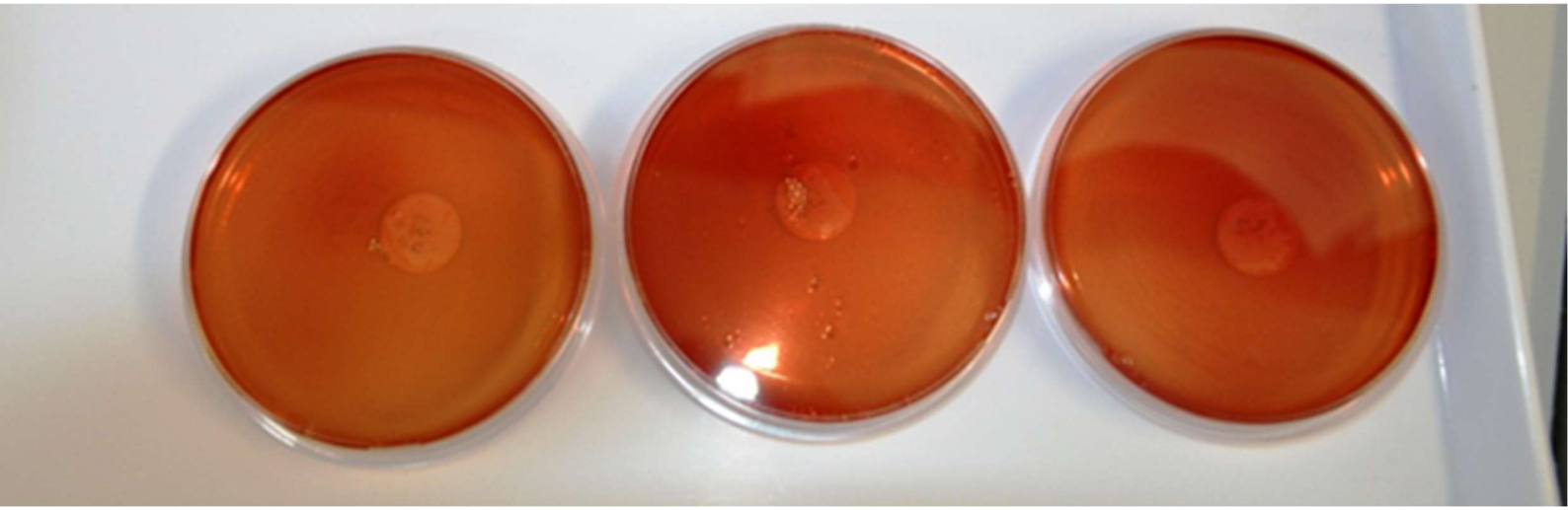
Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

GUIDELINES FOR

BLOOD COLLECTION, PROCESSING AND QUALITY CONTROL FOR TSETSE REARING INSECTARIES

Version 2.0

Food and Agriculture Organization of the United Nations
International Atomic Energy Agency
Vienna, 2019



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The proper citation for this document is:

FAO/IAEA. 2019. Guidelines for Blood Collection, Processing and Quality Control for Tsetse Rearing Insectaries, Parker, A., Abdalla, A.M.M., Argilés Herrero, R. (eds.), Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. Vienna, Austria. 60 pp.

GUIDELINES FOR

BLOOD COLLECTION, PROCESSING AND QUALITY CONTROL FOR TSETSE REARING INSECTARIES

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Vienna, 2019

FOREWORD

Area-wide integrated pest management (AW-IPM) programmes using the sterile insect technique (SIT) depend, on a reliable supply of large numbers of high-quality sterile insects for release. The insects are reared in special large rearing facilities or factories under defined conditions.

Reproduction in tsetse flies is by adenotrophic viviparity, where the female gives birth to live offspring. The larvae are nourished within the mother by secretion from highly modified accessory glands and are larviposited at an advanced stage of development. As a result, the only dietary requirement in a tsetse mass rearing facility is warm vertebrate blood for adult feeding.

Both sexes of tsetse flies are considered to be strictly hematophagous (although hungry tsetse can feed on water and on water with sugar in the lab). Feeding the adults with high quality blood is essential to achieve an efficient colony productivity in a mass-rearing insectary. Bacterial contamination in the blood will infect adults and cause blood mortality and abortions. Individuals infected with blood can become the source of infection of other flies. On the other side, feeding flies with blood with a poor nutritional value will cause a decrease in fecundity, which is already low in tsetse flies.

This document is intended for use in laboratories and institutions that maintain colonies of tsetse flies. It describes the procedures for the collection of animal blood in the abattoir, decontamination through ionizing radiation, preservation and storage, quality control assurance and processing of the blood into diet for feeding tsetse flies.

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Introduction

In the insectaries, tsetse flies are fed on quality-tested fresh defibrinated blood, which has been stored in a frozen condition (Wetzel and Luger 1978).

The aim of these procedures is to ensure the quality of the blood used to feed large colonies of tsetse over long periods and to provide a standard data recording system to store and analyse the information on the quality and quantity of the blood stock.

During collection and processing, blood can become contaminated with bacteria and chemicals. Under the influence of physiology, nutrition and disease, the composition of blood in animals varies among individuals of the same species. Due to these factors, a quality-control procedure was developed, and biological criteria for evaluating the suitability of blood used to feed flies were identified.

After collection and irradiation, the blood from each daily collection batch is bio-assayed to assess its nutritive value through a 25-day feeding test, developed with the aim of having a simple numerical quality factor (QF) that adequately summarizes and combines the various data obtained from pupal production and dissections.

If the nutritional value is acceptable, samples from each container are screened for microbial contamination to confirm the efficacy of the sterilization procedure. On average, 99.6% of microbial contamination is eliminated (2.5 log reduction) by irradiation at 1 kGy. Given that different species of bacteria have different sensitivity to irradiation, this reduction will depend on the species of microbes present in the blood.

The following procedures describe the collection of animal blood in the abattoir, radiation with gamma-rays (decontamination), preservation and storage at -20°C, quality control assurance and processing of the blood into diet for feeding tsetse flies. The procedures aim to provide nutrition of a constant quality to mass-reared fly colonies maintained over a long period for field programmes.

Summary of blood processing procedures

Before the collection day, the containers, stirring material and any other material that will be in contact with the blood must be sterilized in the oven for 24 hours before use (see procedure in Section 1)

The collection of blood is done in the abattoir using buckets (see procedure in Section 2). Collected blood is poured into 25L containers for stirring (see procedure in Section 2) for defibrination. After defibrination, blood is sieved and transported to the insectary trying to avoid delays that would cause bacterial growth (see procedure in Section 3). Upon arrival to the insectary, the blood stored in the 25L containers is bulked into large containers (100-200L) to create a single batch. After homogenization of the blood, 30 samples from the large

container are taken and will be used for the bio assay (see procedure in Section 4 and 9). Then, the blood in the large container is proportioned into storage bottles (see procedure in Section 6), labelled (see procedure in Section 7) and stored in the -20° C cold room (see procedure in Section 8). While proportioning, one blood sample for microbial screening is taken from each bottle and stored with its corresponding bottle.

The blood must now be checked for microbial contamination (see procedure in Section 11) and bio-assayed for its nutritional value (see procedure in Section 9). To do so, the 30 samples of the large container are irradiated and used to run the 25-day feeding test. One single bioassay will be carried out for each blood batch. If the nutritional value is considered acceptable, then the bottles with blood from the same batch will be irradiated together with their respective samples (see procedure in Section10). The samples are now tested for microbial contamination while the corresponding bottle is kept frozen in the -20° C cold room. If the microbial screening shows an acceptable level of bacterial colonies, then the blood in the bottles is ready to be thawed (see procedure in Section 12) and used for feeding the tsetse flies when necessary.

The whole procedures are summarized in the following flow chart.

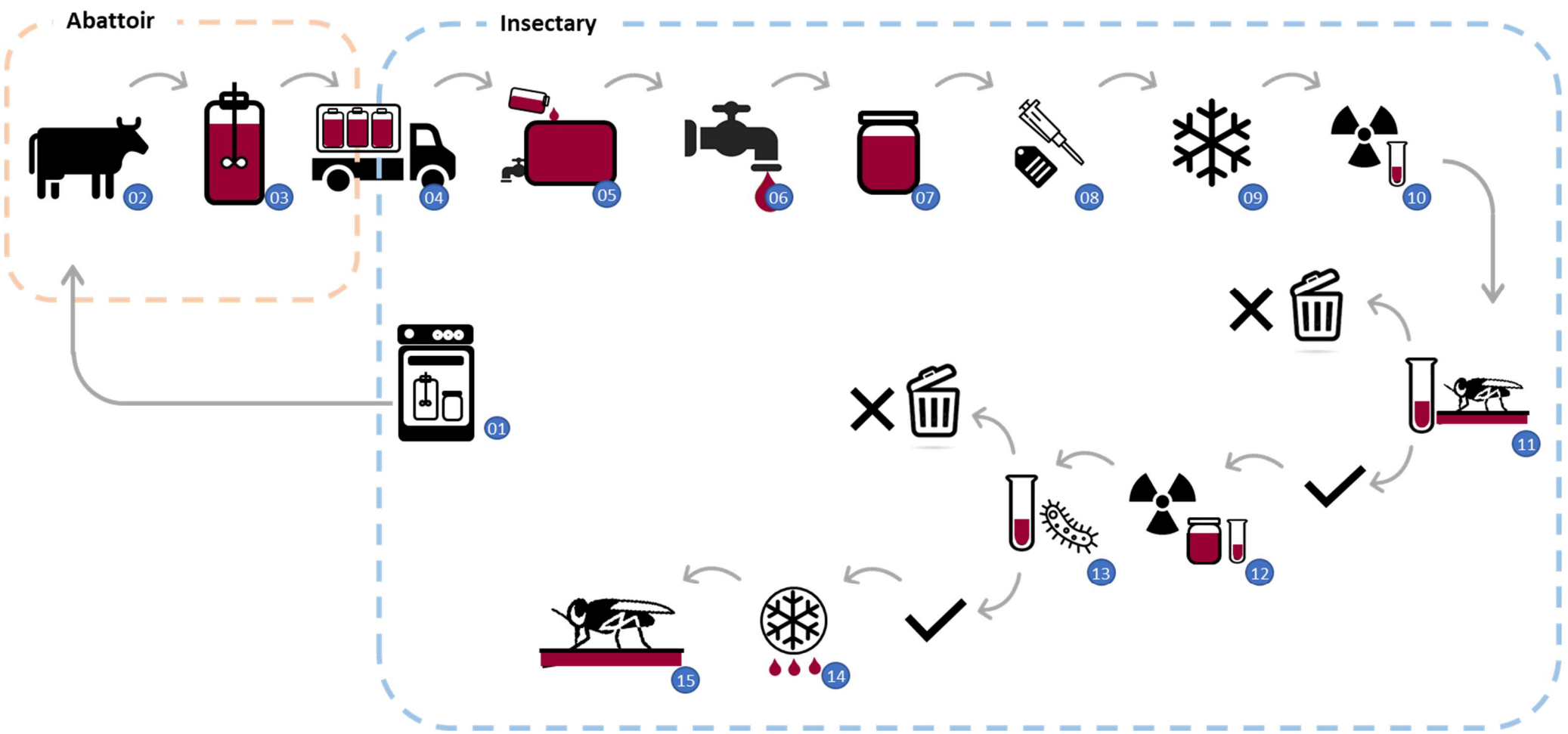

Figure 1. Flow chart of the blood processing procedures for tsetse mass rearing insectaries.

