

**This is an Annex to the article:**

**Practical management plan for invasive mosquito species in Europe: I. Asian tiger mosquito (*Aedes albopictus*)**

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## ANNEX 1

### Standard Operational Procedures for ovitrap field management

Ovitrap should be placed in shady, safe and easily accessible positions on the ground (if it is not possible at a height of not more than 50 cm above the ground) (Figure 1.1). Gravid females of *Aedes albopictus*, attracted by the dark color of the ovitrap and by the water, lay eggs on the oviposition substrate. Ovitrap must be fixed to a support (e.g. trunks, poles, rods, etc.) using a plastic wrapper to avoid accidental spills (the plastic wrap can be passed through the hole of the trap). Alternatively, they should be backed by hard support (e.g. wall, wall corners, massive stones). Experts must find out the choice of exact position at the first set. The position must be kept fixed during the season and possibly over the years, and the precise description of each location must be given in a specific database (DB).

Only operators that have followed a specific annual training course should perform the ovitrap management. It is estimated that in one working day one operator may manage about 40-60 ovitraps, depending on the size of the monitored area.

The application of this procedure does not involve particular risks to the operator who is required to wear standard personal protective gear (e.g. gloves and highly visible vest).

#### 1.1 Suggested materials for the management of ovitraps

- List and map with the description of the station's location.
- Disposable plastic gloves for the operator.
- Handheld GPS receiver to record the geographical coordinates of each ovitrap.
- Black plastic container of about one-liter capacity. The shape may be cylindrical or sub-conical (more comfortable to transport). Overflow hole positioned at about 2/3 capacity. Optionally completed with a protective net at the top to prevent large materials, such as leaves, from entering the ovitrap.
- Warning waterproof label attached on the ovitrap to provide all necessary information about the activity as well as the contact information (e.g. phone number, company/institute responsible for the ovitrap).
- Commonly used oviposition substrates are strips of Masonite, steamed beech wood, tongue depressor with the staples, seed germination paper, and extruded polystyrene block. In case of vertical strips, length will be the same as the ovitrap height, width about 2.5 cm. One side of the stick has to be corrugated to facilitate egg laying by the female (facing inner space of the container).
- Permanent colour for writing the ovitrap code behind the oviposition substrate (on the smooth/other side facing the wall of the container).
- Big paperclip to fix the oviposition substrate, held vertically and adhered to the ovitrap wall, to the top edge of the ovitrap.
- Water tank to fill the ovitraps with tap water and eventually *Bacillus thuringiensis* var. *israelensis* (B.t.i.) solution or B.t.i. granules to be added directly to the ovitrap<sup>1</sup>.
- Cleaning kit (soft sponge and/or paper towel) to clean the inside of the ovitrap at each inspection.
- Plastic bags or cellophane to hold the collected oviposition substrates (each in a separate bag and all from one sampling locality in a common bag). Particularly suitable are the zip lock plastic bags.
- Paper delivery form.

#### 1.2 Management of the ovitraps

The oviposition substrate must be collected the same day of the week, every 7 or 14 days (depending on the objective and the working capacity; see notes for more details), as follows:

1. Write, with a permanent color (white or yellow), the corresponding code on each new oviposition substrate (e.g. BO501 where BO is the city name and 501 is the progressive code of the substrate);
2. Pour on the ground the water inside the ovitraps (and not in catch basins or other places) and place the oviposition substrate in the plastic bag;
3. Refill the ovitrap with the solution previously prepared and insert the new oviposition substrate (for masonite strip and tongue depressor, the smooth part has to be in contact with the ovitrap wall, and the rough part exposed); fix it with a paper clip to the wall;
4. Store the oviposition substrate in the plastic bag;

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<sup>1</sup> The use of B.t.i. is dependent on the periodicity of ovitrap management (see Notes below for more details). If B.t.i. solution is preferred, this must be prepared on the day of collection and can be made in containers that are used for the transport of water, with a concentration of 1 ml/L.

## ANNEX 1

### Standard Operational Procedures for ovitrap field management

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5. Fill in the delivery form;
6. Once the sampling tour has been completed, fill the bins with water (for the next sampling turn) without closing them, in order to allow the evaporation of chlorine;
7. The B.t.i. solution in the bins must be prepared on the day of the new sampling turn;
8. Collected oviposition substrates must be delivered to the laboratory, together with the record of delivery compiled.

Only oviposition substrates that were in operation for 7/14 days should be taken to the laboratory. For spilt ovitraps or ovitraps completely dry at the time of collection, the oviposition substrates should not be provided at the laboratory. For each undelivered oviposition substrate, state the reason in the delivery form next to the ovitrap code.

#### Notes:

- New ovitraps are less effective for egg collection than ovitraps already in use, and may need to be placed outside for some weeks in order to lose their plastic scent.
- Two methods can be followed during the ovitraps sampling:
  - If the collected eggs need to be hatched, the ovitraps should be filled with tap water (without B.t.i.) and left in the field for only 7 days.
  - If there is no necessity for egg hatching, the operators may fill the ovitrap with the B.t.i. solution and leave them in the field for 14 days. For more details regarding this method, see Carrieri et al.<sup>1</sup>
  - If an ovitrap goes missing from its predetermined position, replace with a new one.
- If the ovitrap disappears or is found totally dry for three consecutive times, find another suitable position close by (within no more than 50 meters from the previous location) and write the geographical coordinates in decimal degree format (eg. 45, DD.DDDD N; 11 DD.DDDD E) using a handheld GPS receiver, a GIS software (e.g. QGIS open source - <http://qgis.org>), or Google Earth (open source - <https://www.google.com/earth>); the assignment of a new code will be completed at the end of the monitoring season, when all data about changed trap position will be gathered.

#### References

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**Figure 1.1.** Example of an ovitrap positioned in the field

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**ANNEX 2**  
**Standard Operational Procedures for egg counting**

The present procedure describes how to count the eggs of *Aedes albopictus* deposited on an oviposition substrate collected from an ovitrap. It is utilized for quantitative monitoring of the species. All of the eggs on the oviposition substrate should be counted. The egg detection and counting on an oviposition substrate are done by a stereomicroscope with a magnification of 20-30X.

The application of this procedure does not involve particular risks to the operator, yet personal protective equipment (e.g. gloves) is still recommended to avoid contact with the oviposition substrate.

Other mosquito species (e.g. native *Aedes geniculatus* and *Aedes cretinus* and invasive *Aedes aegypti*, *Aedes japonicus* and *Aedes koreicus*) or other insects (e.g. Psychodidae) might lay their eggs on the oviposition substrate as well (Figure 2.1). To check for the possible presence of other mosquito species, samples of eggs may be hatched periodically, and larvae examined, or analysis using MALDI-TOF or molecular approaches may be conducted.

Personnel for eggs counting must:

- a) Participate in regular interlaboratory training courses;
- b) Periodically submit to blind double-check to assure quality of the results;

The procedure involves the following steps:

1. Oviposition substrates must be transported individually in plastic bags, transferred to the laboratory, and stored at room temperature with the delivery form attached;
2. Before counting, the oviposition substrates should be kept intact for several days in a dry room or at a refrigerated temperature of 2-4 °C<sup>1</sup> to reduce mold growth;
3. The counting of the eggs must be made over the entire surface of the oviposition substrate (e.g. for strips, also on the edges);
4. If the eggs are superimposed on each other, count the ones in the upper layer first, remove them with a needle tip or a brush, and then count the eggs in the lower layer;
5. Group the eggs under following categories:
  - whole and intact
  - hatched
  - dehydrated/collapsed (flattened, slightly raised edges but with the tubercles on the exochorion still intact)
6. Between counting of eggs on subsequent oviposition substrates, it is essential to thoroughly clean the stage plate of the stereomicroscope on which the oviposition substrate is examined to prevent contamination of the new sample with remaining eggs from the last substrate;
7. In case of a report on the sampling DB that an oviposition substrate was found without water (dry), the eggs should not be counted.

Expression of results is as follows:

- The egg number should be expressed as number of eggs/oviposition substrate.
- If there are no eggs, the result is expressed as "0".
- If the eggs could not be counted because they are covered with dirt, the result is expressed as "-1" (not identified).
- If the ovitraps are found without water, the result is expressed as "no value".

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<sup>1</sup>This procedure is recommended only when egg hatching is not required.

