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BEPREP STANDARD OPERATING PROCEDURE

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Related

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1. Abbreviations

SOP	- standard operating procedure
CSA	- case study area
PPE	- Personal Protective Equipment
BACI	- Before, After, Control, Impact
BG-S	- Biogents® Sentinel trap
BG-CDC	- Biogents® Center for Disease Control like trap
DRS	- DNA/RNA Shield

2. Aim of the SOP

The purpose of this SOP is to collect adult mosquito specimens to evaluate the presence/absence of potential mosquito vectors and to detect pathogens in natural population.

3. Safety measures and potential hazards

It is crucial to research the potential danger of exposure to biological agents (viruses, bacteria, protozoa) since the mosquitoes covered by this SOP can be vectors of pathogens to humans, such as Flaviviridae (e.g. West Nile virus, Usutu virus, Dengue virus, Zika virus), Togaviridae (e.g. Chikungunya virus) as well as other viral infections. It is therefore important to adopt the right strategies to avoid contact with the vectors. It is advisable to wear white long-sleeved cotton shirts to cover the skin as much as possible, a hat with mosquito net and closed boots. Spray repellent on clothes and/or on bare skin is recommended.

4. Materials and equipment

Baited suction traps are an efficient sampling tool in mosquito-borne pathogen surveillance where live host seeking/gravid females are needed. During their flight, they are in search of a host for their blood meal and therefore they are attracted by traps baited with a lure and/or CO₂. Gravid females are mainly attracted by traps simulating breeding sites. Therefore, captures are represented mainly by females of many species at various stages of the gonotrophic cycle (unfed, fed, half gravid and gravid). CO₂-baited traps also collect other hematophagous insect families such as *Psychodidae* (sandflies), *Simuliidae* (blackflies), and *Ceratopogonidae* (biting midges).

The most used host-seeking mosquito traps are BG-S trap and BG-CDC trap.

4.1. Traps

4.1.1. Biogents® Sentinel trap (BG-S)

This trap (Fig.1) is designed to attract *Aedes albopictus* with a specific chemical lure (BG-Lure). Their effectiveness can be increased by adding a carbon dioxide source. Carbon dioxide makes the trap attractive to a wide range of mosquito species (e.g. *Culex pipiens* and *Aedes sp.*).

Traps can operate continuously when a power supply is available but can also run on a single 12V-battery (24h).



Figure 1. BG-S type trap.

In EU the link to webpage is this: <https://eu.biogents.com/bg-sentinel/>

The BG-S comes with most things necessary for the mosquito sampling:

- 1x pop-up body
- 1x plastic cover
- 1x intake funnel with shutter
- 1x funnel net
- 2x catch bag
- 1x ventilator (12 V DC, 3.6 W, Type II)
- 1x battery cable (Type II) with connector
- 1x carrier bag

Items you will need additionally, if using this trap, are listed in Table 1 below.

Table 1. Items needed for **capture** of mosquitoes using BGS

Item	Note
Lure	Lasts approximately 5 months (keep it in refrigerator when not used)
Insulated box	For at least 1kg of dry ice (15x15x15cm)
Connection Tube	Inner diameter 3mm
Dry Ice	1 kg
BG-CO ₂ Generator Starter Kit	If you prefer generate CO ₂ through fermentation
CO ₂ cylinder	If you prefer instead of dry ice or fermentation, also a pressure reducer (0.5 kg a day) is needed.

4.1.2. Biogents® CDC-like trap (BG-CDC)

This trap (Fig. 2) is designed to attract mainly nocturnal mosquitoes (e.g. *Culex pipiens* and *Anopheles sp.*). The source of attraction is represented by CO₂. Removing the light source of BG-CDC will eliminate other photopositive insects (e.g., *Lepidoptera*) from the collection and increase the efficiency of identification.

Traps can operate continuously when a power supply is available but can also run on a single 12V-battery (24h).



Figure 2. BG-CDC trap with a light source.

In EU the link to webpage is this: <https://eu.biogents.com/bg-pro/>.

Note! This trap (BG-Pro) can be also used as a BG-S type trap. Please see the website for more information.



The BG-CDC comes with most things necessary for the mosquito sampling:

- 1x pop-up body
- 1x plastic cover
- 1x intake funnel with shutter
- 1x funnel net
- 2x catch bag
- 1x ventilator (12 V DC, 3.6 W, Typ II)
- 1x battery cable (Typ II) with connector
- 1x carrier bag

Items you will need additionally, if using this trap, are listed in Table 2 below.

Table 2. Items needed for **capture** of mosquitoes using BG-CDC

Item	Note
Insulated box	For at least 1kg of dry ice (15x15x15cm)
Dry Ice	1 kg
BG-CO ₂ Generator Starter Kit	If you prefer generate CO ₂ through fermentation
CO ₂ cylinder	If you prefer instead of dry ice or fermentation, also a pressure reducer (0.5 kg a day) is needed.