01 sterilization of blood equipment; **02** blood collection in abattoir; **03** defibrination; **04** transportation; **05** bulking; **06** proportion; **07** storage in bottles; **08** sampling for bioassay and microbial screening and labelling; **09** blood freezing; **10** irradiation of batch samples; **11** bioassay; **12** irradiation of blood bottles together with bottle samples; **12** microbial screening; **13** blood thawing; **14** tsetse feeding.

1. Sterilization of materials in heat sterilization oven or autoclave

Scope:
To prevent bacterial contamination, all equipment that will be in contact with blood during blood collection, blood sampling and blood proportion must be thoroughly cleaned and sterilized before being used.

Equipment and Materials:
The following equipment will be cleaned and sterilized:

- Materials to be used in blood collection:
    - Buckets (5L)
    - Sieves
    - Hose
    - Funnel
    - Stirring containers (10-25L), including caps and side inlet.
    - Paddle / stirrer
    - Bulking container (100-200L)
- Materials to be used in blood sampling:
    - Eppendorf tubes (if not stored in proper sterile conditions)
    - Pipettes and tips
- Materials to be used in blood proportion:
    - Storage bottles (2L), including caps
    - Sieves

The equipment will be sterilized either using a heat sterilizing oven, an autoclave or UV light. Thermal indicators which change their color when exposed to high temperatures will be used as proof to identify and mark the equipment that has been effectively sterilized.

Procedure:

1. Clean the blood-collection equipment.
2. Depending on the dimensions of the equipment, select:
    - heat sterilizing oven for large equipment: Sterilize the equipment by exposing it to dry heat (at 80°C for polyethylene (PE) for at least 3 hours, and glass or metal at 120°C). The large transport container must also be sterilized in the oven. To do so, an oven with a useful inner volume capable of accommodating the container will be required. The external dimensions of a PE container with 100L capacity vary around 81 cm (height) x 45 cm x 45 cm. The external dimensions of a PE container with 200L capacity vary around 103 cm (height) x 55 cm x 55 cm.
    - autoclave for small glass or metal equipment (NO PLASTICS!): 20 minutes at 103.45 kPa (15 psi).
    - The large bulking containers 100L-200L may not fit inside the sterilizing oven. In this case, sterilization will be done by inserting a UV light inside them for at least 1 hour.
3. When not in use, to minimize microbial contamination, store all sterilized equipment in autoclave bags or in an ultraviolet (UV) cabinet.

Figure 2. Heat sterilization of blood collection equipment in oven.

2. Blood Collection and Defibrination

Before collection, arrange with the responsible authorities of the abattoir to ensure that the place, time and amount of blood to be collected are agreed upon. Alert the collection team (a minimum of three persons) to prepare for the work; note that some heavy lifting may be required. It is an advantage for the workers to have a good knowledge of basic biology and tsetse rearing procedures and the ability to handle mechanical equipment. In addition, the workers must be able to make sound judgments regarding the condition of animals (age and health) at the abattoir. The team must wear protective gear (white clothing, rubber boots and hard hat).

Before arrival at the abattoir, all equipment that will be in contact with blood must be thoroughly cleaned and sterilized. The equipment used to collect blood (Figure 3) depends on the amount of blood obtained on one occasion. The frequency of collection depends on the size of the tsetse colony and the amount of space available to store frozen blood at -20°C. In general, during one collection operation, it is economical to obtain as much blood as possible because transport, human labour and quality-control measures become cheaper with increasing quantity. If the ambient temperature is high or the storage facility is a long way from the collection point, it may be necessary to cool the tank of blood during collection and transportation. Using two or three collection sets, blood can be collected from enough slaughtered animals even if the rate of slaughter is high, e.g. 100 animals in 5–6 hours. Freezing slows down, but does not stop completely, the degradation of blood. It has been demonstrated that blood kept frozen at -20°C for at least five years retains its nutritive characteristic.

If animals are slaughtered on the floor, the suitable blood collection equipment used is selected accordingly. Blood is collected from the cut neck of the animal in a mini jerry can, and then immediately poured into a stirring container for defibrination.

Figure 3. Defibrination set (mechanical handheld drill and paddle), 25-litre container with side inlet.

Scope:
Collection of a large volume of blood in an abattoir where animals are hung by their hind legs and pulled up with a chain, with their heads hanging down

Equipment and Materials:

- Stirring containers (screw-capped, 10–25L, polyethylene (PE), with a wide neck 63 to 100mm diameter to allow a paddle/stirrer to be inserted) (The screw cap is equipped with a stand to hold the stirrer in an upright position. The container has a side inlet with a screw attachment for a plastic hose).
- Hose (plastic, made of transparent silicone, with strong walls (4–5 mm thick, inside diameter 4cm, outside diameter 5cm), 1.5m long to lead from the funnel to the inlet on the side of the container, should be autoclavable)
- Funnel (plastic or stainless steel, upper diameter 25–40cm, with an outlet of 4 cm to fit the hose)
- Paddle (consists of a stainless-steel rod of 1cm diameter on which the oar-blade/stirrer is mounted)
- The size of the stirrer is determined by the dimensions of the mouth opening and the depth of the container. The stirrer should agitate the full volume of liquid, reaching close to the bottom of the container. Stirring can be by a manual handheld drill, an electric household drill or a battery-driven drill. If an electric drill is used, it should have a speed regulator and switch to control the driving speed. The drill should be strong enough to agitate a volume of 10–20L of highly viscous liquid.
- When using electric stirring equipment, a ≥50m length of waterproof extension cable is necessary to reach a power supply in the abattoir. Mains powered electrical equipment must only be used with a residual current device (RCD) of maximum 30 mA rating.

Procedure:

1. On arrival at the abattoir, assemble the collection equipment and check that it functions.
2. Negotiate with the butcher for permission to skin the neck of the animal before the jugular vein is cut.
3. When the butcher has cut the throat of the animal, direct the flow of blood into the funnel attached to a hose leading to the buckets or directly to the stirring container.
4. Collect as much blood as possible before terminating the procedure.
5. While one person controls the position of the funnel, the second controls the rotation of the stirrer, adjusts it to a moderate number of revolutions, and observes the filling of the container. Up to 15L of blood can be expected from a bull. Due to the formation of foam, only 2/3 of the total volume of the stirring container should be filled with blood.
6. After 10 minutes of stirring, the blood is defibrinated, and the stirring machine is stopped. (Meanwhile, if practical, use a second set of blood-collection equipment to collect blood from additional animals).
7. Open the lid of the stirring container and remove the paddle with all the clotted fibres.
8. Close the lid and rinse spilled blood if any from the outside of the 25L container.
9. Rinse the paddle of the stirrer with clean water to free it from fibrin clots.
10. As more animals are slaughtered, continue collecting blood. Using two or three sets of blood-collection equipment and enough 25L containers, blood can be collected from many animals, even if the slaughtering speed is high (100 animals in 5–6 hours).
11. After the blood collection is over, rinse all equipment with cold water before loading it into the vehicle.
12. Carry the 25L containers full of defibrinated blood and the stirring equipment to the vehicle and place them in the trunk of the vehicle. In order to minimise bacterial multiplication, the vehicle should be equipped with a cooling device (fridge or ice-box) to cool down the defibrinated blood.

3. Transport to the insectary

Once the blood collection is completed and the blood in the 25L containers are loaded in the back of the transport vehicle, the drive to the mass-rearing insectary should be done without delay to minimise the multiplication of the bacteria.

Scope:

To transport the blood collected at the abattoir to the mass rearing insectary.

Equipment and Materials:

- Vehicle (for transport to the abattoir, carrying equipment): ideally, the vehicle should be equipped with a cold room capable of maintaining a temperature of –20°C, specially if abattoir is at long distance from the mass-rearing facility . The capacity of the trunk of the vehicle must be sufficient to accommodate several 25L containers (see estimated dimensions in ANNEX III Special Equipment and Materials with Specifications)
- 25L containers: the number of containers will depend on the amount of blood to be collected.

4. Creating blood batches

Scope:
Before feeding the flies in the colony, the nutritional value of the blood must be assessed though a bioassay. This test is lengthy and time consuming and therefore can't be conducted for every single 20L container.
In order to have a manageable workload in operational programmes, all the blood collected on the same day can be tested with a single bioassay test. To do so, the blood in the 25L containers is poured into a large 100L or 200L bulking container and homogenised through mixing. This batch will be sampled for the bioassay test. By merging the blood from different animals, the nutritional value of the batch will be averaged. Also, if the blood from one of the animals is contaminated with residues of a veterinary treatment, the residues will be diluted in a larger volume which will reduce the risk of affecting the flies.
This operation must be done by the Lab Technician in aseptic conditions. The bulking container must have been sterilized previously with the UV light or other suitable sterilizing method.
Ideally, the creation of batches, proportion and sampling should be done immediately upon arrival to the insectary of the blood from the abattoir. However, this might not be possible in case of late schedules. In these situations, the blood in the 25L containers must be stored overnight in the cold chamber or chest freezers at -20°C and the procedures must be resumed early next day before the blood freezes in the 25L containers. If the bulking is done in the evening, proportioning and storage in the cold room must be done immediately after to minimise the time for freezing.

Equipment and Materials:
- Large container for blood bulking: the volume of the container must be at least 100L, although, depending on the amount of blood to be collected, a container of 200L capacity is recommended. The container must have a tap at the bottom to allow being emptied for handling when needed.

5. Sampling for 25-day feeding test (bio assay)

Scope:
To take an overall sample of the blood collected on a certain day. This overall sample will be used to run the 25-day feeding test, whose result will define the average nutritional value confirm the absence of any toxic component of the blood collected on that day.
This task must be done by the Lab Technician.

Equipment and Materials:
- Scintillation vials (30, each 20mL, polyethylene)
- Pipette of 25mL and pipettor
- Sieve (to collect floating clots)
- Graduated cylinders (1L)
- Funnel

- Pre-printed labels
- Transport container
- Laminar flow bench
- Irradiator

Procedure:

1. Knowing that 600mL of blood are needed to run the 25-day feeding test (30 samples x 20mL) and the number of containers that will be used to pool the blood, calculate the amount of blood that will be sampled from each container (i.e., if 2 large containers have been used for blood bulking, then 300mL of each will be sampled and mix it to make one bioassay test).
2. Place a graduated cylinder under the tap at the bottom of the transport container with a funnel if necessary, open the tap and pour to the quantity that has been previously calculated.
3. Take the graduated cylinder to the laboratory and place it in the laminar flow bench, which during the previous night had a UV lamp turned on to sterilize the interior; turn off the UV lamp but keep the clean air flowing.
4. Shake the blood gently in the cylinder. Working with your hands and all blood and equipment inside the bench, use the 25mL pipette to portion into 20mL aliquots in 30 sterile vials.
5. Clean thoroughly the graduated cylinder, sieve and funnel before using them again and place them inside the bench.
6. Label the vials as described in Section 7 *Labelling bottles and* samples with the following information:
    a. Collection date: should be the same as the sampling date.
    b. Vial number / total number of vials of the sample (example: 1-30, 2-30, …)
7. Record the previous data electronically (in a database or spread sheet) and in a hardcover record book.
8. Store the vials in deep freeze at -20°C before irradiating them the next day. After irradiation, the vials are ready to start the 25-day feeding test.