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Standard Operational Procedures for egg counting

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**Figure 2.1.** Eggs of *Ae. albopictus* (a), *Ae. geniculatus* (b) and psychodids (c) (20X magnification)

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**ANNEX 3****Standard Operational Procedures for quality control of *Aedes albopictus* monitoring**

The quality control procedure involves two successive steps of data control.<sup>1</sup>

The first step is based on two evidences:

- a. During the summer months the *Ae. albopictus* population is stable and widespread, thus the chances of an ovitrap with no eggs are rare. A threshold of traps, expected to be negative during the summer, should be established (e.g.  $\leq 5\%$ );
- b. When a similar mosquito control strategy is applied, there is quite a stable relationship between the egg density in a specific city and the egg density on a larger scale (i.e. at district or prefecture level). It is therefore possible to comparatively analyze data to check possible deviation from the expected correlation. To do this, a good historical data set for the particular region is necessary (several years of standardized surveillance).

Specific analysis in quality control should be used for individual ovitrap data obtained from the urban areas, in a case where the areas are larger (LA) or smaller (SA) than 600 ha. Both LA and SA ovitrap data are required for data analysis at the province and regional levels (first step validation), while the LA data alone are processed as mean values per city and help in data analysis at the municipality level (second, third and fourth step validation).

The data prerequisites are as follows (Figure 3.1):

1. Individual ovitrap data collected fortnightly<sup>1</sup>, at least 10 collections per season in the period May-October (raw data);
2. Individual ovitrap data (both LAs and SAs) validated; this is the first validation step (these data will also be used for analysis at the province and regional level);
3. Mean number of eggs/ovitrap in LA (pre-validation); the SA data which satisfy the first step of validation should be kept on standby until the second step of validation;
4. Data of cities LA (validated: second validation step (automatic) and third and fourth validation steps (non-automatic));
5. Data of cities LA (not validated and therefore not included in the historical series);
6. Data of cities LA (validated definitively and included in the historical series).

**Quality control procedure**

- a) Data collection and first validation step

The raw data reported every 14 days by the personnel responsible for the egg counting are submitted for first validation. Only data obtained by ovitraps in normal conditions at the time of inspection are retained (e.g. ovitraps without water at the time of inspection or not in the regular position or missing are not included on the database).

- b) Second validation step

Automatic checks of the valid data produced by the LAs involve two simple algorithms:

1. Calculate the percentage of ovitraps with no eggs,
2. Compare the mean number of eggs/ovitrap in the specific city with the mean number of eggs/ovitrap at the regional level.

The system considers the data valid if:

- the percentage of ovitrap without eggs is below the pre-determined threshold of 5% during the summer months (June, July, August, September). In this period, the *Ae. albopictus* population is widespread, and ovitraps with no eggs are considered rare. In a case where the percentage of negative ovitraps to the total number of ovitraps in a specific city is higher than 5% for two consecutive turns, the data need to be submitted for further validation before being added to the DB;
- the ratio of the mean no. of eggs/ovitrap in a given city to the district/regional mean no. of eggs/ovitrap should not increase above a certain threshold in the following years. This is based on the relationship between the egg density in a city and the egg density on the larger geographical scale, which is usually constant across the years if no important change in the mosquito control strategy has been adopted. The equation of the automatic validation system is:

$$AM = (AM0-AM1) / AM0 > \pm 0.5 \quad (2)$$

<sup>1</sup>The following procedure is based on the method where ovitraps remain in the field for 14 days (see Annex 1 for more details)



## ANNEX 3

### Standard Operational Procedures for quality control of *Aedes albopictus* monitoring

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where

$AM1 = Mc1/Mr1$  (Mc1 is the mean number of eggs/ovitrap in the city obtained at time t1, and Mr1 is the mean number of eggs/ovitrap in the region at time t1).

$AM0 = Mc0/Mr0$  (Mc0 is the mean number of eggs/ovitrap in the city registered at time t0 and Mr0 is the mean number of eggs/ovitrap in the region at time t0).

The value of Mc1 is considered invalid if the value of AM is  $\geq \pm 50\%$  of the value for the previous year for two consecutive turns in the summer period. In that case, the data need to be submitted for further validation before being added to the DB.

#### c. Third validation step

The data considered invalid in the second step are submitted for further validation in the third step. The third step is conducted manually by a skilled technician according to the following specific procedures:

- i. Analysis of the possible local weather conditions which might cause data anomaly (e.g. prolonged adverse weather conditions, big storm, or wind). In a case where the data anomaly is as a result of the weather conditions, the data are considered valid and included on the DB, while in a case where the anomaly is not caused by the weather conditions, the data need to be submitted for further validation.
- ii. Field inspection to check if the correct ovitrap management procedures are observed. This involves checking the exact ovitrap position, checking the quantity of water in the ovitrap, and checking if the oviposition substrate is placed accurately in the ovitrap. In a case where the inspection shows incorrect field management, the data are considered invalid and excluded from the DB. If the inspection shows correct field management, the data need to be submitted for further validation.
- iii. Double-check of the number of eggs on the oviposition substrates. This is conducted by independent technicians on random samples of oviposition substrates. If the double-check shows significant errors in the egg counting, the data are considered invalid and removed from the DB. In a case where the double-check shows correct egg counting, the data are submitted for further validation (fourth step of validation).

#### d. Fourth validation step

This last step is performed only in a case where the previous steps showed evidence of invalid data. In this step, human landing collections (HLC) are conducted in the city to check the correlation between the number of biting females and the number of eggs. This is possible because previous studies confirmed the good correlation between these two population indices (Carrieri et al. 2012). The HLC is organized on the same grid which serves the ovitrap positioning. On each grid cell, a technician finds a suitable position to conduct the HLC session for 15 min, usually in a green public area. The HLC sessions are conducted in the late afternoon on a day the weather conditions are good, during the period of maximum female activity, by five technicians using a manual aspirator. Each technician performs four HLC sessions on the different cells during the 90 min of *Ae. albopictus* peak activity to obtain 20 HLC data. From the mean number of females collected in the 15 min, it is possible to estimate the expected number of eggs using the equation (2):<sup>2</sup>

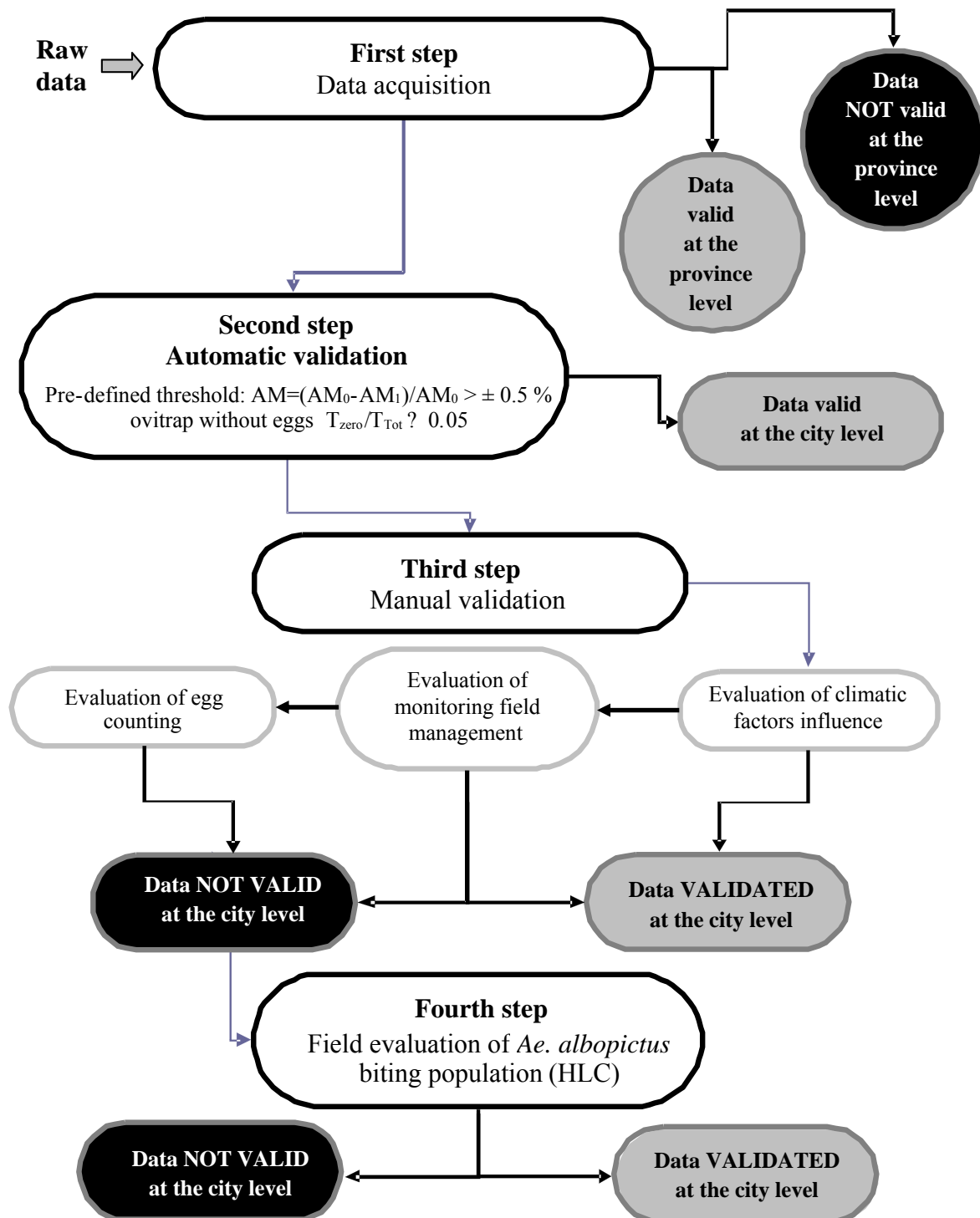
$$\text{Expected N. eggs} = 111.52 \text{ N. Females} - 6.33 \quad (2)$$

