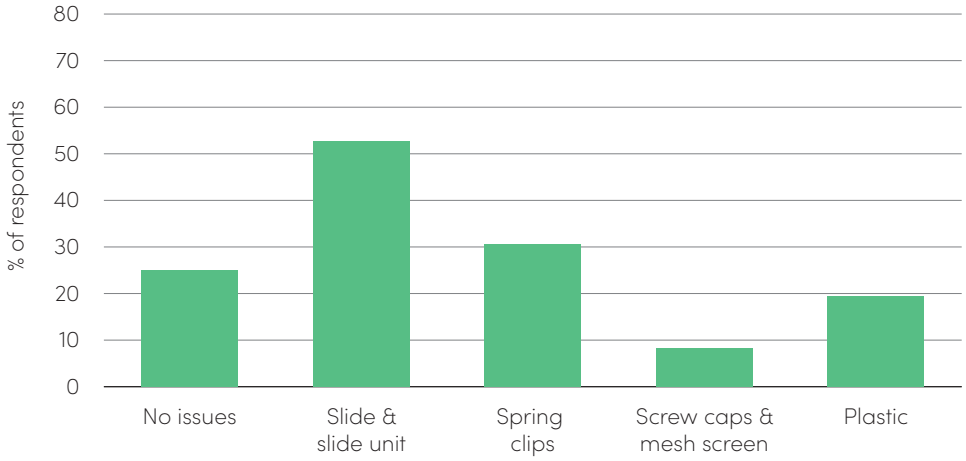
2.2 Issues with durability and usability of WHO test kits

In response to the question "Have you noticed any issues with the durability and usability of the plastic tubes (exposure and holding tubes) or any other items in the WHO susceptibility kits?" a range of responses were given.[1]

The main areas of response included the slides and slide units, the plastic used for the tubes, the spring clips used to hold papers in place, the screw caps and mesh screens at the ends of the tubes, and other issues (Fig. 2). Some respondents reported no issues with the kits (25%).

1 Note that some respondents provided more than one comment, so the percentage of comments for each part of the test kit may exceed 100%.

Fig. 2. Issues with parts of the WHO test kits reported by respondents



Slides and slide units. The main issues with the slides and slide units were the slides getting stuck (28% of respondents) and the slide units breaking into two pieces along the welded seam (25%). In addition, some respondents mentioned that the screw mechanism did not work well (19%), the slide came out (3%) or the kit did not work well for sand flies (3%).

Plastic of tubes. The plastic used for the tubes was also mentioned by 19% of respondents. Some respondents mentioned that the plastic became cloudy over time (11%), whereas others noted issues with durability (6%) and cracking (3%). However, the length of time for which these kits had been used was not mentioned.

Spring clips. A number of respondents (31%) mentioned the metal spring clips, including that the copper spring clips were too hard and did not have enough flexibility (11%), there was a general "clip problem" (6%), there were not enough clips in the test kit (6%), the spring clips had rusted (3%), the clips were difficult to handle (3%), the tangs of the spring clips were too short (3%), the metal coating had come off after washing (3%), and there were issues with replacing the spring clips after inserting the test papers (3%).

Screw caps and mesh screens. Of the respondents, 8% noted issues with the screw caps and mesh screens. The comments included the poor quality of the caps and screens (6%). In addition, one participant mentioned that the dark mesh screen made it difficult to see the mosquitoes in the tubes (3%), and another mentioned that the mosquitoes got stuck in the screen material (3%).

Other issues reported that were not related to the plastic test kit included the following: the paper did not match the size of the tube (3%), and the aspirator tubes were bigger than they had been previously and were uncomfortable to use (3%).