This operation must be done by the Lab Technician. All the equipment used must have been sterilized previously in the oven/autoclave, following the appropriate procedure described in Section 1.

Figure 4. Scintillation vials.

6. Proportioning and sampling for microbial screening

Scope:
To proportion blood pooled in large containers into storage bottles (usually 2L or 4L based on the size of the irradiator canister) so that they can be irradiated and handled conveniently.
During the proportioning of blood from the large transport container, samples for the bioassay and for the microbial screening must be taken. All samples and bottles will be labelled in the same operation.

Equipment and Materials

- Funnels (metal, small)
- Storage bottles (usually 1L, 2L or 4L volume), preferably made of heat- and cold-resistant plastic, that are heat-sterilized before use and able to withstand prolonged storage at -20°C. The lid must have a screw cap with a rubber gasket that can be tightly closed and an inner push-fit cap to accommodate the Eppendorf tube with the sample for microbial screening.
- Pipette
- Sterile tips, 1mL
- Microtubes, 1.5mL
- Pre-printed labels
- Laminar flow bench
- Surface sterilant (70% ethanol)
- Gloves

Procedure:

1. After the sample for the 25-day feeding test has been taken, place an open 2L bottle under the tap of the transport container with a funnel if necessary.
2. Open the tap at the bottom of the transport container and fill the bottle with blood.
3. Close the blood bottle and take it to the laboratory. Do not shake it. Place it in the laminar flow bench, with the clean air flowing.
4. Open the bottle and take a sample (1mL) in a disposable Eppendorf tube of 1.5mL using a pipette with sterile tip.
5. Label both the Eppendorf tube and the bottle as described in Section 7 with the following information:
    a. Collection date: should be the same as the sampling and proportion date.
    b. Bottle (Eppendorf) number / total number of bottles (Eppendorf) in the blood batch

    Both the bottle and the sample must be labelled with exactly the same information. The information in the labels has been previously prepared and printed.
6. Close the inner push-fit cap and fix the Eppendorf to the centre upper part of the push-fit cap with tape
7. Close the screw tap tightly.
8. Deep-freeze the bottles and their bacteriology samples together immediately at -20°C. Sampling and proportioning must be done in the same operation.

9. Thoroughly clean and sterilize all equipment that was in contact with blood and keep until required again.
10. Record the data electronically (in a database or spread sheet) and in a hardcover record book.
11. This operation must be done by the Lab Technician. All the bottles and equipment used must have been sterilized previously in the oven/autoclave, following the appropriate procedure described in Section 1 *Sterilization* of materials in heat sterilization oven or autoclave.

Figure 5. Some of the blood proportion equipment (funnel, sieve, 100L container, storage bottles of 4L, 2L and 1L capacity, Eppendorf tubes).

7. Labelling bottles and samples

Scope:
Labelling the samples and the different blood bottles used is key to ensuring traceability of the blood in the store and to ensure the correspondence between samples and bottles.
To minimize typing mistakes on the labels while handwriting and to avoid difficulties when reading hand written labels, labels will be produced using a label printer and specific software. Ideally, special labels for cold storage should be used to ensure that the ink will not fade and the label will stick properly in the frozen storage.
All items that will be stored in the cold room need to be labelled:
A. 20mL-vials containing the samples for the bioassay
B. Storage bottles, usually 2L or 1L based on availability, after proportioning

C. 1mL Eppendorf samples for microbial screening.

Printed labels must be ready before initiating the sampling and proportioning operation. This task must be done by the Lab Technician.

Equipment and Materials:

- Label printer
- labels

Procedure:

A. Labelling the vials containing the samples for bioassay

30 samples are taken into 20mL vials for the bioassay. Each vial must be labelled with the following information:

a. Collection date: this should be the same date of the sampling and proportion.
b. Vial number / total number of vial of the sample (example: 1-30, 2-30, …)

Col.: 22-03-2015 / BA: 1-30

Collection date: 22nd of March 2015

First vial out of 30 from the same collection day.

B. Labelling the storage bottles after proportioning

After the proportioning, storage bottles (1L or 2L) will be labelled with the following information:

a. Collection date: this should be the same date of the sampling and proportion.
b. Bottle number / total number of bottles proportioned from the same batch (example: 1-64, 2-64)

Col.: 22-03-2015 / B: 1-64

Collection date: 22nd of March 2015

First bottle out of 64 from the same batch.

C. Labelling the Eppendorf with the samples for microbial screening

During the proportioning, individual samples of the blood of each bottle are taken in Eppendorf tubes for later microbial screening. These Eppendorf tubes must be labelled with exactly the same information as the original bottle from which the blood sample comes.

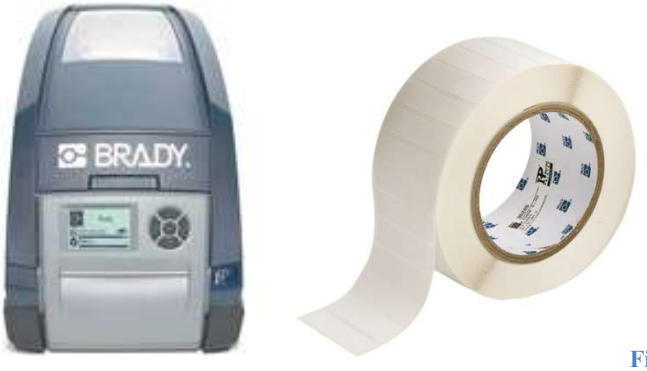

Figure 6. Thermal label printer and labels.

8. Freezing at -20°C

Scope:
To preserve and store the animal blood for processing into diet for feeding tsetse flies frozen at -20°C to avoid the development of microbial contamination.
The blood will be frozen after proportioning into the storage bottles (usually 1L or 2L) and stored until the results of the quality control tests are available.

Equipment and Materials:
- Chest freezers or walk-in rooms at –20°C.
- Storage bottles, usually with a volume of 1L or 2L.

Procedure:
1. After proportioning, clean any residue of blood from the outside of the bottles.
2. Bring all the bottles to the chest freezers or walk-in rooms and store them in an orderly manner, with the labels easily accessible.
3. Protective clothing must be used inside the deep-freeze walk-in rooms.
4. Freeze the bottles immediately at -20°C. Make sure that the corresponding Eppendorf tube with the sample for microbial screening is kept together with the bottle.

9. Bioassay: 25-day feeding test

The 25-day feeding test was developed with the aim of having a simple numerical system that adequately summarizes and combines the various data obtained from pupal production and dissections. It is expressed as the quality factor (QF). For irradiated blood, a QF value of 1.1 is the minimum acceptable value, although higher values (QF >1.2) are recommended. The assumption is that the larva/pupa will develop into a viable fly. Since both the female adult

and the larva within the uterus are dependent on the same source of food, it follows that the quality of the blood is one of the most important factors affecting reproductive physiology in tsetse.

For the blood quality feeding test, a minimum quantity of 600 mL of blood aliquoted in 30 vials is necessary. These vials will be irradiated and one of them tested for microbial contamination. Three small (11 cm) cages of thirty teneral females will be mated and fed daily for 25 consecutive days using this blood. The number and size of pupae produced, number of abortions and the number and reproductive status of the surviving females will be used in an empirical formula to assess the nutritional value of the blood. The highest of the three results is taken as the quality factor for the blood collection.

This test is lengthy and labour intensive. Due to this, only one bioassay is conducted for each collection day. The value of the QF obtained represents the average nutritional value of the blood collected on the same day from different animals.

Scope:
25-day feeding test

Equipment and Materials:
- Scintillation vials of blood for testing (30, each 20mL, polyethylene)
- Deep freezer
- Laminar-flow bench (hood)
- Foil (aluminium, to cover autoclaved or sterilized glassware)
- Bunsen burner
- Weighing balance (resolution 0.01 g or better)
- Magnetic stirrer/hot plate
- Incubator
- Bacterial-colony counter/viewer
- Petri dishes (glass and autoclaved or disposable and new)
- Distilled water (autoclaved)
- Nutrient agar
- Erlenmeyer flasks (various sizes)
- Eppendorf with the blood corresponding to the bottle to be tested
- Syringes (1mL, sterile, disposable)
- Incubator
- Bacterial colony-viewing/counting device
- Teneral female flies (90, mated in the appropriate way, and caged at 30 per standard 11cm-diameter cage)
- Radiation source
- Stereomicroscope
- The blood used for the bioassay must also be screened for bacterial contamination. Therefore, the list of equipment and materials should be completed with those described in the procedure of Section 11.

Procedure:

1. Thoroughly clean and sterilize all parts of equipment that have come into contact with blood.
2. Sterilize in an oven by exposure to 80°C (polyethylene) or 120°C (glass and metal) for 3 hours or in an autoclave at 120°C (glass and metal only) for 30 minutes, and then store properly to minimize the risk of microbial contamination.
3. At emergence, put 30 teneral females and 15 teneral males each in three standard 11cm-diameter cages. After 10 days, males can be removed to avoid disturbance to the females.
4. Mate flies in the appropriate way.
5. Thaw one vial of blood each day. Using a syringe and needle, take a 1mL sample from the vial and perform microbial screening of the blood as in Section 8.
6. Feed flies daily for 25 days, using the samples from the batch to be tested.
7. Check for mortality and dissect any dead female — examining for uterine content, insemination, and mating scars where applicable, and record.
8. After 8 days, place the cages on individual dishes, and thereafter examine the dishes for abortions under the stereomicroscope on days 10, 15, 20 and 25 from the time of emergence.
9. Collect larvae and pupae.
10. Allow larvae to pupate, and record individual weight.
11. If a pupal size-sorting machine is available, at the termination of the test, sort by class all pupae, count, and note the various size classes.
12. Dissect all females surviving 25 days, and examine for mating scars, insemination, uterine content, follicle next in ovulation sequence (FNOS), and reproductive abnormalities such as blockage of the oviduct. ANNEX II describes the procedures for uterus dissection.
13. Calculate the quality factor following the formula provided in the following box.
14. Record the previous data electronically in the spread sheet or database prepared for this purpose and in a hardcover record book.

Parameters used to calculate the blood quality factor (QF):

Parameter Explanation

First reproductive cycle:

FS_{18}	number of females surviving on day 18
FS_{25}	number of females surviving on day 25
P_T	total number of produced pupae
P_A	number of A-class pupae
P_B	number of B-class pupae
P_C	number of C-class pupae
P_D	number of D-class pupae
P_E	number of E-class pupae

Second reproductive cycle (dissection results):

$F_{(E+I)}$	number of inseminated females on day 25 with early pregnancy stage *in utero*
$F_{(II+III)}$	number of inseminated females on day 25 with late pregnancy stages *in utero*
F_{BL}	number of inseminated females on day 25 with oviduct blockage
F_{AB}	number of inseminated females on day 25 that aborted, empty uterus, follicle next in ovulation sequence (FNOS) is not mature

Calculation of the blood quality factor (QF):

QF = (positive parameters from first reproductive cycle + positive parameters from second reproductive cycle – negative parameters from first reproductive cycle – negative parameters from second cycle) / number of females

$$QF = \frac{\left(FS_{25} + P_T + 0.3P_B + 0.4P_C + 0.5P_D + 0.6P_E + 0.3F_{(E+I)} + 0.6F_{(II+I\ )}\right) - (0.3P_A + 0.5F_{AB} + F_{BL})}{FS_{18} + FS_{25}}$$

A QF value of 1.0 is the minimum acceptable value. For laboratories where tests are run regularly, experience shows that dissections can be omitted at day 25 without affecting the overall value, but the formula for calculating the QF value is modified (see below).