Before being used, this equation should be validated and eventually adjusted to the local condition.

In a case where the expected number of eggs are compatible with the mean number of eggs collected (in the confidence limits of 95%), the egg data are considered valid and included on the DB. If there is significant difference between the expected number of eggs and the mean number of collected eggs, the data are considered invalid and definitively removed from the DB.

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**Figure 3.1.** Procedure for the quality control of *Aedes albopictus* ovitrap-based monitoring data (Carrieri et al. 2017, modified).

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**Practical management plan for invasive mosquito species in Europe: I. Asian tiger mosquito (*Aedes albopictus*)**

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**ANNEX 4**

**Standard Operational Procedures – Template for mayor ordinance**

Subject: Measures for the prevention and control of diseases transmitted by insect vectors, particularly by the Asian tiger mosquito (*Aedes albopictus*)

WHEREAS: *[add relevant documents/information]*

**ORDER**

**To owners, managers, and all responsible and involved who have structured areas with rainwater collection systems (private citizens, condominium managers, companies that manage areas of shopping centers, operators of multiplex cinemas, etc.):**

1. In open public and private spaces, including terraces, balconies and flat roofs, remove containers of any kind and size in which rain and irrigation water may collect and avoid any collection of stagnant water even temporarily.
2. If containers are not abandoned but under the control of those in ownership or in actual use, proceed to empty any water contained in them and relocate them to avoid accumulation of water from rain and irrigation. Otherwise, proceed with placing either a mosquito netting or a tight-fitting lid over the containers. Alternatively, the containers should be empty daily; avoiding discharging the water in the drains (these requirements do not apply to ovitraps).
3. In the presence of storm drains, drain grates, and/or catch basins of storm water, treat them with products with proven larvicidal efficacy. The frequency of treatment must be in accordance with the type of product used, i.e. as indicated on the label; irrespective of the periodicity of the treatment, it should be applied after every heavy rain. Alternatively, cover the manhole lids, drain grates, and/or catch basins of stormwater with mosquito netting, which must be suitably maintained and kept free of leaves and debris to allow water runoff.
4. Keep courtyards and open areas free of weeds and rubbish of all kinds and take actions to prevent accumulation of rainwater or any other water source.
5. Empty fountains and pools when not in operation or perform adequate treatment with larvicides.

**To the public and private operators responsible for embankments, slopes and roadsides, waterways, wasteland, and abandoned areas:**

1. Keep the areas free of weeds, trash or other materials that might facilitate the collection of stagnant water.

**To all gardeners:**

1. Perform watering in such a manner as to avoid any accumulation of water. If watering is done with a container, the container itself must be wholly emptied after use.
2. Store all containers and other materials (e.g. plastic sheets) in a position they cannot collect water from rain and irrigation.
3. Close any water tank with tight-fitting lids.

**To owners, managers, and all those who are responsible for warehouses and industrial activities, crafts and trade, with particular reference to the events of destruction in general and materials stored for recycling:**

1. Take all effective measures to prevent materials from collecting water, such as by storing the materials indoors where they are protected from the rain, or by covering them with tarpaulin fixed and made firm to avoid collection of water in the folds and depressions. Otherwise, empty the collected water after each rain.
2. For materials stored outdoors, ensure that control treatments are performed within 3 (three) days of any rain during summer months.

**To managers of stores for tire repair, recycling and sale, and to holders of tires in general:**

1. Store the tires, after being emptied of any water collected in them, indoors or in containers with lids, or if outdoors, protect them with plastic sheeting in such a way as to prevent the collection of water on the sheetings themselves.
2. Empty the tires of any water before handing them over for recycling and marketing.
3. For tires stored outdoors, ensure that control treatments are performed within 3 (three) days of any rain during summer months.

**To managers of building sites:**

1. Avoid water collection in areas of excavation, cans and other containers; if the activity requires the availability of containers with water, they should be provided with hermetic covering or must be emptied at regular intervals not exceeding five days.

## ANNEX 4

### Standard Operational Procedures – Template for mayor ordinance

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2. Secure the equipment necessary for the activity to avoid water collections.
3. If work is suspended on the construction site, ensure the management of land and materials to prevent the accumulation of rainwater.
4. For the materials stored outdoors, ensure that control treatments are performed within 3 (three) days of any rain during summer months.
5. The bases of the temporary fences of construction sites should not collect rainwater in spare holes.

#### **To the public and private operators, managers or whoever is responsible for containers (bins) and the collection of municipal solid or other waste:**

1. Store bins, after being emptied of any water collected in them, indoors or in containers with lids, or if outdoors, cover them with plastic sheeting such that no water collects on the sheets themselves.
2. Empty the bins of water before handing them over for repair and marketing.
3. Ensure, in situations where the measures mentioned above are not applicable, the removal of potential larval breeding sites and adequate mosquito control treatments. The frequency of control treatments and removal of breeding sites depends on the product used, though in rainy periods they should be carried out within 3 (three) days of each rainfall.

#### **To all owners, operators and managers of nurseries, greenhouses, plants storages, and farm sites near towns:**

1. Perform watering such that there is no accumulation of water; for manual watering, the container must be filled from time to time and completely emptied after use.
2. Keep all containers and other materials (e.g. plastic sheets) such that they do not collect water from rain and irrigation.
3. Close any water tank with tight-fitting covers.
4. Carry out appropriate checks and treatments of the containers for plant cultivation and marketing.

#### **In the cemetery:**

1. If possible, fill the fake flower vases with sand.
2. Make available a container of water containing larvicide product.
3. Maintain all containers used occasionally (e.g. small watering cans) such that they do not collect water from rain and irrigation.

#### **RULES ON PENALTIES**

Failure to comply with the provisions of this ordinance is sanctioned by the municipal regulation *[add relevant documents/information]*

#### **OBLIGATIONS OF THE SERVICE OFFICERS**

It is the responsibility of the municipal police force, the public health authority, and other agents and judicial police officers duly authorized by the provisions in force to ensure execution of and compliance with the provisions of this Ordinance, to supervise and investigate the execution, and to impose sanctions where applicable.

#### **ADDITIONAL OBLIGATIONS OF ALL INVOLVED**

That in the presence of suspected or confirmed cases of dengue, chikungunya and Zika, or situations of localized infestations of particular consistency, with associated health risks, particularly in areas around sensitive sites such as schools, hospitals, homes for the elderly or the likes, the City Council must be allowed to carry out adulticide and larvicide treatments and removal of larval breeding sites in public and private areas, providing, where appropriate, separate and additional orders and urgent actions to enforce the these treatments against recipients specifically identified.

#### **BENEFICIAL ACTIONS FOR COMMUNITY ENGAGEMENT**

Regular information campaigns using the most appropriate channels and methods should be considered for the mayor ordinance empowerment. Table 4.1 summarizes actions that could help improve community participation.