4.1.3. Gravid traps

This trap (Fig.3) is designed to attract mainly gravid females searching for breeding sites for eggs oviposition. There is a higher probability of collecting virus-infected mosquitoes in a gravid trap than in other baited suction trap because gravid traps attract female mosquitoes that have already taken at least one blood meal. The source of attraction can be represented by a mixture of water and grass infusion poured in a basin above which a fan sucks up the mosquitoes that visit the bait container into a catch bag. The species collected may vary by where the trap is set and/or what formula is used to make the infusion bait.

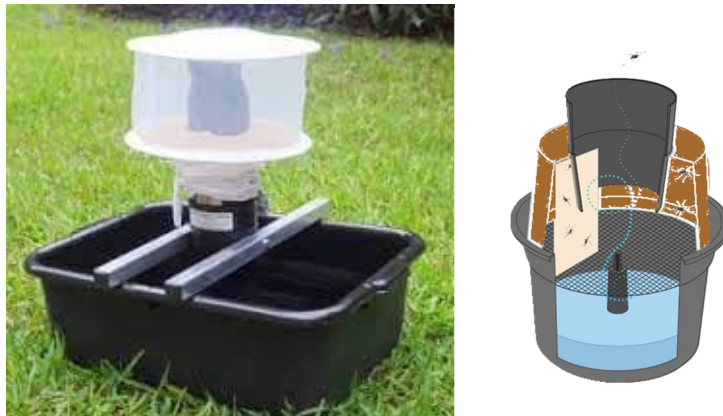


Figure 3. Gravid traps.

Traps can operate continuously when a power supply is available or run on a single 12V-battery (24h). Some gravid traps rely on passive catch methods (No fan).

The recommended trap: <https://www.johnwhock.com/products/mosquito-sandfly-traps/cdc-gravid-trap/>

It comes with all the necessary for mosquito sampling:

- 1x Trap
- 1x Power Cable
- 1x Double-Ring Collection Bag
- 1x Oviposition Pan
- 1x Instructions

Though additionally, you need water with grass infusion.

4.1.4. Backpack aspirator

Blood fed mosquitoes can be also captured by a motorized/electric backpack aspirator (Fig.4) used in mosquito resting places (e.g., shaded areas under vegetation, shaded dry-stone walls and inside catch basins).

The recommended aspirator: <https://www.johnwhock.com/products/aspirators/modified-cdc-backpack-aspirator>

It comes with all the necessary for mosquito sampling:

- 1x electric aspirator with hose
- 1x built-in 12 VDC 17 AmpHr sealed electrolyte battery
- 1x 5 universal and automatic battery charger
- 1x extra screened-collection cups
- 1x switched suction motor
- 1x lightweight welded aluminum backpack frame
- 1x instructions



Figure 4. Backpack aspirator used for mosquito collection.



4.2. Items needed for mosquito sorting and storage

Item	Purpose
Tweezers	For sorting the mosquitoes
Chill table	Ice tablet can be used to maintain cold chain when sorting and identifying mosquitoes
Stereomicroscope	Magnification: 80x
Identification keys	Relevant to your sampling area
Tubes or vials	
Pen or pencil	
Paper	
External battery	To purchase the right battery (W, V, Ah), follow manufacturer's instruction
Charger	for what?
Plastic bags	To protect batteries from the rain
Petri dish	

5. Sampling procedures

For all traps and sampling:

- take coordinates of each trap (using WGS84 system)
- mark each trap with its own unique ID
- all traps should have contact information and a short description of the project
- Traps must not be operated during windy and/or rainy days.
- Traps must be placed in sites with good accessibility but out of public sight and not accessible by children.
- Traps must be placed in shaded places, reach in vegetation, moist and protected from wind (avoid open terrain like meadows or parking lots). Possibly choose places sheltered from the rain. Some models have their own rain shield or build an *ad hoc* rain shield.
- Temperature and relative humidity should be recorded throughout the season



5.1. Trap placement

5.1.1. BG-S trap

The trap should be placed at ground level. Remember to insert the odorous bait (BG-LURE) inside the trap. Dry ice can be added in a separate insulated box, connected to the trap by a small tube (inner diameter ca. 3mm, Fig.1).

5.1.2. BG-CDC trap

This trap should be hung at 1.5-2m height. No odorous bait is necessary. Dry ice can be added in a separate insulated box connected to the trap by a small tube (inner diameter ca. 3mm). Some CDC models come with their own dry ice container just above the fan.

5.1.3. Gravid traps

If gravid trap is used (targeting gravid females) the trap should be placed far away from other possible natural/artificial breeding sites to avoid competition. No odorous bait or dry ice is necessary.