An EXCEL spreadsheet to calculate the QF is available at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria.

To calculate the QFC (calculated quality factor), use the following formula:

$$QFC = \frac{(11P_A + 17P_B + 19P_C + 20P_D + 22P_E)}{(23.86FS_{18} + 0.616)}$$

An EXCEL spreadsheet to calculate the QFC is available at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria.

Entomology Unit, Joint FAO/IAEA Programme

IAEA Agriculture Laboratory Seibersdorf
Record Sheet for Tsetse Blood Diet Quality Control

BLOOD CODE: **BC06076** date collected 20.04.06 storage

date collected storage

Test-No.	6
Year	2006

date processed **pH**

additives **osm. pr.** mOsm

MICROBIAL SCREENING			(CFU/ml)
cont. A	cont. B	diet before γ	diet after γ
0 Sp/ml	Sp/ml	Sp/ml	Sp/ml

SURVIVAL & FECUNDITY

G. pallidipes
Test species

DISSECTIONS

day	DATE	dead st	dead bl	fem. surv.	No. of. pupae prod.	No. of. abortions in dish
1	5.5.06			30		
2	6.5.06			30		
3	7.5.06			30		
4	8.5.06			30		
5	9.5.06			30		
6	10.5.06			30		
7	11.5.06			30		
8	12.5.06			30		
9	13.5.06			30		
10	14.5.06			30		
11	15.5.06			30		
12	16.5.06			30		
13	17.5.06			30		
14	18.5.06			30		
15	19.5.06			30		
16	20.5.06			30		
17	21.5.06			30		
18	22.5.06	2		28	15	
19	23.5.06	1		27	3	
20	24.5.06			27	1	
21	25.5.06			27	1	
22	26.5.06			27		
23	27.5.06			27		
24	28.5.06			27		
25	29.5.06			27	2	
Σ		**3**		**27**	**22**	
26	30.5.06	1		26	1	
27	31.5.06			26	2	
28	1.6.06			26	7	
29	2.6.06			26	6	
30	3.6.06			26		
Σ		**4**			**38**	

females: **males:**
date EM date MAT date SEP date EM

other remarks:

	weight class distribution				
	A	B	C	D	E
Σ	**0**	**0**	**0**	**0**	**0**
Σ	**0**	**0**	**0**	**0**	**0**

N	Sp	UT
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
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22		
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25		
26		
27		
28		
29		
30		

Day 25

FS 18	28	PT	22	PB	0	PD	0	E + I	0	AB	0
FS 25	27	PA	0	PC	22	PE	0	II + III	0	BL	0

QF	
QFC	1.24

Day 30

FS 18	28	PT	38	PB	0	PD	0	E + I	0	AB	0
FS 30	26	PA	0	PC	38	PE	0	II + III	0	BL	0

QF	
QFC	1.70

Figure 7. Template of the excel file to record and calculate the blood quality factor.

10. Decontamination by irradiation

This procedure describes the decontamination of animal blood, after collecting blood at an abattoir, to reduce bacterial contamination for processing into diet for feeding tsetse flies.

Access to a gamma radiation source is a prerequisite for decontamination at 1 kGy.

After the bioassay is completed and if the nutritional value of the blood batch is acceptable, blood must be decontaminated and tested for microbes before being used to feed the flies. The 1L, 2L or 4L bottles with the proportioned blood and its corresponding sample for microbial screening are taken from the storage in the cold room and irradiated in a frozen state.

The samples for the bioassay must also be irradiated before initiating the feeding test.

Scope:
Decontamination of frozen blood stored in 1L, 2L or 4L bottles and of samples for bioassay and microbial screening using gamma radiation.

Equipment and Materials:

- Gamma radiation source (commonly, a Gamma Cell irradiator is used)
- Blood (frozen) in 1L, 2L or 4L bottles with their samples for microbial screening in Eppendorf tubes. The samples must never be treated separately from their corresponding bottles.
- Blood samples in 20 mL vials for bioassay
- Irradiation basket (cylindrical metal box) for bioassay samples

Procedure for blood irradiation

1. Transport the frozen blood (in 1L, 2L or 4L or other size bottles) from frozen storage to the irradiation facility. The Eppendorf tube with the sample for microbial screening must be properly fixed to the bottle. Both the sample and the bottle must be irradiated in the same operation, to ensure that both receive the same dose.
2. Open the Gamma Cell, put the 1L, 2L or 4L or other size bottle into the irradiation chamber and close the chamber.
3. Close the doors of the irradiation chamber with special care with the sliding latch.
4. Set the exposure time (according to the activity of the source) for 1 kGy.
5. Press the start button.
6. After the exposure is finished, retrieve the bottle. Label the irradiated bottle with the word: IRRADIATED. Irradiation indicators can also be used.
7. Put the bottle together with the other irradiated bottles and place a new non-irradiated bottle in the chamber.
8. Repeat until all bottles have been irradiated.
9. Transport the blood still frozen back to the store.
10. Store the blood frozen until the results of the microbial screening show that the blood can be used for thawing and feeding.

Special attention is required to avoid mixing the non-irradiated bottles with the irradiated ones during the operation. In the cold room, irradiated bottles must not be stored on the same side as non-irradiated.

The elapsed time with the bottles out of the freezer must be kept as short as possible. If thawing of the external layer of blood is observed, reduce the thawing time by irradiating fewer bottles at a time.

Irradiation must be done under the appropriate supervision of the Radiation Protection Officer (RPO) and following the strict regulations of the National Radiation Protection Authority of the country.

Irradiation must only be done by authorized personnel.

Procedure for sample irradiation

1. Take the sample vials for the 25-day feeding test from the chest freezer, put them in the irradiation basket and bring them to the irradiation facility.
2. Check that vials are properly labelled.
3. Open the gamma cell, put the basket into the irradiation chamber and close the chamber.
4. Set the exposure time (according to the activity of the source) for 1kGy.
5. Press the start button.
6. Retrieve the vials when irradiation is complete.
7. Store the vials in a chest freezer in the lab. Use one of the vials for microbial screening and, if acceptable number of CFU, the rest of the samples are ready to start the bioassay.

11.Microbial screening of the blood

Scope:

Microbial screening of blood to decide if the blood is suitable for tsetse feeding. Microbial screening must be done for each feeding bottle (usually 2L or 4L bottles) that has been stored in the walk-in freezer to ensure that the bottle is not contaminated.

The blood sampled for the bioassay will also be tested for bacterial contamination before the bioassay is started. In this case, only one test will be conducted for each set of bioassay samples.

In both cases, the samples are irradiated to reduce the bacterial load before the microbial screening is conducted.

Equipment and Materials:

- Laminar-flow bench (hood)
- Bunsen burner
- Weighing balance (resolution 0.01 g or better)

- Magnetic stirrer/hot plate
- Incubator
- Bacterial-colony counter/viewer
- Petri dishes (glass and autoclaved or disposable and new)
- Distilled water (autoclaved)
- Nutrient agar
- Erlenmeyer flasks (various sizes) with cotton wool
- Eppendorf with the blood corresponding to the bottle to be tested
- Pipette and Tips or 1mL syringe to take 1 mL blood
- Spatula
- Ethanol 70% or other antiseptic solution
- Printed Label

Procedure:

1. Mix 2g of agar with 100mL distilled water in a 200mL glass Erlenmeyer flask closed by cotton wool, stir gently and boil.
2. Autoclave the dissolved agar at 125°C for 15 minutes.
3. Put the flask of agar in a water bath at 40°C.
4. Clean working bench and hands with Ethanol 70%.
5. Place sterile Petri dishes on the bench.
6. Wipe the outer surface of all containers with ethanol 70% before placing them on the bench.
7. Ignite the Bunsen burner.
8. Label the Petri dishes on the bottom of the smaller half with the blood code and have one Petri dish for air and one Petri dish for nutrient agar.
9. Open the Petri dishes briefly, flame the open parts, and close them immediately.
10. Make sure the agar in the Erlenmeyer flask is between 40–45°C.
11. Lift the lids of the Petri dishes and add 1mL samples of blood from the Eppendorf tubes to the pert dish
12. Again, lift the lids slightly from the Petri dishes and pour on 10- 13mL agar to each Petri dish. Aim at an agar-layer thickness of ¼-⅓ of the internal depth of the dish. Ensure that the temperature of the agar is under 45°C, which would kill bacteria and give false results.
13. Stir gently by hand for about 15 seconds to mix and homogenate the distribution of the blood samples and the agar, put the lid of the plate close to the petri-dish, but ensue you leave the plate open.
14. Wait until the mixture of agar and blood has solidified, and then close the petri-dish by the lid and turn the Petri dish upside down.
15. Incubate the inoculated Petri dishes in the upside-down position at 37°C for 24-72 hours.
16. Check the agar layers for bacterial colonies after 48 and 72 hours.
17. Record the number of bacterial colonies found per sample.
18. If, after 72 hours, the irradiated blood contains NOT more than 10 colonies, the corresponding bottle of blood is ready to be thawed, provided the result from the 25-day feeding test of its corresponding batch is satisfactory.
19. If the blood has more than 10 colonies, discard this bottle of blood. In case of shortage of blood and if the number of CFU is between 10 and 20, the bottle of blood can be

exposed to an additional irradiation dose of 0.5 kGy instead of being discarded. A new microbial screening will be conducted to ensure that the number of CFU has been reduced below 10. The accumulated dose should not exceed 2kGy because of the accumulation of toxic methaemoglobin.

20. Record the previous data electronically in a database or spread sheet prepared for this purpose and in a hardcover record book.

Figure 8. Petri dishes with nutrient agar ready for incubation.

Figure 9. Laminar flow bench with the equipment used for the microbial screening.

12.Blood thawing

Scope:

Thawing the blood stored in the abattoir bottles is required before mixing it in one single batch for the bioassay sampling and proportioning.

The blood will also be thawed after all the processing is completed and the quality control tests are satisfactory.

Blood freezing and thawing operations should be kept to the minimum necessary since the quality of the blood is reduced after each operation while the chances of contamination increase.