**ANNEX 4**  
**Standard Operational Procedures – Template for mayor ordinance**

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**Table 4.1** Summarized beneficial actions for the community engagement

<b>Tool</b>	<b>Description</b>
Brochure	To provide information on the biology of mosquitoes, methods of control and personal protection
Posters and flyers	To be posted in the public health units, pharmacies, garden shops, waiting rooms of public places, bus stops, allotment garden complexes, cemeteries, etc.
Web page (or website)	Usually a specific web page (or a website if possible) within the websites of responsible public administrations
TV spots (video clips)	Themed public meetings held by experts should be broadcast on local stations.
Involvement of neighbourhood associations	Mosquito control activities may be included in local management actions
Teaching in school	Specific intervention with practical field experience in primary and secondary schools
Stands in local markets and in public events	Useful for direct contact with people and for the distribution of materials and products

## ANNEX 5

### Standard Operational Procedures for emergency vector control in case of dengue, chikungunya and Zika detection

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## ANNEX 5

### Standard Operational Procedures for emergency vector control in case of dengue, chikungunya and Zika detection

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### Standard Operational Procedures for emergency vector control in case of dengue, chikungunya and Zika detection

In areas where *Aedes albopictus* is present and imported cases (suspected or confirmed) of chikungunya, dengue or Zika fever have been detected, it is necessary to perform immediate and capillary mosquito control activities, with the aim of possibly eliminating already infected mosquitoes, starting the activities within 24 hours of reporting the case (see Table 5.1 for all the actions/activities).

#### Area definition

- For single case detection (identified and defined by the Department of Public Health), the area to be disinfested corresponds to a buffer with a radius of **100 meters** around where the case has recently spent most of his/her daytime;
- For cluster cases (two or more cases), identified and defined by the Department of Public Health, the area to be subjected to mosquito control should be extended to **300 meters** buffer from the most peripheral cases of the outbreak itself, as well as the rest of the entire outbreak area (area delimited by the cases).

**Mosquito control execution:** Mosquito control activities include three actions to be conducted in a synergistic way: treatments with adulticide(s), treatments with larvicide(s), and removal of larval breeding sites.

The optimal sequence in which these processes should be conducted is:

- Adult treatments in public areas during the early morning hours;
- Adult and larval treatments and source removal in private areas (door-to-door);
- Contextual larval treatment in public catch basins.

**Products:** Pyrethroids are particularly suitable for adulticide interventions. For larvicide interventions, diflubenzuron, *Bacillus thuringiensis* var. *israelensis* and liquid surface films (e.g. Aquatain™) may be used.

**Equipment:** Depending on the accessibility of the areas to be treated, atomizer for vehicle-mounted spraying (when inaccessible) or handheld sprayer (when accessible) can be used.

**Places to treat:** Treatment must be directed at the vegetation (hedges, bushes, shrubs), up to three to four meters high, in public and private areas. For roads, treatments for both the left and right sides, possibly with the double pass, should be carefully conducted.

**Repetitions:** Adulticide treatments on public roads should be repeated for three consecutive early mornings. In case of heavy rain, the three repetitions should be completed at the end of the precipitation.

#### Warning:

- The treatment must be performed in the absence of people and animals.
- During a storm or breeze with wind speed more than 2-3 m/sec, treatments should be suspended until suitable weather conditions. Fog or rain of low intensity do not affect the outcome of the intervention.
- The treatments are conducted guaranteeing the absence of people; therefore, in public areas early morning hours are preferable.
- The inhabitants of the target areas should be forewarned of the date and time of the intervention, to allow them take measures to reduce exposure to insecticides and to facilitate the entrance of operators in their properties.

In a case that a municipality does not have any operational service in place, legislation of hiring the PCO under emergency should be included in the bill of the administrative unit/region/country as soon as possible before the need for the vector control is evident and, preferably, before an outbreak.

If the local density of *Aedes albopictus* is not known, mosquito surveillance using BG traps baited with attractant and CO<sub>2</sub> should be conducted immediately. Results will inform of the need for mosquito control treatments, and mosquito samples should be submitted to be analyzed for arboviruses.



## ANNEX 5

### Standard Operational Procedures for emergency vector control in case of dengue, chikungunya and Zika detection

**Table 5.1.** Emergency control measures for imported cases (suspected or confirmed) of DENV, CHIKV and ZIKV in areas with *Aedes albopictus*

Measure	Process	Description
Determination of area to be treated	Single case detection	The area to be disinfested corresponds to a buffer with a radius of at least 100 meters around the place/s where the case has recently spent most of his/her daytime.
	Cluster case detection	When a cluster of two or more cases is identified by the responsible authorities, the area to be subjected to mosquito control should be extended to 300 meters buffer from the peripheral cases of the outbreak, as well as inside the entire outbreak area.
Mosquito control activities	One turn of adult and larval treatment and source removal in private areas (door-to-door)	Treatment should be conducted by a team of two operators, one equipped with a portable atomizer producing aerosol droplets of size 50-100 $\mu\text{m}$ or hot fogging atomizer producing more effective droplets of size 5-30 $\mu\text{m}$ using pyrethroids, the other equipped with portable manual pump performing larviciding and source removal.
	Up to three adulticide treatments in public areas in consecutive days	Treatment by truck-mounted atomizer 30-60 HP, producing cold aerosol with droplets of size 50-80 $\mu\text{m}$ , using pyrethroids, during early morning hours.
	One larvicide treatment in public areas	Treatment by portable manual pump using larvicide with immediate action mechanism (i.e. surface layer).
Biocidal products	Adulticide & larvicide	It is essential to use only biocides with marketing authorization in the country, in accordance with the instructions of use that provides efficacy already demonstrated and proven in local conditions.
Precaution	Adulticide	Before starting the adulticide treatments, the resident should be notified to take adequate precautions (i.e. flyers or letters describing the details of timing should be disseminated in the target area). The treatments in public and private areas should be applied on the foliage (bushes, hedge) up to approximately 3-4 meters high. In addition, for treatments applied from the roads, the biocide should be spread consistently on both sides. Special attention should be paid in cases of adulticide treatment in the vicinity of apiaries and surface waters. In accordance with certain national legislations, note that the presence of the synergist piperonyl butoxide in pyrethrin-based formulations is prohibited on organic crops and a 50 m no-treatment zone (reduced to 25 m for foot treatment) must be respected. Operators should be protected to avoid being bitten by mosquitoes.

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## ANNEX 6

### Standard Operational Procedures for quality control on larval treatment of road drains

It is suggested to conduct quality control on the treatments by pest control companies (PCOs) or public employees. Typically, quality control activity is undertaken by an organization that has knowledge of and the know-how in entomology and pest control science, and should be performed by skilled technicians, independent (with no conflict of interest) of the entity performing the mosquito control.

#### Number of catch basins to control

The minimum significant number of catch basins to assess in a municipality is proportional to the total drains present and covered in each turn of treatment, as follow:

- Municipalities or sectors with the number of public drains  $\leq 2,000$  = control of 20 drains
- Municipalities with the number of public drains  $>2,000$  = control of 1.0 % of drains

Technicians appointed for quality control should not consider catch basins that prove to be dry or those that cannot be opened (the record of dry drains will nevertheless be of some usefulness in order to correctly estimate the percentage of manholes that, as regularly with the water, have the potential to contribute to mosquito infestation).

To limit the costs, quality control can be conducted on selected turns of treatment (at least 2 (two) turns of treatment should be subjected to quality control during the season). For the chosen turns of treatment, the quality control should be planned according to when there is a higher density of larvae (mid-summer months).

#### Location of drains to control

The treatment of the target area must be completed in no more than two consecutive working days. For large target areas, it is essential to define operational unit areas to allow better planning of the quality control session (usually each operational unit should include 1,000-4,000 drains).

The number of catch basins to be inspected should be defined accordingly with the overall number of catch basins present in each operational unit (usually 20-40 drains should be inspected per operational units).

For the inspection, technicians should select the catch basins distributed on both sides of the road and not too close to each other. It is recommended to inspect catch basins in shaded positions to optimize sampling effort (e.g. under vegetation).

The PCO or the public operator in charge of treatments is required to provide timely reports about the operational units completed and the list of streets treated by each operator. This organization of information flow is necessary in order to keep a known interval between treatment and quality control operations.

#### Timing of sampling

The entity performing larval control is required to provide daily reports to the Municipality with detailed data about the operational unit areas and the list of streets treated by each operator. In this way, it will be possible to plan the appropriate time for the quality control inspection.

The correct timing for the quality control will be established based on the insecticide used.

In the case of larvicides with immediate action such as B.t.i., the inspection of the drains can be scheduled in the time frame of 2-7 days post-treatment. In the case of Insect Growth Regulator-based products (IGR) such as diflubenzuron, methoprene and pyriproxyfen, B.t.i. + *Lysinibacillus sphaericus* (L.s.) based products, or surface layer, inspection should be performed in the time frame of 10-20 days post-treatment.