For gravid traps, the bait infusion for *Culex* mosquitoes can be made on the spot. The materials needed are:

- large plastic trash can (100 Liters) with a tight-fitting lid
- water
- 0.5 kg of straw or hay
- 5 grams of brewer's yeast

Mix all the ingredients in the trash can, place a lid on it and let the mixture brew for at least five days under direct sun light, stirring at least once each day. After about three weeks, this bait becomes slightly less attractive to *Culex pipiens* and becomes more attractive to *Ae. albopictus* and container-breeding *Aedes* species.

5.1.4. Aspirator

This is considered a complementary activity. The purpose of aspiration is to collect more blood fed and gravid females, so it is recommended in case of poor trapping collection.



5.2. Number of traps

It is recommended to use at least 2-3 traps each trapping site (i.e., 1 BG + 1 CDC +1 gravid trap). Following the rodent sampling scale (see small rodent SOP), one can deploy 2-3 traps in each trapping site (within BACI framework).

5.3. Trap operation

Traps should be placed and activated once a month from May to October. To reduce viral decomposition and damage to the mosquitoes, **all traps should only be run for a single day (24h)**.

As BG-CDC traps target nocturnal mosquitoes, they can be set at least one hour prior to dusk until one hour after dawn.

The CO₂ container should be filled with at least 800/1000 g of dry ice for a 24h run and the tube from the insulated box stuck close to the opening of the trap. Alternatively, CO₂ can be obtained from a mixture of water, sugar and yeast (fermentation – see below recipe) or using CO₂ cylinders (recommended a flow rate of about 1 kg of CO₂ per day). With high temperatures add more dry ice (1.5 kg).

A label with trap code, date of capture, and trap type should be placed inside the catch bag.

Connect the trap to a power supply or to a battery and make sure the fan is working.

Traps must not be operated during windy and/or rainy days.

CO₂ is obtained using fermentation, the recipe is:

- 1 mixing bag with a tube to be stuck close to the opening of the trap
- 2 liters of warm water (38°)
- 500 g sugar
- 20 g yeast powder

Always set up the yeast mixture about 2 hours before the monitoring starts. One can also use the BG-CO₂ generation kit: <https://eu.biogents.com/bg-co2-generator/>



5.4. Mosquito collection, identification and storage:

When removing the collection net, blow from above through the fan cylinder to move the mosquitoes down. Close the collection net with the label inside and store it in a refrigerated box (e.g., dry ice left from the traps).

In the lab, under a stereomicroscope, sort mosquitoes from the rest of the insects. Start with keeping the collections in -20 °C for 20 minutes to ensure that the mosquitoes (and all other insects) are dead and do not try to escape.

To maintain cold chain during all procedures a chill table could be used under petri dishes during mosquitoes sorting operations.

Morphological keys that can be used all over Europe are:

- Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., & Kaiser, A. (2010). *Mosquitoes and their Control*. (2 ed.) Springer. <https://doi.org/10.1007/978-3-540-92874-4>
- Schaffner F, Angel G, Geoffroy B, Hervy JP, Rhaim A, Brunhes J. 2001. The mosquitoes of Europe. An identification and training programme. CD-ROM, IRD and EID Méditerranée: Paris and Montpellier.
- Günay F, Picard M, Robert V. 2022. MosKeyTool, an interactive identification key for mosquitoes of EuroMediterranean. Version 2.4. Available at www.medilabsecure.com/moskeytool.

It is preferable to identify the mosquitoes, and divide them by species, trap, date and sex and gather in pool of max 10 specimens in a 2 ml tube. Engorged mosquitoes must be placed in individual tubes for diet analysis.

If identification and sorting is not possible in the field or soon after collection, mosquitoes can be sorted in laboratories. Pay attention to storage temperature (preferably -80 °C) during transport to keep specimens in a good shape, otherwise it will be hard to identify them. Mosquitoes can be placed in 70% and transported uncooled. **As soon as the mosquitoes are in ethanol, no further species determination can be performed, as important identification features will then vanish.** Therefore, the species determination and pooling must be done in prior. Storage in AVL buffer (or DNA/RNA Shield) for 1 to 2 weeks unrefrigerated at room temperature is also possible **after species determination and pooling.**

Alternatively, for microbiota analysis, and/or exportation, store each mosquito individually in DNA/RNA Shield (1.5 ml).



BEPREP_SOP-13: mosquito sampling

It could be also useful to store different species in different boxes. Likewise, engorged mosquitoes should be stored in a separate box. Males are not useful to detect pathogens in natural population, so they can be discarded after identification and data recording.

Write on each vial an ID referred to ID trap, date, species, sex and n° of specimens

Store vials preferably in a -80° freezer for following molecular analysis