Equipment and Materials

- Chest freezers or walk-in freezers maintained at -20°C)
- Abattoir bottles (usually 5L) or storage bottles (usually 1L or 2L)
- Refrigerator (at +4°C)
- Boxes (plastic, for storage of bottles with blood)

Procedure:

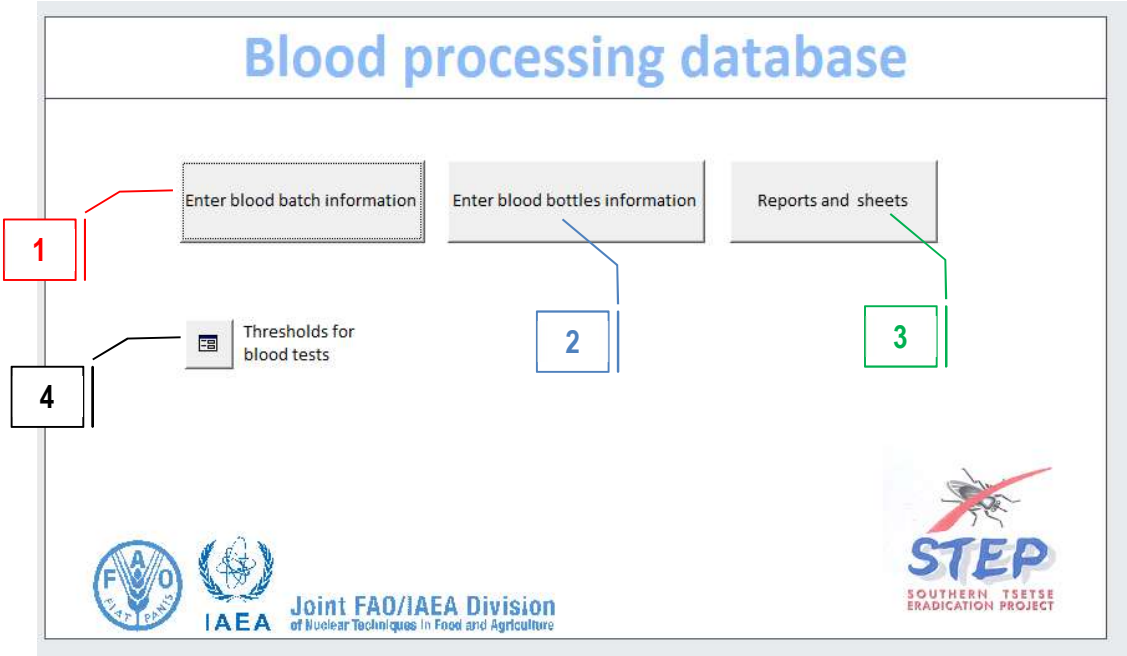
1. Estimate the amount of blood that will be thawed.
   If the goal is to proportion it into storage bottles, all the abattoir bottles from the same collection day must be thawed at the same time to obtain a single batch for the bioassay sampling.
   If the goal is to prepare the blood for feeding the flies after all the processing has been completed, the amount needed for the following 3-4 days will be estimated. This procedure is performed twice per week. Verify that the results of both the microbial test and the 25-day feeding test for the batch of blood that is intended to be used are satisfactory.
2. Transfer an appropriate number of frozen bottles of blood to a refrigerator to thaw SLOWLY at +4°C. For example, to thaw blood for feeding flies, transfer the bottles on a Friday, planning to use some of the bottles of blood on the following Monday, and the remaining bottles on Wednesday.
3. For bottles stored at +4°C until Monday, do not place the bottles tightly together to permit them to thaw. For bottles stored at +4°C until Wednesday, place the bottles together tightly (which will slow down the rate of thawing) until Monday, but on Monday separate them so that they thaw more quickly. The blood must be completely thawed when the next step in processing begins.
4. This blood is not thawed rapidly (as was once done under running cold water) because there is a risk with rapid thawing that the blood near the surface of the bottle warms up beyond +4°C before the middle of the bottle is fully thawed, reducing the quality of the blood. Instead, the blood is transferred from -20°C frozen storage and kept at +4°ºC in a refrigerator for several days, allowing the blood to thaw slowly.
5. When the blood has thawed, it is ready to use in feeding flies. However, keep the bottles of blood at +4ºC until the blood is to be poured onto feeding trays (under feeding membranes). If thawed blood is not used, it can be stored for a maximum of 3 days at +4ºC. If, after 3 days, this blood has still not been used, discard it.
6. If some of the thawed blood in a bottle is used to feed flies but the remainder is not used, this unused blood can be stored at +4ºC **in a separate place** for up to 3 days. Discard the blood if it is not used within these 3 days.
7. If there is an urgent unforeseen need for blood, frozen bottles can be thawed in cold water. Make sure the water does not contact the cap or enter the bottle. Rapid thawing should not become a routine procedure.

ANNEX I: **Use of a database for storage and analysis of the blood processing information**

A user-friendly database has been developed in MS Access 2010 to allow a proper storage and management of the information of the processing of the blood following the current procedures. This database is available at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria.

It has one main menu and three submenus (blood in, blood out and reports)

Main Menu



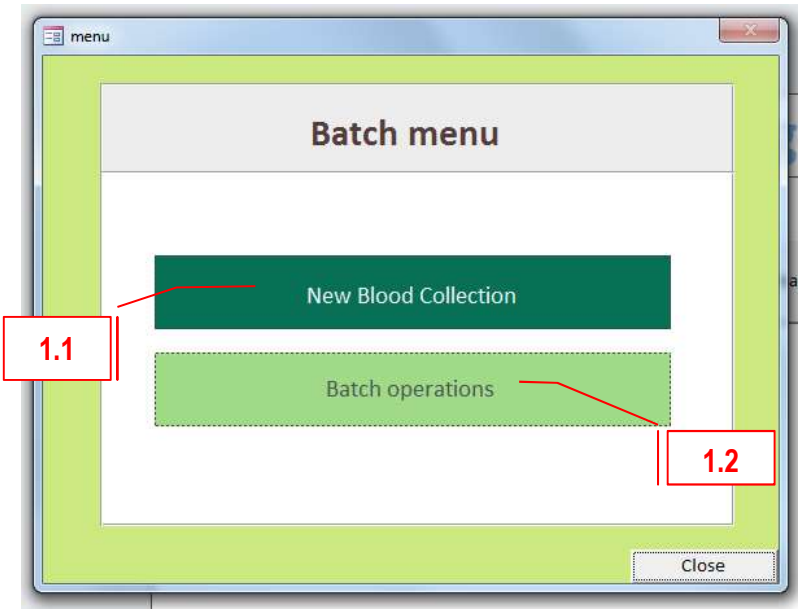
1 Enter blood batch information: Use this option to start introducing the information on the blood collection operations at the abattoir and the subsequent batch operations in the insectary, including thawing, proportioning and batch testing (both microbial screening of the batch and bioassay)

2 Enter blood bottles information: Use this option to introduce the information related to the blood bottles in the facility: irradiation, microbial screening of the individual bottles after irradiation and blood delivery for flies feeding.

3 Reports and sheets: this option allows on one side to check and print the current status of the blood storage and, on the other side, to print blank forms that can be used to record manually the information of the different operations. These forms incorporate updated fields according to information from precedent operations.

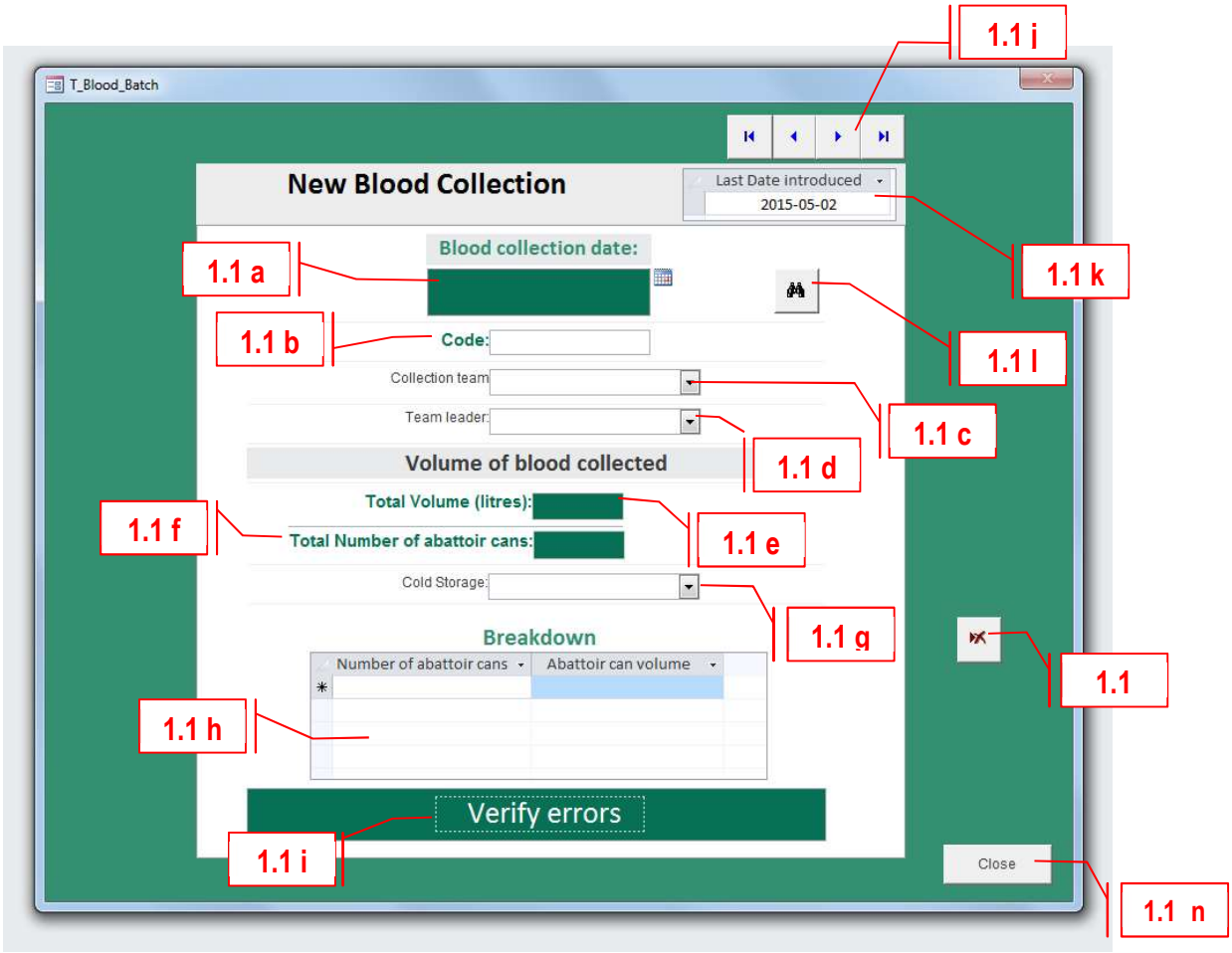
4 Thresholds for blood tests: Use this option to modify the threshold values for the microbial screening and bioassay

1. Batch Menu



1.1 New blood collection

Use this option to enter the information on the blood collection operations at the abattoir. After clicking on the button, the following data entry form will appear:



1.1.a Blood collection date: enter the date of blood collection. This field is mandatory

1.1.b Code: enter the code as given in the laboratory until now. This field is not mandatory

1.1.c Collection team: using the drop down list, select the team that collected the blood in the abattoir on the selected date. If the team is not listed, additional teams can be added by clicking in the icon with a pencil at the bottom of the drop-down list. This field is not mandatory.

1.1.d Team leader: using the drop down list, select the name of the team leader that collected the blood in the abattoir on the selected date. If the team leader is not listed, additional team leader can be added by clicking in the icon with a pencil at the bottom of the drop down list. This field is not mandatory.

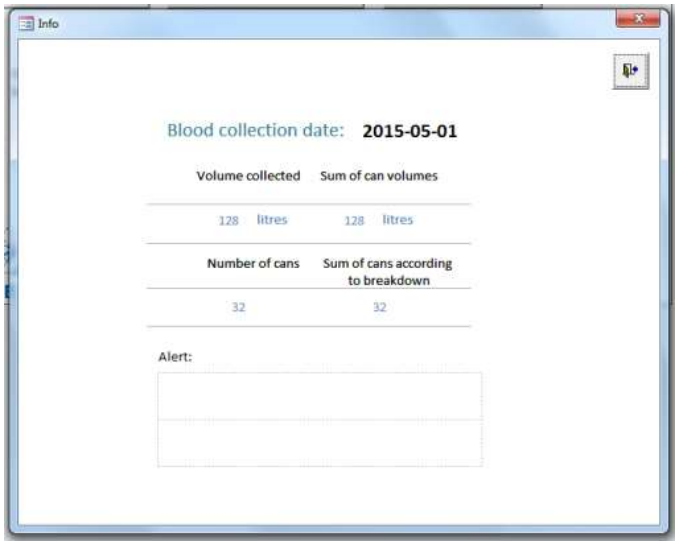
1.1.e Total volume: enter the total volume of blood (in litres) collected on the selected date. This field is mandatory.