#### Sampling techniques

*In the case of treatments performed with larvicides with mode of action expressed on the immature phases (Diflubenzuron, Bacillus thuringiensis var. israelensis, or water surface film formulations).*

Once the catch basin has been opened, wait 30-60 seconds before starting the sampling, to give time for any larvae/pupae that have escaped to the bottom, as a result of the disturbance caused by the opening of the manhole, , to come back to the water surface.

During sampling, the technician must avoid shadowing the water, since this might disturb the larvae/pupae and cause them to move to the bottom.

The immature collection will be conducted with an aquarium net, which will be held upright and moved under the water surface according for a complete circle.

After the sampling, a volume of 500-1000 ml of water must be taken with a dipper from the same catch basin and poured in a white plastic tray, where the content of the water net will be emptied. The large surface area and the white background of the tray will facilitate the observation of the larvae even for very turbid water.

The treatment will be considered well done ("drains not infested") when no larvae/pupae are found or when only first-stage larvae are present.

In a case where larvae and/or pupa are still present after treatment, reporting the species should be considered.

## ANNEX 6

### Standard Operational Procedures for quality control on larval treatment of road drains

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*In the case of treatments performed with larvicides with delayed action (Methoprene or Pyriproxyfen).*

When using products that determine delayed mortality, especially at the pupal stage, it will be necessary to take a sample of 20-30 late L<sub>4</sub>-pupae from each catch basin. This sampling must be performed gently using first a dipper and then a pipette, avoiding the use of the water net that would damage the sample and thus influence the subsequent rate of adult emergence. Washing the equipment after each sampling is recommended to avoid contamination. The specimens collected should be placed in a plastic cup with screw cap filled with 100-150 ml of water from the same catch basin. The samples should then be kept in insulated containers and transported to the laboratory where adult emergence will be observed daily. The plastic cups have to be signed with a reference code and covered with tulle. Adults only partially freed from pupal exuviae, or freed but unable to fly away, should be considered as inhibited.

The treatment will be considered as well done ("drains not infested") when no flying adults are observed.

If larvae and/or pupae are present, the species should be determined and reported.

#### Data collection in the field

Data collection in the field should be assisted by a paper form, or an electronic database if smartphones or tablets are used.

#### Outcome of the quality checks

Field data recordings will be better supported by electronic databases, especially when smartphones or tablets are used. The presence of larvae/pupae can be expressed only in terms presence/absence and not quantitatively. The data should be reported separately for *Ae. albopictus* and other mosquito species that can be found in the drains (i.e. *Culex pipiens* and *Culiseta*). A threshold of the maximum allowed rate of drains found with third-/fourth-stage larvae and/or pupae (untreated drains) should be pre-fixed as a mandatory requirement in the PCO tender (i.e. 5%). In the case of treatment with B.t.i., B.t.i. + L.s., or diflubenzuron, this percentage should be calculated as the number of drains found with old larvae/pupae to the total number of drains with water inspected in a sector.

In the case of methoprene and pyriproxyfen treatments, a maximum rate of emerging adults from the total number of larvae/pupae collected must be pre-fixed as well for each drain (i.e. 5%). The drains showing adult emerging rate above 5% should be regarded as untreated and included in the calculation of the allowed threshold.

Above the permitted percentage value, the catch basins must be considered untreated or inadequately treated, and corrective actions agreed by parties involved during the tender procedures should be executed. SOP for quality control on larval treatments of road drains are summarized below:

- The evaluation of the larval presence should be expressed only in terms of quality (presence-absence) and not of quantity.
- The data should be reported separately by species (*Aedes albopictus*, *Culex pipiens*, etc.).
- The maximum admissible percentage of infested drains is equal to [.....5%]. This percentage should be calculated for each operational unit as:

$$\frac{\text{N. drains infested}}{\text{N. total drains sampled}} \times 100$$

#### Safety aspects and prevention

In order to reduce the risks associated with specific activities of quality control, technicians must comply with the following requirements:

- Work in pairs.
- Use personal protective equipment, such as safety shoes with soles and toes reinforced, sturdy gloves, high visibility vest.
- Utilize a specific and approved tool to lift and replace the grate.
- Open and sample just one catch basin at a time, always positioned by the sidewalk.

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**ANNEX 7****Standard Operational Procedures for quality control on emergency treatments**

Quality control on emergency treatments performed by Pest Control Operators (PCOs) or public employees, following the detection of imported case(s) of dengue, chikungunya or Zika, is recommended. Typically, quality control activity is undertaken by organizations that have knowledge and expertise in entomology and pesticide science, and should be performed by skilled technicians, independent (with no conflict of interest) of the entity performing the mosquito control.

**Procedures for QC on larval treatments in public areas:**

- The number of catch basins to be inspected in the 100 m buffer treated public area should be  $\geq 10$  (apply the same procedures given in Annex 6).
  - The drains to control should be in shadow and less visible.
  - The sampling can take place any time during the day by applying the same procedures given in Annex 6.
- Outcome of the quality checks of road drains: no road drain is admitted if it harbors larvae/pupae.

**Procedures for QC on larval control of private properties:**

- The number of properties to be inspected in the 100 m buffer treated area should be  $\geq 10$  (by choosing properties with vegetation and large backyards).
- All the breeding sites in the selected properties should be checked.
- The checking and sampling can take place any time during the day.

Outcome of the quality checks of private properties: no breeding site is admitted if it harbors live larvae/pupae.

**Procedures for QC on adult treatments (of public and private areas):**

For adulticide treatments, inspectors should check if residents were notified of the emergency situation in the target area and ask if they were adequately informed prior to the mosquito treatments. To check if there are more adult mosquitoes, BG-Sentinel® traps (Biogents, AG Regensburg, Germany) baited with dry ice, or the human landing collection (HLC) method, may be used. The HLC method is usually more efficient in terms of collection by unit of time, but technicians should be skilled to avoid being bitten. In more details, the following requirements should be considered:

- Adult mosquitoes are able to fly by definition and may move into the treated area from the outside.
- *Aedes albopictus* adult collection should be conducted by BG-Sentinel® traps and/or human landing collection (HLC) in the 100 m buffer treated area.
- At least three (3) BG-Sentinel traps and three (3) HLC sessions (each session lasting for at least 15 min) should be performed by choosing locations with vegetation and in full shadow.
- During HLC, the technicians should avoid being bitten.
- Sampling of adults can take place any time during the day.

Outcome of the quality checks of private properties: no adult mosquito is admitted being collected (in the case *Ae. albopictus* adults are collected, analyzing them for the presence of the imported virus is recommended).

**Safety aspects and prevention**

In order to reduce the risks associated with specific activities of quality control and the risk of acquiring diseases, technicians must comply with the following requirements:

- Work in pairs.
- Use personal protective equipment such as safety shoes with soles and toes reinforced, sturdy gloves, and high visibility vest.
- Use a specific and approved tool to open the catch basin.
- Open and sample just one catch basin at a time, always positioned by the sidewalk.
- Take precautions against being bitten by the mosquitoes while performing HLC.

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## ANNEX 8 Standard Operational Procedures for bioassays

The goal of this activity is to verify the sensitivity status of the *Aedes albopictus* (insecticide resistance) target population to larvicidal products such as *Bacillus thuringiensis* var. *israelensis*, *Lysinibacillus sphaericus*, or Insect Growth Regulators (diflubenzuron, pyriproxyfen or methoprene) and to assess the quality of the products used.

### Materials and methods

It is necessary to collect about 2,000 eggs in each designated locality, to hatch the eggs, and to rear the larvae in standard laboratory conditions. Old L<sub>3</sub>/early L<sub>4</sub> larvae should be used. They should be placed in transparent plastic glasses (PET) of 300 ml capacity. The temperature should be kept at 27 ± 1°C, with a photoperiod 16:8 h and relative humidity of RH 90 ± 5%. The larvae should be fed 10 mg/l of cat biscuit FRISKIES® (typically used in rearing). Deionized water at the laboratory temperature should be used. Four glasses, each containing 25 larvae, are prepared for each dose to be tested and for the non-treated control. The glasses should be marked externally with a pen at the level corresponding to 100 ml; then about 20 ml of water is added together with the larvae that were previously removed from the field containers. Finally, additional water is added to reach 100 ml.