2.3 Ease of use

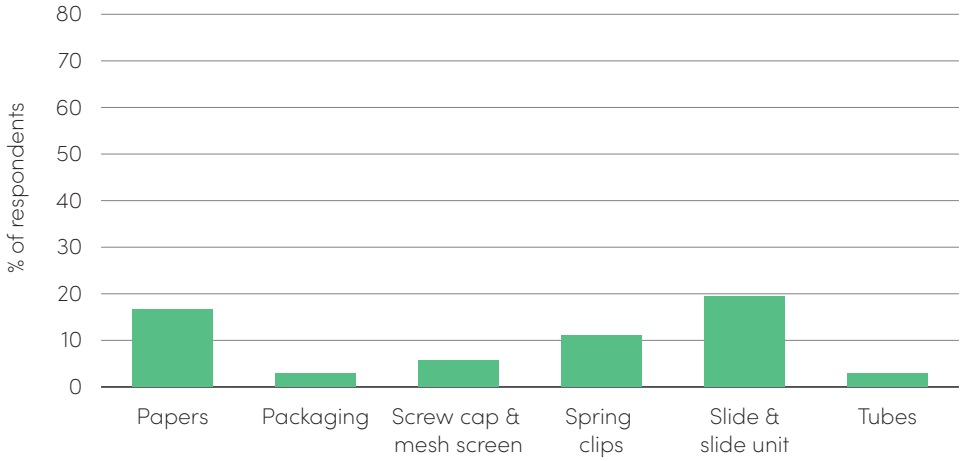
When respondents were asked whether the test kits were easy to use, 97% (35/36) responded "Yes". Of those respondents, 12% qualified their response, mentioning difficulties related to training, transferring the mosquitoes into the tubes or fixing the mesh screen on the tube. One respondent stated that the test kits were "not always" easy to use.

2.4 Aspects of the kits hindering assessment of susceptibility

Participants were asked "Do any aspects of the kits hinder you from a proper assessment of the susceptibility of the species you are working with?" There was a wide range of responses. The primary response was "No" (50%). Grouping the "Yes" answers by the

aspects of the kits mentioned showed that the most common aspects were the papers, slides and slide units (19%) (Fig. 3). Specific comments included the time required for the papers to arrive (8%), issues with the sliding mechanism (8%), and the need to adapt the slide unit for sand flies (6%). All other responses were provided by a single respondent each: no fixing paper; poor quality of the insecticide paper; difficulty putting papers into place because there is space between the paper and tube wall; poor quality of the packaging; change of material used for the mesh screen from wire to plastic, which needs replacement; poor quality of the screw cap and slide in the test kit; need for the copper and silver spring clips to be more flexible with longer tangs on both ends to hold, press and insert; spring clip coating wearing off; insufficient number of spring clips; subpar quality of the copper and steel spring clips; difficulty transferring the mosquitoes between the tubes; tubes not easily sliding into the screw flanges; recommendation against using the tube test kits for chlorfenapyr; and warping of the tubes when washed with hot water.

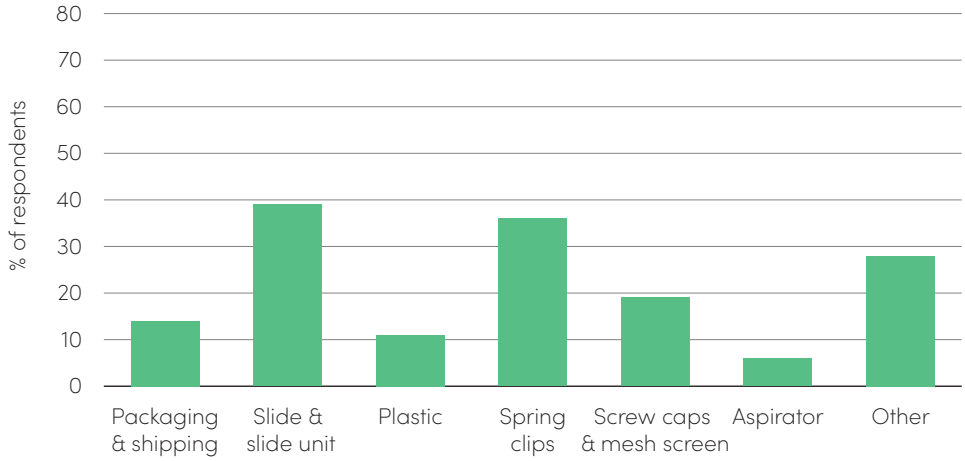
Fig. 3. Aspects of the kits hindering respondents from properly assessing susceptibility