1.1.f Total number of abattoir cans: enter the total number of abattoir cans used to collect the blood on the selected date. This field is mandatory.

1.1.g Cold storage: select from the drop down list the cold room where the abattoir cans are stored until the thawing. This is not a mandatory field.

1.1.h Breakdown: this option enables the user to enter different types of abattoir cans with different volumes used to transport the blood to the insectary. This field is mandatory.

1.1.i Verify errors: this button checks if the number of abattoir cans entered in field 1.1.f match with the sum of cans entered in the breakdown. It also checks if the total blood volume in the breakdown matches with that of the field 1.1.e. An pop-up window will display the message with the confirmation or matching errors if any.



1.1.j Surf through records: if required, use these buttons to go to the first, previous, next or last record.

1.1.k Last date introduced: gives the useful information to the user on the last collection date for which data has been entered in the database. The field must not be edited.

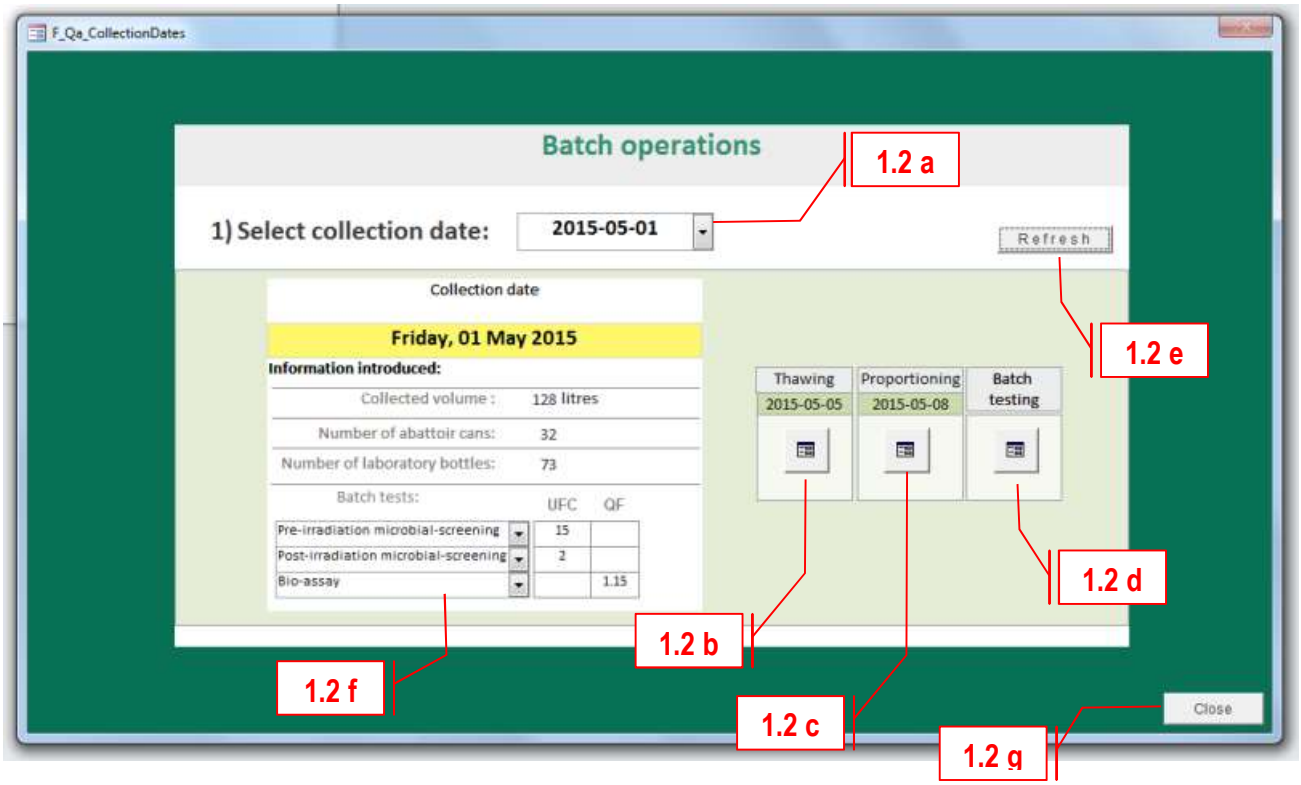
1.1.l Search specific date: allows the user to search collection records entered for a specific date.

1.1.m Delete present record: use this button only when the information entered is not correct and must be deleted**. If additional information on the selected collection date has been entered using the menus described later in the manual, it will also be deleted.**

1.1.n Close: close the current window and go back to previous menu.

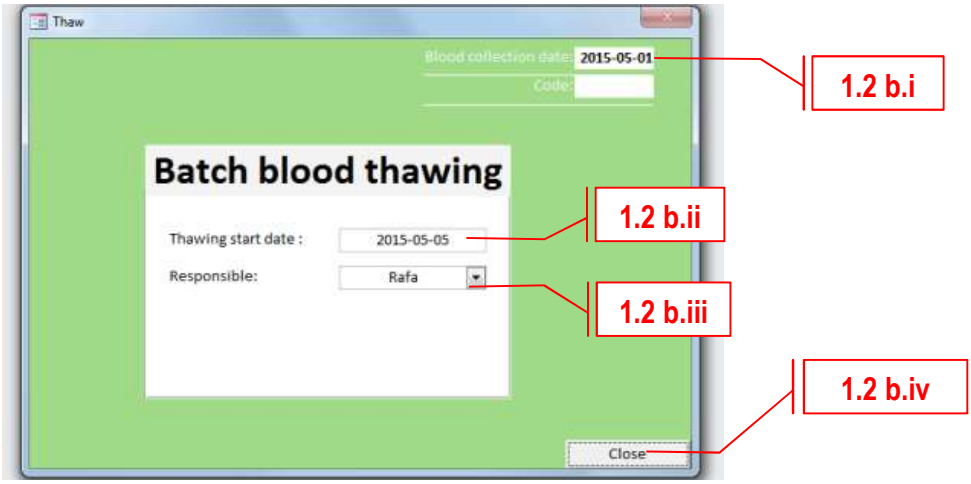
1.2 Batch operations

Go to this submenu to enter any information related to the batch operations, including thawing, proportioning and batch quality testing (microbial screening of the batch and bioassay)



1.2.a Blood collection date: enter the date of blood collection from the options available. Only dates with previously filled information on blood collection are available in the drop-down list. This field is mandatory.

1.2.b Thawing: click this button to enter the info related to the thawing operation. A new window will be displayed.



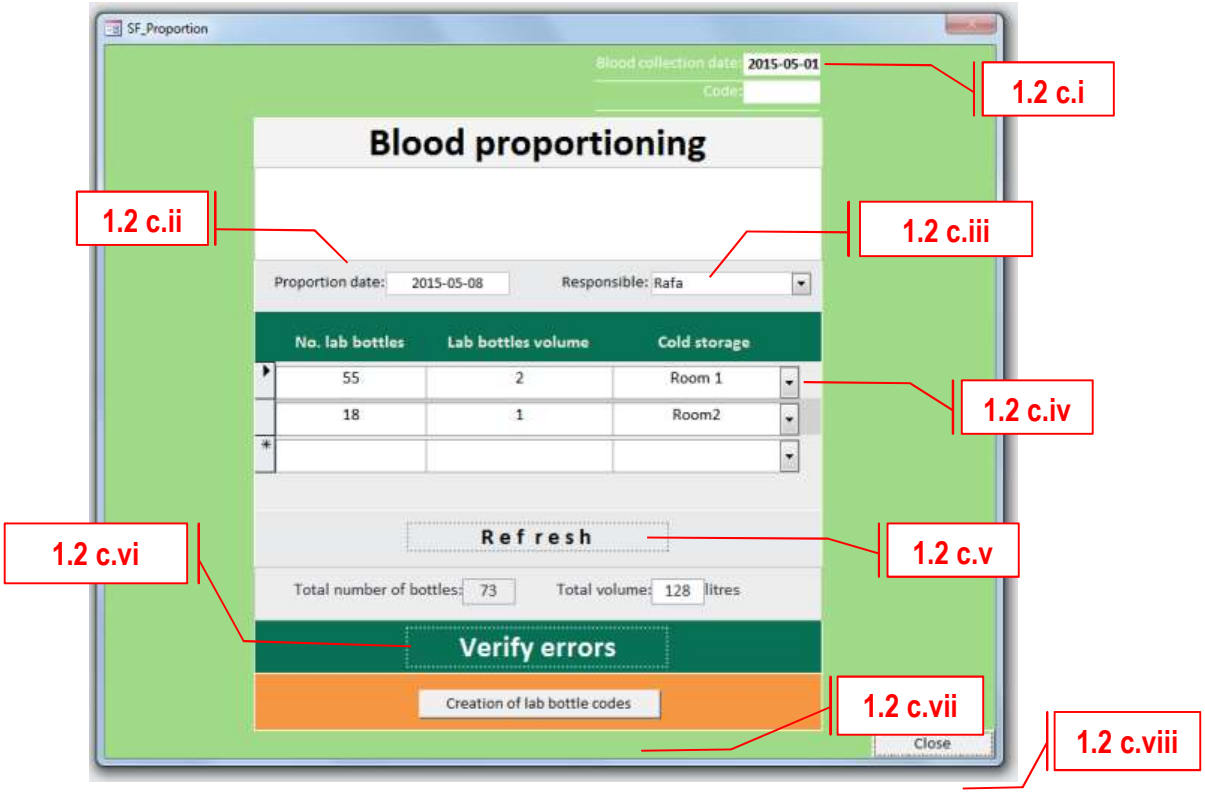
1.2.b.i Blood collection date: this is just an informative field, showing the date that was selected in the previous menu "Batch operations". It cannot be changed.

1.2.b.ii Thawing start date: select the date when the thawing operations have started. Note that this date will be different than the date displayed in the upper left field "Blood collection date". This field is mandatory.

1.2.b.iii Responsible: select the laboratory technician responsible for the thawing from the drop-down list. If not found on the list, it can be added to it using the pencil icon at the bottom. This field is not mandatory.

1.2.b.iv Close: close the current window and go back to previous menu.

1.2.c Proportioning: click this button to enter the info related to the proportioning operation. A new window will be displayed.



1.2.c.i Blood collection date: this is just an informative field, showing the date that was selected in the previous menu "Batch operations". It cannot be changed.

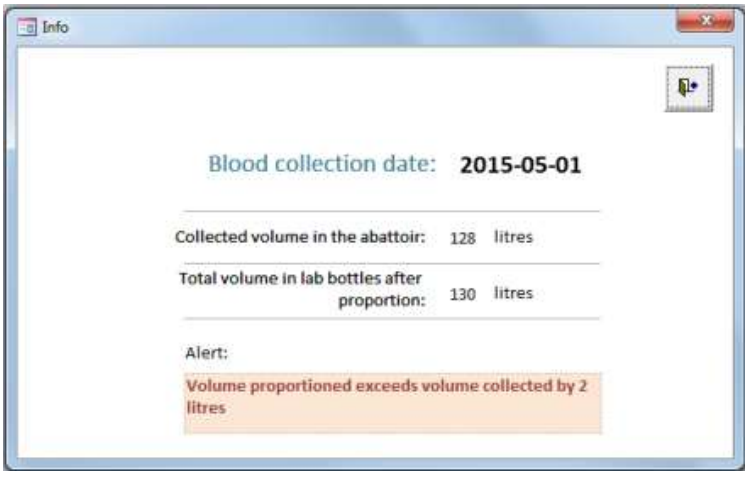
1.2.c.ii Proportion date: select the date when the proportion operations is done. Note that this date will be different than the date displayed in the upper left field "Blood collection date". This field is mandatory.