The same procedure is applied in testing larvae from the colony known to be fully sensitive to the product. In any case, it is necessary that the product(s), against which the sensitivity must be tested, are of certified quality and exact concentration.

### Assay preparation for microbiological products

A minimum volume of 1 ml or weight of 1 g should be used for the preparation of the stock solutions to minimize risks of poor homogeneity of the sample. The following example uses 1 g of product at 1200 ITU.

#### MOTHER SOLUTION (MS)

Add 1.000 g of the product (1200 ITU) in 250 ml of deionized H<sub>2</sub>O

Mother solution is: MS 4 mg/cc (or 4 g/L)<sup>1</sup>

#### DAUGHTER SOLUTION (DS1)

Add 10 cc of MS (40 mg) in 250 ml of deionized H<sub>2</sub>O

Daughter solution is: DS1 0.16 mg/cc (or 160 mg/L)<sup>2</sup>

#### NEPHEW SOLUTION (NS, DAUGHTER DS2)

Add 25 cc of DS1 (4 mg) in 250 ml of deionized H<sub>2</sub>O

Nephew solution is: NS 0.016 mg/cc (or 16 mg/L)<sup>3</sup>

### DOSES

In glasses containing 100 ml of deionized H<sub>2</sub>O with 25 larvae III – IV

Doses prepared with the NS:

A – 1.5 cc	= 0.024 mg ↔ 0.24 mg/L <sup>4</sup>
B – 1.0 cc	= 0.016 mg ↔ 0.16 mg/L
C – 0.75 cc	= 0.012 mg ↔ 0.12 mg/L
D – 0.5 cc	= 0.008 mg ↔ 0.08 mg/L
E – 0.25 cc	= 0.004 mg ↔ 0.04 mg/L
F – 0.1 cc	= 0.0016 mg ↔ 0.016 mg/L

The six doses should provide a mortality rate between 1% and 99%, of which at least four doses must be between 10% and 90%. The dose is administered in each glass using micropipettes of variable volume. The larval mortality is determined by counting live and death larvae 24 h after treatment.

If pupation occurs or if control mortality exceeds 5% at 24 h control, the test is considered invalid.

When the observed mortality is within the expected range, no more test is necessary. When observed mortality is out of the predicted range, up to three replications should be conducted on different days to determine the sensitivity of the product tested. Every time, a new suspension must be prepared starting with the original product.

The three replications are considered valid if the power values obtained give to the analysis of variance a coefficient of variation <20%. If the ratio of variation is higher, two more replications should be done, and so on.

---

<sup>1</sup> 1000 mg/250 ml = 4 mg/cc

<sup>2</sup> 40 mg/250 ml = 0.16 mg/cc

<sup>3</sup> 4 mg / 250 ml = 0.016 mg/cc

<sup>4</sup> (0.024 mg / 100 ml) x 1000 = 0.24 mg /L



## ANNEX 8

### Standard Operational Procedures for bioassays

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The LD<sub>50</sub> is calculated for each replication through data processing with Log Probit.

The resistance rate (RR) is determined using the formula:

$$RR = \frac{LD_{50} \text{ (mg /L) field population}}{LD_{50} \text{ (mg /L) sensitive colony}} \quad \text{or} \quad \frac{LD_{90} \text{ (mg /L) field population}}{LD_{90} \text{ (mg /L) sensitive colony}}$$

#### Assay preparation for IGR products

Because of the delayed effect, the efficacy of IGR-based products (i.e. diflubenzuron, methoprene, pyriproxyfen) must be considered based on their ability to inhibit adult emergence (IE) instead of to cause direct larval mortality.

#### MOTHER SOLUTION MS

Add 1.000 gr of water-soluble formulation (i.e. 15% active ingredient) in 1000 ml of deionized H<sub>2</sub>O

Mother solution is: MS 0.15 mg /L (or 4 g/L)

#### DAUGHTER SOLUTION DS1

Add 10 cc of MS in 1000 ml of deionized H<sub>2</sub>O

Daughter solution is: DS1 A 0.0015g/L

#### DOSES

In glasses containing 100 ml of deionized H<sub>2</sub>O with 25 larvae L<sub>3</sub>-L<sub>4</sub>

Doses prepared with the DS1:

A – 0.01 cc = 0.00015 mg /L

B – 0.02 cc = 0.0003 mg /L

C – 0.05 cc = 0.00075 mg /L

D – 0.15 cc = 0.00225mg /L

E – 0.4 cc = 0.006mg /L

F – 1 cc = 0.015mg /L

Six prefixed doses are used to provide IE between 1% and 99%, of which at least 4 must be between 10% and 90%. The dose to be tested is administered in each glass using micropipettes with a variable volume. The IE is estimated by counting and removing from the glasses death larvae, death pupae and death adults, as well as live adults, every 24 h post-treatment until no more specimens are present.

When control mortality exceeds 5% at 24 h control, the test is considered invalid.

When the observed IE is in the expected range, no more test is necessary. When the observed IE is out of the predicted range, up to three replications should be conducted on different days to determine the sensitivity of the product tested. Every time, a new suspension must be done starting with the original product.

The three replications are considered valid if the mortality rate values obtained give to the analysis of variance a coefficient of variation <20%. If the coefficient of variation is higher, two more replications will be executed, and so on.

The LD<sub>50</sub> is calculated through data processing with Log Probit.

The resistance rate (RR) is determined using the formula:

$$RR = \frac{IE_{50} \text{ (mg /L) field population}}{IE_{50} \text{ (mg /L) sensitive colony}} \quad \text{or} \quad \frac{IE_{90} \text{ (mg /L) field population}}{IE_{90} \text{ (mg /L) sensitive colony}}$$

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## ANNEX 9

### Standard Operational Procedures – Public tender template for the selection of Pest Control Operators

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## ANNEX 9

### Standard Operational Procedures – Public tender template for the selection of Pest Control Operators

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**Article 1**

## Types of intervention

- a) Periodic larvicide treatments of catch basins in public areas, including those pertaining to public buildings as indicated by the Client.
- b) Adulticide treatments in specific sites, according to the opinion of the local public health district.
- c) "Door-to-door" interventions, including larvicide treatments of permanent breeding sites, removal of active and potential larval breeding sites, to be conducted within private properties.

**Treatments referred to in point (a) (larvicide)**

Interventions should be made based on the list of urban areas, agglomerations and fractions provided by the "Customer"<sup>1</sup> (see *maps and list of streets*). Each treatment cycle must be completed in [*number of working days*] from the start date, except in the case of a duly documented "force majeure".

Treatments should be performed in the period [*e.g. April to October*] of each calendar year. There will be [*number of larvicide cycles for each year*] covering a number of catch basins, presumed equal to [*number of catch basins*], susceptible to update even during the work. The organization of the service and all contracts necessary for its proper conduction are entirely the responsibility of the Company in charge. The operational units must be equipped with a hand sprayer and GPS device able to transmit in real time the position of the unit during the working time. A liquid or granular formulation of [*registered product for mosquito control in country/EU*] is required. All the public drains located in streets, squares, parks, along road, around perimeters of roundabouts, along bicycle and pedestrian paths, and in public facilities must be treated (included those apparently dry). Furthermore, the catch basins, apparently closed but collect water, must be treated. A daily report indicating the list of streets treated the same day must be described by email to the "Customer" within 24 hours from the end of the treatment. The report should include: the date of intervention, the name of the operator(s), the list of streets treated with the respective number of drains treated, the name and the dose of the insecticide. The "Company" must promptly report all the situations that have hindered the normal course of work<sup>2</sup>.

It is the responsibility of the "Customer" to update the list of streets, squares, parks, and structures pertaining to the municipality, according to the actual needs, in the service of the pest control contractor and on the basis of new territorial evidence. In this case, the estimated value of the contract will be changed proportionally.

(See also "Article 2" for the composition of the operating units and equipment required).

**Treatments referred to in point (b) (adulticide)**

Adulticide treatments will be performed on the specific request of the "Customer" only, targeting specific sites or areas where cases of dengue, chikungunya or Zika have been reported. The request will indicate precisely the locations to be treated and the time of execution of the operation.

The area to be treated may vary in size and characteristics (i.e. parks, gardens, nursery schools, gardens and private courtyards, etc.), thus requiring specific treatment methods.

(See also "Article 2" for the composition of the operating units and equipment required).

The "Company" is required to enter into service within 24 hours from notification, 7 days/week, even on pre-holidays and public holidays, unless otherwise requested by the "Customer" via email.