2.5 Suggestions for improving the quality or usability of the test kits

When respondents were asked "Do you have any suggestions to improve the quality or usability of the test kits?" comments were received in a few categories. These included the packaging and shipping, the slide and slide unit, plastic, clips, mesh screens and screw caps, aspirators, and a few other topics (see Fig. 4).

Fig. 4. Aspects of the test kits mentioned during a survey of areas for improvement



Packaging and shipping. A few of the comments were related to the packaging and shipping of the kits and papers. Suggestions for improvement included making the kits easier to obtain (6%), shipping papers in a timelier manner (3%), and improving the packaging (3%).

Slides and slide units. Several suggestions were made to improve the slides and slide units, which were the parts the most frequently mentioned with respect to the durability and usability of the kits. Suggestions included ensuring that the transferring assembly can slide easily to open and close (19%), developing a slide unit that is resistant to breakage (14%), improving the screw mechanism (3%), and adding more binding material to the screw flanges to avoid easy separation (3%).

Plastic of the tubes. Suggestions related to the plastic of the tubes included using higher quality plastic to prevent scratching/cracking (8%), and changing the material or amending the standard operating procedure for washing to prevent tubes from becoming warped when washed in hot water (3%).

Metal clips. In terms of the spring clips used to hold the paper in place inside the tubes, suggestions for improvement included making the spring clips more flexible so that they can easily be clipped (14%), redesigning the spring clips to improve functionality (11%), providing more spring clips (8%), and exploring alternative coatings that do not wear off quickly (3%).

Screw caps and mesh screens. There were only a few suggestions related to the screw caps and mesh screens, including making the mesh screen white on the top to improve visibility (6%), improving the size of the mesh and stability of the mesh screen so that it is appropriately sized and fits in the cap (6%), ensuring that the mesh and cover are very smooth while tightening (3%), making the mesh metal and sturdy (3%), and enhancing the design and construction of the screw cap (3%).

Aspirators. It was noted that the suction tube mouthpiece should be flat or in the shape of a blunt cone that can be held with the lips (3%), or the aspirator should be smaller (3%).

Other suggestions. Other suggestions for improvement included developing guidance on larval susceptibility testing (3%), regularly seeking feedback from users and collaborating with experts (3%), providing a wooden box for the holding tubes for 24 hours (3%), making it easier to separate live and dead mosquitoes (3%), offering the kit instructions in different languages besides English (3%), and improving the kits for testing with sand flies (3%). Some respondents noted that "others provided feedback which includes my recommendations" (3%), while three respondents made no suggestions.

2.6 Final thoughts on improving the test kits

When respondents were asked if they had any other thoughts on improving the test kits, there were a number of suggestions. These included using a tougher transfer housing (possibly metal), adding a lock system to keep the slide in place, making the test procedure easier but with the same principle, ensuring frequent training, including more tubes in the kit, ensuring that the aspirator tube and jointer are of good quality, making the mesh screen finer for sand flies, making the tube a standard size and flexible, clarifying the minimum number of samples per test in the guidelines, developing guidance on larval susceptibility testing, conducting field trials to validate and troubleshoot the test kits, exploring alternative materials and designs, actively seeking feedback, providing detailed instructions, standardizing the kit components across batches, developing a user-friendly mobile app, developing tools to fit the spring clips, engaging with the 3D printing of kit parts, adding more screen pieces to the kit, using colours that are more permanent

for the red/yellow/green dots, so that they do not wash away easily, and making tube test kits easier and faster to procure.