1.2.c.iii Responsible: select the laboratory technician responsible for the proportioning from the drop-down list. If not found on the list, it can be added to it using the pencil icon at the bottom. This field is not mandatory.

1.2.c.iv Table: fill as many rows in this table as different types of bottles have been used in the proportioning operation. Each row has to include the number of bottles, their volume and the cold room where they have been stored.

1.2.c.v Refresh: click on the refresh button after filling the table. The total number of bottles and total volume will be displayed in the fields below.

1.2.c.vi Verify errors: this button checks if the sum of laboratory bottles and their blood volume entered in table 1.2.c.iv match with the information of blood collected entered in the data entry form "Blood collection". An emerging window similar to the one below will display the message with the confirmation or matching errors if any.



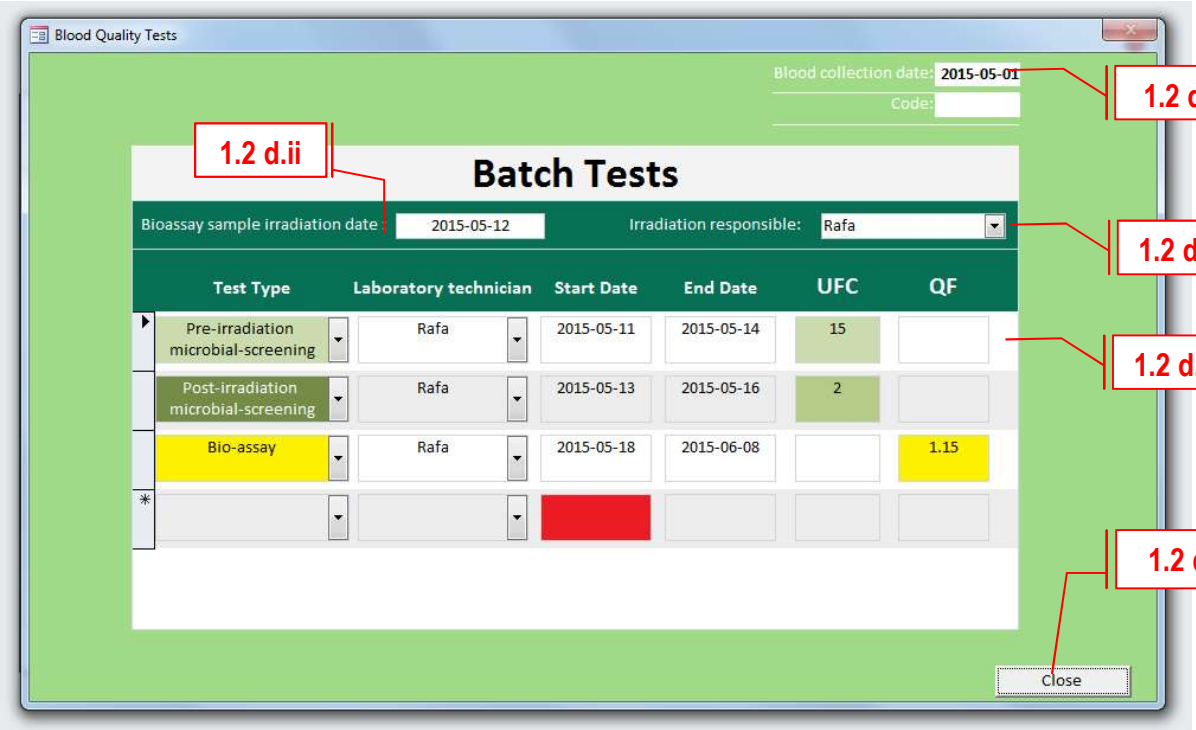
1.2.c.vii Creation of lab bottle codes: by clicking on this button a new record is generated in the database for each bottle used in the proportioning. **This is a mandatory button.**

Microsoft Access will display a warning message with the following text: "You are about to run an append query that will modify the data in your table? Are you sure you want to run this type of action query?" Respond yes and confirm the following message.

After the codes have been created, a message with the number of bottles that have been added to the database will be displayed under the title of the window. If any modification to the information entered is needed to correct eventual mistakes, the records on blood collection corresponding to this day must be deleted and the information must be re-entered again, starting with the menu on "Blood collection".

1.2.c.viii Close: close the current window and go back to previous menu.

1.2.d Batch testing: click this button to enter the info related to the batch testing operations. A new window will be displayed.



1.2.d.i Blood collection date: this is just an informative field, showing the date that was selected in the previous menu "Batch operations". It cannot be changed.

1.2.d.ii Bioassay sample irradiation date: enter the date when sample for the bioassay was irradiated. This date will differ from the "Blood collection date" and from the dates when the tests are conducted.

1.2.d.iii Irradiation responsible: select the operator responsible for the irradiation of the bioassay. If not found on the list, it can be added to it using the pencil icon at the bottom. This field is not mandatory.

1.2.d.iv Test table: fill one row for each test that has been conducted. On the first column, the test type has to be selected from the drop down list. The laboratory technician responsible for the test can be selected in the second column. Third and fourth column enable the user to add the dates when the tests were started and finished. For the microbial screenings, the resulting number of UFC will be filled in column 5. For the bioassay, the value of the QF will be filled in column 6.

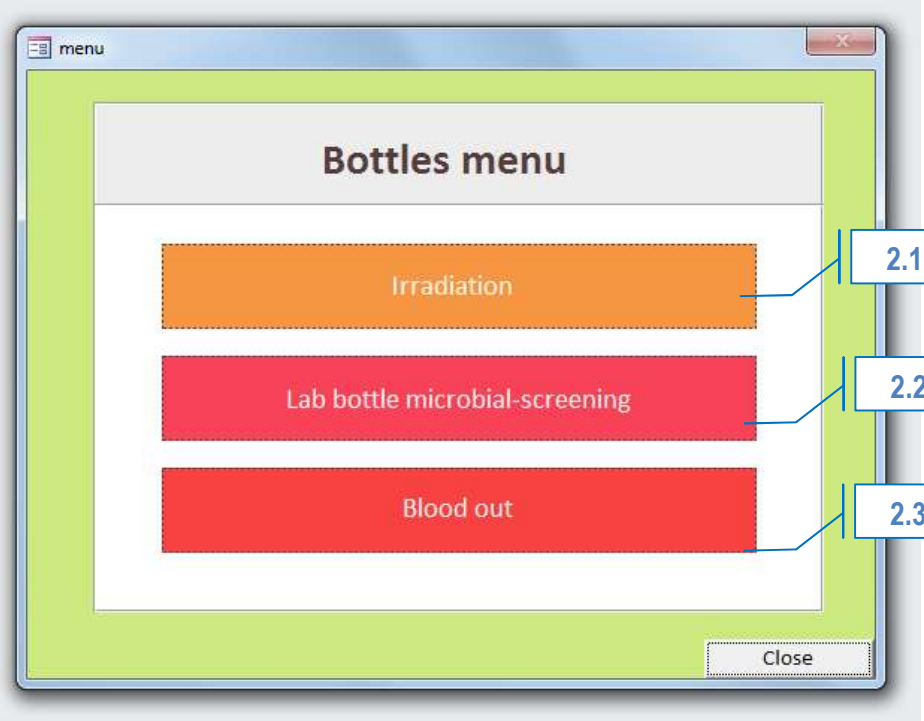
1.2.d.v Close: close the current window and go back to previous menu.

1.2.e Refresh: click this button to refresh the info displayed in the table “Information introduced” on the left side of the window. If new info has been entered using the buttons “Thawing”, “Proportioning” or “Batch Testing”, the table will be updated accordingly.

1.2.f Information introduced: after clicking on the refresh button, the table displays all the available information on the blood batch selected date.

1.2.g Close: close the current window and go back to previous menu.

2. Bottles Menu



2.1 Irradiation

Go to this submenu to enter the information related to the blood bottles irradiation.

2.2 Lab bottle microbial screening

Go to this submenu to record the information related to the blood bottles microbial screening.

2.3 Blood out

Go to this submenu to enter any information related to the delivery of stored blood bottles for flies feeding.

2.1 Irradiation

2.1.a Step 1: Select blood collection date and bottle volume

The drop down list offers the bottles for which records on collection, thawing, proportioning and batch tests have been entered. Select the desired bottles from the available options. The bottom table will show the rows corresponding to the selected option.

2.1.b Step 2: Enter the common information to all selected bottles

Enter the information on irradiation date, irradiator, exposure time, irradiation responsible, irradiation operator and room where irradiated bottles have been stored. This information will be assigned to all the bottles selected in the table below.

2.1.c Step 3: Click on the bottles that will be updated with the above info

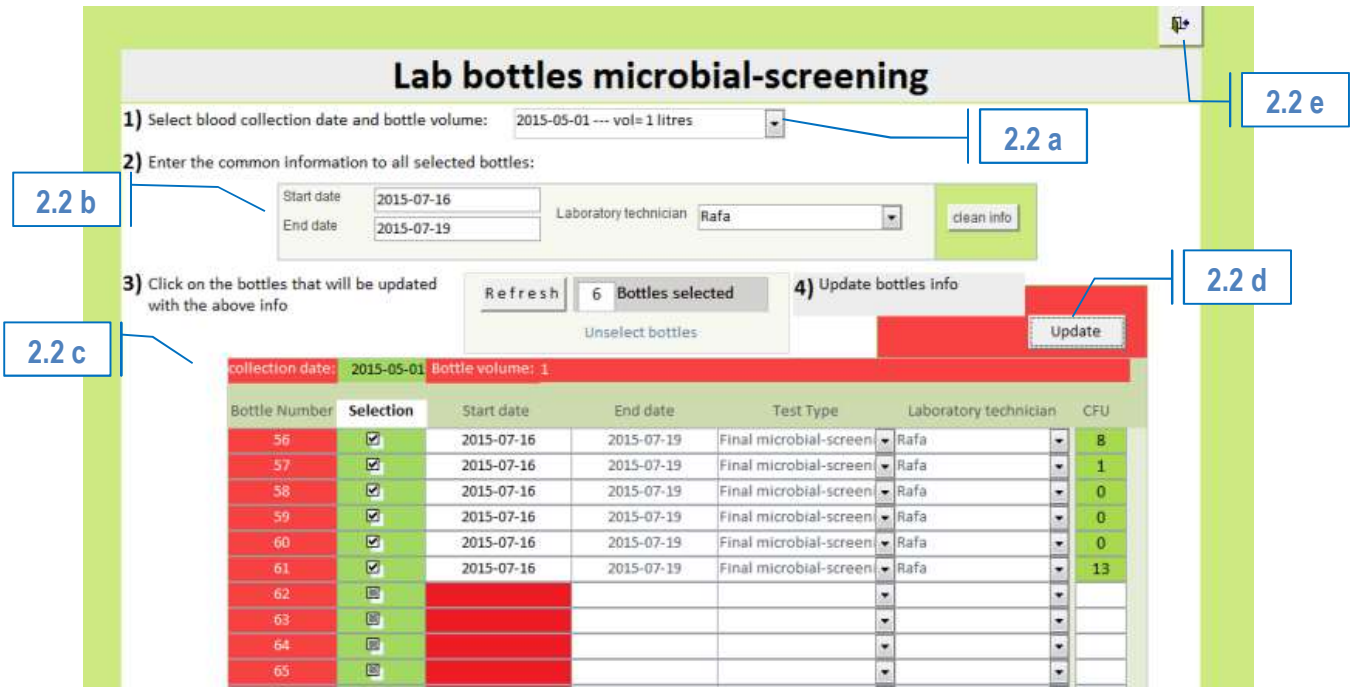
Click on the selection case of the bottles that will be updated with the information entered in step 2. The number of bottles selected is displayed after clicking on the “Refresh” button. There is also an option to unselect previous selections.
Data can also be amended for individual bottles by editing the respective row.