Before the treatment, the "Company" is required to put up a notice in the area mapped out for treatment, with information about the exact time of treatment and the product to be used in order to allow residents to take measures to reduce exposure to the insecticides. The operational units must be equipped with a GPS device which is able to transmit in real time the position of the group during the working time. The operational unit speed should be in the range of 5-8 km/h, and treatment should be stopped in case of wind speed higher than 8 km/h.

A specific report indicating the list of streets treated during the treatment must be sent by email to the "Customer" within 24 hours from the end of the treatment. The report should include:

- the date of intervention,
- the list of streets/areas treated,
- the name and the dose of insecticide,
- the name of the operator(s),
- the number of hours required, and
- the equipment used.

The "Company" must promptly report all the situations that have hindered the ordinary course of work.

<sup>1</sup> "Customer" could be the Municipality, the Regional Unit, the Region, or other institution/organization responsible for mosquito control in the targeted area

<sup>2</sup> "Company" could be any company awarded the contract of service

**Treatments referred to in point (c) ("Door-to-door" interventions)**

Door-to-door (DtD) interventions consist of larvicide treatments of active and potential breeding sites, source removal, and direct information given to the citizens on the private properties on which the treatment would be carried out (yard, garden, vegetable garden, parking, etc.).

These interventions will be performed in areas specified by the "Customer" with a notice of [number of days] days.

A specific report indicating the list of streets and house numbers treated must be described by email to the "Customer" within 24 hours from the end of the treatment itself. The report should include:

- the date of intervention,
- the list of streets/areas treated including the house numbers,
- the name and the dose of the insecticide,
- the name of the operator(s),
- the number of hours required, and
- the equipment used.

The "Company" must promptly report all the situations that have hindered the ordinary course of work.

Moreover, the daily report must include the signature of the citizen who has approved access to the property.

The organization of the service and all contacts deemed necessary for the proper conduct of the treatment are entirely the responsible of the "Company" (See also "Article 2" for the composition of the operating units and equipment required).

**Article 2****Operators, products, equipment**

The "Company" will have to provide to the "Customer" with the names and telephone numbers of the Technical Director in charge of coordinating the work in question and of the operators involved in the mosquito control campaign. The Company must ensure maximum staff stability and promptly inform in case of possible replacement of operators. The "Customer", upon justified request, may demand the replacement of some operators with other operators with proven experience and ability.

Interventions must be executed in accordance with the law, ensuring minimal environmental impact. All operators should be equipped with personal identification cards, use personal protection devices and high visibility vests (waistcoats), with the name of the "Company" and the words – "*Treatment against the Asian tiger mosquito*" or "*Treatment against mosquitoes*" – explicitly indicated on them.

**Treatments referred to under larvicide**

The products used for the larvicide treatments should be based on [*diflubenzuron or other registered products for mosquito control in the country/EU*] formulation(s) in [*tablet, granular or liquid form*]. In the offer, the larvicide formulation that the "Company" intends to use must be indicated, attaching label, data sheet and safety data sheet for each formulation offered.

Suitable equipment such as pumps, pressure tanks of the portable pumps with a minimum capacity of 10 litres for the spraying of larvicide in liquid form, or proper equipment for the distribution of granular formulations must be specified. A number of [*number of larvicide cycles for each year*] turns of treatment is required during the period [*e.g. April to October*]. Depending on the situation, it will be possible to perform the operation on foot or with the aid of the bicycle or motorcycles.

**Treatments referred to under adulticide**

The adulticide formulations acting against mosquitoes must be based on natural pyrethrin and/or pyrethroid, mixed with piperonyl butoxide, to be applied at the doses indicated on the label for the specific use against mosquitoes.

Therefore, in the offer, the adulticide must be indicated by attaching label, technical data, and safety data sheet for each of the proposed formulations.

In compliance with local regulations, the formulation(s) proposed must be registered as biocide for the outdoor adult mosquito control.

Formulations must be without any phytotoxicity at the standard doses of use.

It is the faculty of the "Customer" to request the replacement of the formulations used in case of adverse effects such as unpleasant odor or irritation of the mucous membranes.

For the adulticide treatments, the availability of at least [*required number of operating units*] operating units is required, each consisting of a specialized operator equipped with a vehicle with ultra-low volume (ULV) generator or low volume (LV) nebulizer to be used in the treatment of large areas (e.g. city streets and public parks), and/or dorsal atomizer motor for the treatment, by feet, of areas of limited size and/or areas impossible to reach with the sprayer on the truck.

The operational units must be equipped with a GPS apparatus which is able to transmit in real time the position of the unit during the working time. It is mandatory to keep the GPS equipment in good condition.

**Treatments referred to under “door-to-door.”**

The insecticide formulations are the same as requested before under larvicide and adulticide treatments.

Larvicide treatments must be performed with a portable pump, with a minimum capacity of 10 litres for the distribution of larvicide in liquid form.

Source removal must be conducted following permission obtained by the owner<sup>3</sup>. The operator must check carefully for possible active and potential breeding sites present in private areas and explain the reason for the source removal to the owner.

Adulticide treatments are required only when dengue, chikungunya or Zika cases have been detected. The operator should be equipped with back pack motor atomizers for the treatment by feet, in order to spray the vegetation and any resting area in the property accurately. Attention should be paid to reduce exposure of the residents to the insecticide.

**Article 3****Common requirements for all interventions**

The “Company” is required to give timely notice of the existence of any causes, not attributable to itself, impeding or limiting the correct conduct and/or accomplishment of the conduct of the services covered by this specification.

The “Company” will have to obtain access to municipal/public facilities. The “Company” will have to comply with the procedures and time of access agreed with the heads of the structures and respond to any issues or problems associated with access to the areas in question.

The “Company” has to request for permission and/or license required to perform the service covered by this specification.

Interventions not conducted for reasons attributable to the “Company” will not be paid for, and the failure will be considered in the application of penalties based on the provisions of “Article 5” of this Tender.

**Article 4****Quality control procedures**

The “Company” will have to perform the services of mosquito control with diligence and the necessary professionalism, and the “Customer” will conduct the appropriate quality control, also appointing independent external technicians who have no conflict of interest with the “Company”.

In particular, for the institutional control on competence, the technicians appointed by the “Customer” will have free access at any time to the headquarters of the “Company”, the products store, and its territorial base of operations for inspecting equipment. They may attend the field operations, and they may withdraw product samples from the containers and subject them to laboratory analysis.

The technicians, at their discretion, may conduct spot checks to verify the efficiency and effectiveness of the services and the fulfilment of the obligations of these specifications, while observing the daily program and integrity of the report that the “Company” must have produced.

The technicians in charge of the quality control will produce regular reports to the “Customer”. In case of detected faults and/or failures in the performance of services, the “Customer” will apply penalties to the extent of and based on the criteria outlined in this specification, and/or will draw up specific requirements for the appropriate conduct of the business contract.

The “Customer” and/or the technicians appointed for quality control will verify that the activities of the “Company” and the staff employed correspond to daily schedules posted, and that the behavior, clothing and technical equipment of the personnel during the service are suitable and in accordance with the standards for good practice as provided in this specification. Representatives of the “Company” will have to attend to the demands of the controls, depending on the wish of the “Customer”.

The technicians appointed by the “Customer” may conduct two types of quality control:

- a) Checking the information produced by the GPS mounted on the operational units to control the movement of the operational units in the area indicated by the “Company” in the daily report. If an area indicated in the intervention program is not covered appropriately, or if the treatment did not happen according to the required speed, this area will be considered as not treated and subject to the penalties provided by “Article 5”.
- b) Verifying the effectiveness of the larvicide treatments by taking samples of water in the treated larval breeding site (i.e. catch basins). Indicatively, the number of catch basins to be subjected to quality control following each treatment will be proportional to the total number of drains present in the urban area with a minimum threshold of 20 drains per locality. The catch basins that, once opened by the technicians, contain mosquito

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<sup>3</sup>“Owner” is the proprietor of the target private area (e.g. house)

larvae and L<sub>3</sub>-L<sub>4</sub>-pupae will be considered as not properly treated and subject to the penalties provided by “Article 5”. Representatives delegated by the “Company” may be present at the controls, provided the “Company” asks for permission from the “Customer”. The larvicide treatment will be considered as properly done when less than or equal to [5-10%] of breeding sites checked have L<sub>3</sub>-L<sub>4</sub>-pupae (calculated on the single control session). In a case where the threshold result is exceeded, the penalties in “Article 5” will apply.