3. Summary and key findings

A review of the issues and suggestions made by the respondents provided some key inputs for the improvement of the WHO test kits and papers.

Slides getting stuck. Numerous respondents mentioned the fact that the slides of the WHO test kit become "sticky" after repeated use. It is unclear whether the use of different plastics, as suggested (HPDE, nylon or polypropylene), would prevent this stickiness.

Slide unit coming apart. The slide manifold coming apart was another issue that was mentioned repeatedly. A better glue might ensure a stronger connection between the two halves of the slide unit. Indeed, after further consultation with the VCRU following this survey, it was discovered that the glue used to combine the two parts of the slide unit had been replaced with an ultrasonic transducer glue in 2019. It will be useful to see whether this continues to be an issue in new kits.

Sand flies. There were two areas of concern related to the testing of sand flies in the WHO test kits. The first aspect was the mesh of the screens that are put inside the screw cap of each tube. This mesh needs to be fine to ensure that sand flies cannot pass through. The other aspect mentioned was the space between the slides and the tubes. A mesh screen with a finer mesh would help to prevent sand flies from escaping from one end of the tube, while the addition of thin plastic strips or pieces of paper on the slide would prevent sand flies from escaping between the slide and the slide unit *(3)*. In addition, the 3D-printed slide unit appeared to provide a tighter fit, preventing sand flies from escaping (Vincent Corbel, personal communication, 2024) (Fig. 5).

Fig. 5. Examples of modifications that may improve WHO test kits for use with sand flies: using fine mesh screen and the 3D-printed slide unit (left) and inserting thin strips of plastic or paper at the sides of the slide to prevent the escape of sand flies (right)

Plastic getting cloudy. Plastic tubes can become cloudy due to abrasive washing, which creates small scratches in the plastic, or due to the use of acetone to clean the tubes. Instructions for the care and cleaning of tubes have been provided to users, specifically to "soak the tubes overnight in a 20% alkaline solution (TFD4 or Decon 90) for equipment in direct contact with the insecticide (e.g. treatment tubes with copper clips, etc.) or in a 10% solution for equipment that did not come in contact with the insecticide but was used for handling the mosquitoes (e.g. holding tubes, steel clips, etc.)... On the following day, rinse the equipment 3 times with tap water and dry at room temperature" *(4)*.

Plastic breaking. While there were a few comments on the durability of the plastic tubes, this does not seem to be a widespread phenomenon. The tubes are made of poly(methyl methacrylate), also known as plexiglass or perspex, which is generally a strong polymer used for many purposes. While the users did not specify how long these tubes had been in use, further investigation is warranted to understand the conditions under which the tubes cracked (e.g. water temperature) and how long the tubes were in use before cracks developed.

Number of clips. Current WHO susceptibility test kits come with 12 tubes (sufficient for testing four replicates of an insecticide with two controls). These come with six metal clips (silver colour) and six copper clips. The current WHO standard operating procedure *(4)* recommends using two steel clips for each of the six holding tubes, two copper clips for each of the two control tubes, and two copper clips for each of the four insecticide exposure tubes. This requires a total of 12 steel clips and 12 copper clips. Since the current kits come with only six steel clips and six copper clips, it does seem reasonable to increase the number of clips to at least 12 steel clips and 12 copper clips. Alternatively, additional clips can be ordered from Universiti Sains Malaysia.

Metal coating of clips. One respondent noted that the coating of the clips had been coming off over time in one facility where tubes were machine washed and dried (at a maximum temperature of 50 °C). The metal clips are made of stainless steel without a coating, so it is unclear what this comment referred to. In any case, standard cleaning procedures should be followed (as noted above).