## Article 5

### Penalties

In a case of inadequate or unsatisfactory performance as detected from the quality control procedures, the “Customer” will be entitled to apply, in his/her sole judgment, the penalties listed below:

1. **Reports not delivered.** The failure to report daily the works performed is equivalent to the non-fulfilment of the planned intervention, with the following penalties for non-compliance and non-economic settlement of the treatments themselves: €*exact amount*].
2. **Failure to perform intervention as planned or requested.** Any changes to the programs must be communicated in advance. Failure to do so will be attract a penalty of €*exact amount*].
3. **Delay in the execution of an operation** (mosquito control). Delay of more than [*hours*] in conducting planned interventions, unless changes are motivated and approved by the “Customers”, will attract €*exact amount*] for each day of delay.
4. **Refusal by the “Company” to cooperate in the execution of quality controls.** Evidence of non-collaborative behaviour by the “Company” will be subjected to the penalty of €*exact amount*] per event until the termination of the contract.
5. **Percentage of non-treated breeding sites** (i.e. catch basins). In a case where the appropriate quality control procedures show areas where the rate of breeding sites not treated exceed [*number in %*], the “Company” is required to perform the treatment in the involved area immediately without any extra cost to the “Customer”.
6. **Incomplete coverage of the area indicated by the intervention program** or its treatment at a pace in disagreement with the required pace, as verified by the GPS device from [*date*] to [*date*], depending on the severity of the failure, will lead to repetition of the treatment without any extra cost to the “Customer”.
7. **No activation of the GPS receiver.** For every hour of failure to activate the GPS system [*date/period*] penalty of €*exact amount*].
8. **No specific notice of adulticide treatments, or unsuitable exposure to adulticide, in public areas** (warning the residents about the formulation used and the exact time of treatment for security purposes): penalty of €*exact amount*].
9. **Ascertained unauthentic reports** sent after interventions: penalty of €*exact amount*] for each false statement until the termination of the contract.
10. **Non-compliance with the proposed offer** by the organizational structure used for the execution of the performance, such as personnel, equipment and products: penalty of €*exact amount*].
11. **Non-compliance of the dilutions and/or dosage of insecticides applied** with respect to the requirements of the technical product: penalty of €*exact amount*] for the first challenge, €*exact amount*] for each additional dispute.
12. **Failure to meet the requirements for the use of equipment and personal protection equipment** i.e. garment as per art. 2: penalty of €*exact amount*] for the first challenge, €*exact amount*] for each additional dispute.

### Notes:

The delayed implementation of a planned intervention becomes non-execution after 48 hours of delay. The delayed implementation of an emergency treatment becomes non-execution after 24 hours of delay. The penalty provided for failure covers that provided for the delayed execution.

### Formulation of the offer, unit prices-based auction service, and award criteria

The service will be assigned according to the principle of the most economically advantageous tender.

The total score (technical and economic) is available for 100 points, divided into:

- A. [number from 1-100] points for curriculum, reliability and functional capacity of the Company.
- B. [number, 100-number in A] points for economic offer.

#### A. Curriculum, reliability and technical capacity of the Company

For the evaluation of the curriculum, reliability and technical capacity, the following parameters will be considered:

1. Provision of equipment and vehicles to be used in each type of service required precisely correspond to the details in the description in “Article 2”. The number, the technical characteristics of the equipment and their compliance with the types of services required according to “Article 1” will be evaluated.  
Maximum points [*exact number of points*].

2. Experience in similar services and its turnover in the last three years. Similar services are those related to mosquito control involving the use of the same equipment described in the preceding paragraph, in local and/or regional mosquito control projects. The “Company” for this purpose must provide a list of the main services provided in the last three years with addresses, year and amount. Together with the list, “Company” should also provide contact information (persons) for recommendation upon request.  
Maximum points [*exact number of points*].
3. Evaluation of the proposed products being offered pursuant to “Article 2” of these regulations.  
Maximum points [*exact number of points*].
4. Experience of personnel in similar services. Technical/operational staff’s professional background, expertise and experience will be evaluated. For this purpose, it is necessary to submit proof of the organizational skills of each operator to be engaged in the service, the training courses already taken, any updating task. The Technical Director’s curriculum and professional work experience in the field will also be evaluated.  
Maximum points [*exact number of points*].
5. Localization of the operational base necessary for the permanent provision of the tools/means, the equipment, and storage of larvicide and adulticide products. Points will be awarded according to the positioning of the strategic logistics base, verifiable by inspection on the spot in the award stage.  
Maximum points [*exact number of points*].

The offer for the evaluation of the "Reliability and technical capacity" of the Company must be submitted following the track indicated in the Annexes [*include relevant documents, depending on the rules of each organization/prefecture/country*].

Companies who have not achieved a score on "Reliability and technical capacity" of at least [*a number of points out of number*] will be considered ineligible.

At the request of the “Customer”, the participants (“Companies”) will be required to provide any additional information, deemed necessary, in order to formulate a more precise evaluation of the offer.

#### ***B. Economic offer***

Once the scoring of "A. Curriculum, reliability and technical capacity of the Company" is complete, the commission, in a public session, will consider the bids that reach or exceed, during the technical evaluation, the established minimum threshold [*points*], will open the envelope containing the economic offer, will calculate the score, and proceed with the allocation of final scores, as well as with the final award.

The economic offer must be expressed as a percentage reduction of the single unit prices specified in the tender for the three types of operational service using the facsimile.

(Annex [*include relevant documents, depending on the rules of each organization/prefecture/country*]).

It is allowed to submit offers including only one or two types of intervention as specified in “Article 1” and discounts variation between the three different types of service.

If during the execution of the contract an increase or a decrease in the number and/or size of the interventions become necessary, the selected “Company/ies” is/are obliged to accept the variation at the same conditions, up to 20% of the total contract price.

#### ***Notes for the preparation of the technical annex***

1. Pay particular attention to the identification of parking lots or private land for public use and include them in the list of areas to be treated.
2. Please note that adulticide interventions, outside of public health emergency, should be conducted only in the case of particularly intense infestations in sensitive sites such as schools, gardens, hospitals areas, protected residential structures, etc. These interventions should be performed always, only on request and upon obtaining an approval from the local Department of Public Health.
3. The door-to-door intervention is not always feasible because of the cost. It may be necessary in particular situations, in which case it can be costed hourly without the need for prior estimation.
4. The maximum duration of a larval treatment turn of intervention should be determined based on the number of catch basins to be treated and the larvicidal product used, and should not, in any case, exceed two (2) weeks. The date of the beginning of the first turn of intervention must be carefully decided based on the seasonal meteorological conditions in order for the intervention to be completed before the end of the larval cycle of the first mosquito generation.
5. Local climatic conditions determine the duration of the mosquito breeding season and therefore the period to be covered by larval treatments. It is reasonable to assume that the number of treatment turns per season is in relation to the lasting activity of the larvicide used. Attention must be paid to the product used and, in particular, to its formulation and concentration of active ingredients; products with extended lasting action make it possible to reduce the number of turns of treatment. The lasting action declared on the label of the larvicidal formulation must be checked and validated locally before being considered right.



6. *It is recommended that the municipalities should develop communication channels with the aim of letting the citizens know about the mosquito control campaign and about their active involvement, in each case according to the requirements of the Public Health Districts and by the legislation in general.*
7. *In the bid evaluation “A. Curriculum, reliability and technical capacity of the Company”, it is expected that in the contract procurement of public works, the condition of use of vehicles is at least Euro....., favoring eco-friendly vehicle parks (electric, hybrid, natural gas, and propane).*
8. *The third type of quality control, such as the marking of catch basins treated with different colours depending on the treatment cycle, may be considered. In case the municipality decides to activate this control, it can be inserted as: “Systematic inspections to detect the presence of the marking, as required, to be conducted during the larval treatment. The verification of the presence of the markings will be made in days, in areas chosen by the Client (or by the technicians appointed). A percentage of catch basins adequately marked at or above [.....%] of the total catch basins present is required. In case of failure to achieve the established threshold, the sanction will be applied as in Art. 5.*
9. *The recommended speed of vehicles mounted with adulticide equipment in the street may be considered 8-10 km / h.*
10. *The service of larvicidal treatment of Art. 1 (a) can be paid for a singular catch basin at a time if the number of catch basins is known, but can also be paid for per block of urban areas or per hour.*
11. *The service of adulticide treatment in Art. 1 (b) is paid for per hour, but please note that you may also opt for an estimation of the cost for a single intervention, especially in the case of localized operations and related areas of limited extension.*