Colour and material used for the mesh screen at the end of the tube. Some comments related to the use of different colours of mesh at the end of the tubes (held in place by the caps) and the ease of observing mosquitoes. Generally, a lighter colour was favoured for better visibility. The use of a plastic mesh as part of an entirely plastic screw cap/mesh unit was proposed as an alternative. The suitability of this method for ensuring the visibility of mosquitoes (and sand flies, see above) should be assessed.

Chlorfenapyr. Several participants noted that the WHO tube test kits should not be used for testing susceptibility to chlorfenapyr. Currently, WHO recommends the use of the WHO bottle bioassay for the assessment of susceptibility to chlorfenapyr *(5)*, and chlorfenapyr has already been deleted from the WHO tube test catalogue *(6)*.

Larval susceptibility test kit. One participant noted that a larval susceptibility test kit was received with no liquid in the bottle. This is likely a rare occurrence, but larval susceptibility kits should be carefully closed before shipping. Furthermore, the WHO guidance on larval susceptibility testing (for *Anopheles* mosquitoes) has not been updated since 1981 *(7)*, so this should be done in the near future. Interim guidance for entomologists to monitor resistance in *Aedes* mosquito populations (adults and larvae) was published in 2016 *(8)*.

Hot water washing. One of the respondents noted that the tubes warped when washed and dried at high temperatures. The highest temperature in the drying cabinets was 50°C. The respondent suggested either using a plastic that is more temperature-resistant or providing a standard operating procedure that notes the upper limit for temperature during the washing of test kits. It should be noted that high-temperature washing is not the recommended method for cleaning WHO test kits (see above).

Screw mechanism (tightness/glue). One suggestion was made to deepen the threads or increase the number of threads to tighten the fit of the tubes to the slide unit. It is unclear whether these modifications would improve the tightness. Another possibility is to include some information about how much pressure should be applied when attaching the tubes to the slide unit.

Shipping of papers. The timely shipping of papers was mentioned by a few participants. The VCRU of Universiti Sains Malaysia has made considerable effort to improve the shipment of WHO susceptibility test kits, particularly since an external review in 2018. It is unclear whether these comments were based on previous experience or current experience, but it is important for the VCRU to continue shipping test kits and papers as quickly as possible. At the same time, procurers are advised to place orders using the WHO order form downloaded from the WHO website only *(9)* and make advance payment. The VCRU produces papers once payment has been made.

3D printing of pieces. Two respondents mentioned the possibility of 3D printing of test tubes and/or test kit parts. One of these methods has been published *(2)*. The authors noted that WHO kits printed using polylactic acid were able to be used with similar results as the original test kits, and several bioassay solutions did not degrade the printed tubes. While the printing did not allow the tubes to be transparent, this was not a big issue, as the insects are observed through the mesh at the ends of each tube.

4. Conclusion

While survey respondents raised many issues and made many suggestions, the key points that have been addressed or may be addressed in the near future include the following:

- Plastic. It may be that other types of plastic materials could result in higher durability or better movement of the slide in the slide unit. The costs of alternatives will be investigated in the near future.
- Glue. The adhesive used to join the slide unit pieces has been replaced with an acoustic transducer glue, which should result in improved adhesion. Attention should be paid to slide units obtained after 2019 to see whether the change of adhesive has led to improvement.
- Mesh screen. A finer mesh or plastic cover on the screw cap might better prevent the escape of sand flies or mosquitoes. It is also important to ensure that the mesh screen is durable and of a lighter colour/transparent material. As this part of the kit can be easily changed, different materials will be considered for improving the mesh screens.
- Spring clips. At the moment, WHO kits come with six steel clips and six copper clips. It may be better to increase this to 12 steel clips and 12 copper clips to ensure that two clips can be used to hold the paper in each tube.

WHO realizes that the surveillance of insecticide resistance is an essential part of the control of vector-borne diseases. The recommendation to continue seeking feedback on the kits will be followed, and any further suggestions may be emailed to: gmp-vcr@who.int or vve@who.int.

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