2.1.d Step 4: Update bottles info

Click on the button “Update” to assign the information to all selected bottles. The changes will be saved on the database.

2.1.e Exit to previous menu Close the current window and go back to previous menu.

2.2 Lab bottles microbial screening



2.2.a Step 1: Select blood collection date and bottle volume

The drop down list offers the bottles for which records on collection, thawing, proportioning, batch tests and irradiation have been entered. Select the desired bottles from the available options. The bottom table will show the rows corresponding to the selected option.

2.2.b Step 2: Enter the common information to all selected bottles

Enter the information on start date, end date and laboratory technician. This information will be assigned to all the bottles selected in the table below.

2.2.c Step 3: Click on the bottles that will be updated with the above info

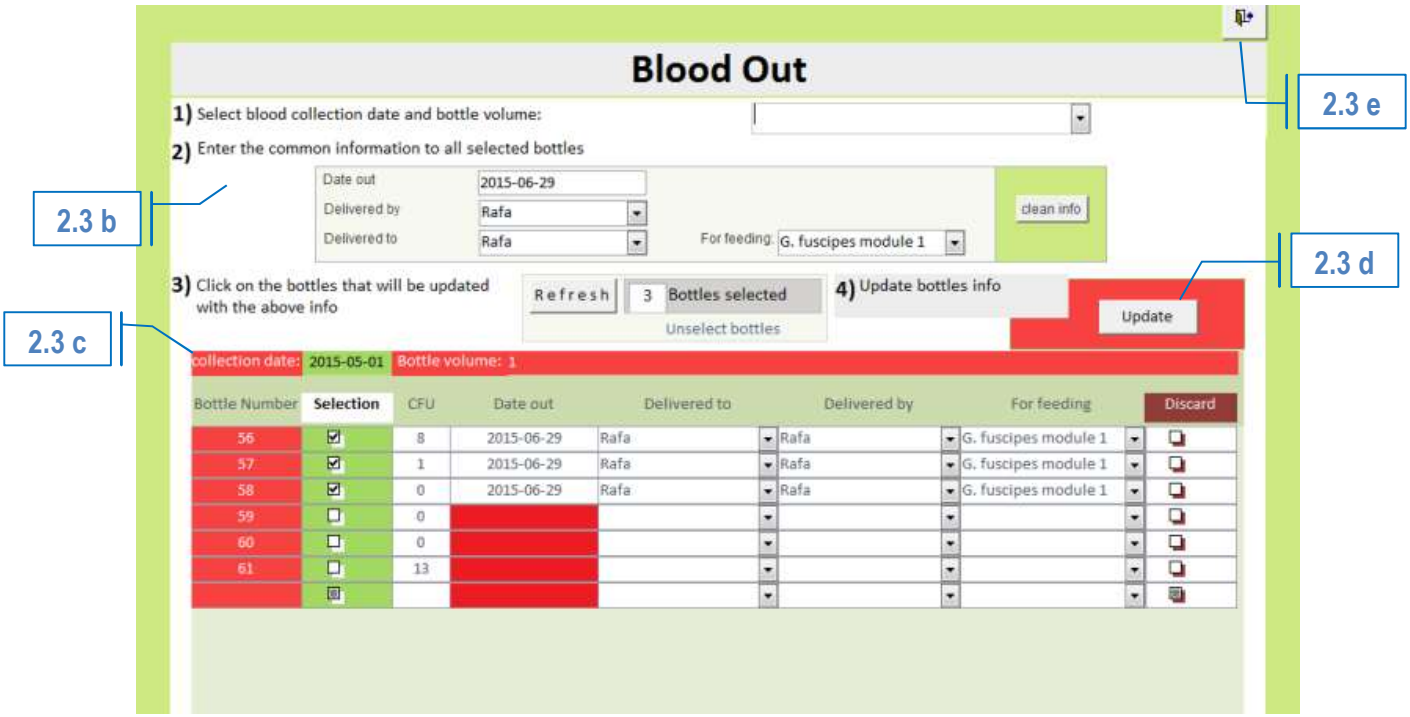
Click on the selection case of the bottles that will be updated with the information entered in step 2. The number of bottles selected is displayed after clicking on the “Refresh” button. There is also an option to unselect previous selections. Data can also be amended for individual bottles by editing the respective row. On the right column, enter the information on the number of CFU obtained after the test for each bottle.

2.2.d Step 4: Update bottles info

Click on the button “Update” to assign the information to all selected bottles. The changes will be saved on the database.

2.2.e Exit to previous menu Close the current window and go back to previous menu.

2.3 Blood Out



2.3.a Step 1: Select blood collection date and bottle volume

The drop down list offers the bottles for which records on collection, thawing, proportioning, batch tests, irradiation and microbial screening have been entered. Select the desired bottles from the available options. The bottom table will show the rows corresponding to the selected option.

2.3.b Step 2: Enter the common information to all selected bottles

Enter the information on delivery date, who is delivering and receiving the bottles and which colony will be fed with. This information will be assigned to all the bottles selected in the table below.

2.3.c Step 3: Click on the bottles that will be updated with the above info

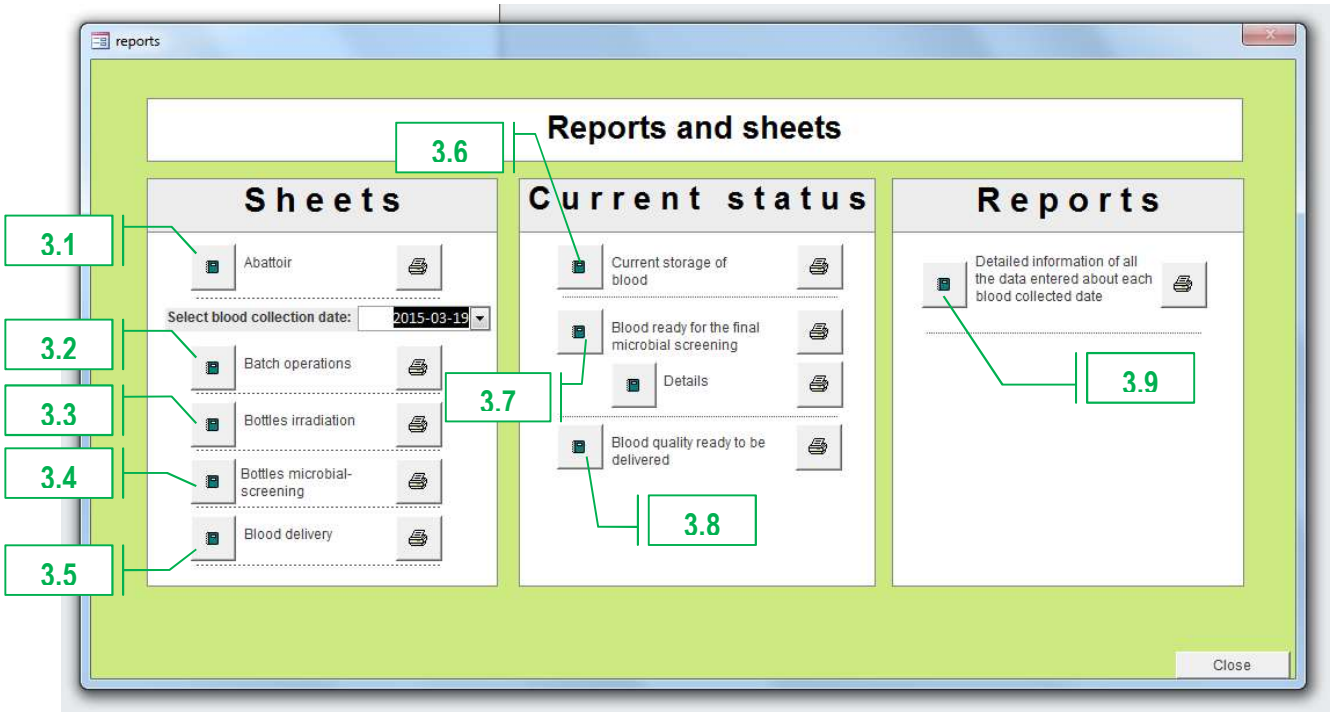
Click on the selection case of the bottles that will be updated with the information entered in step 2. The number of bottles selected is displayed after clicking on the “Refresh” button. There is also an option to unselect previous selections. Data can also be amended for individual bottles by editing the respective row. On the right column, the user can click on the “Discard” case if the blood intended for disposal.

2.3.d Step 4: Update bottles info

Click on the button “Update” to assign the information to all selected bottles. The changes will be saved on the database.

2.3.e Exit to previous menu Close the current window and go back to previous menu.

3 Reports and Sheets Menu



Sheets

The goal of the sheets is to make available tailored template forms that can be printed in advance for each of the blood processing operations that require manual record keeping. For each operation, the corresponding form includes and updated number of rows with the bottle codes for each collection date, in accordance with the information that has been entered previously in the database.
In order to facilitate the data entry into the database, the information to be recorded in the hardcopy has the same structure than the data entry form.

3.1 Sheets for the abattoir

A sample of the form is reproduced below. The date of the form must be selected before printing.

3.2 Sheets for batch operations

A sample of the form is reproduced below. The batch operations include the thawing, proportioning, batch microbial screening and bioassay result. The date of the form must be selected before printing.

3.3 Sheets for bottles irradiation

A sample of the form is reproduced below. The date of the form must be selected before printing. The form includes one row for each bottle that has been proportioned with its corresponding code.

3.4 Sheets for bottles microbial screening

A sample of the form is reproduced below. The date of the form must be selected before printing. The form includes one row for each bottle that has been irradiated with its corresponding code.

3.5 Sheets for blood delivery

A sample of the form is reproduced below. The date of the form must be selected before printing. The form includes one row for each bottle that has gone through microbial screening with its corresponding code.

Close

Abattoir sheet

Blood collection date:_____

Code:_____

Collection team:

Team leader:

Total Volume from abattoir(litres):

Total Number of abattoir cans:

Cold Storage:

Breakdown:

Number of abbatoir cans	Abbatoir cans volume

Close

Batch operations sheet

Blood collection date: **Friday, 01 May 2015**

Code:

Thawing

Thawing start date : _____

Responsible: _____

Proportioning

Proportion date: _____

Responsible: _____

Number of lab bottles	Lab bottles volume	Cold storage

Batch testing

Sample irradiation date: _____

Irradiation Responsible: _____

Test Type	Lab technician	Start Date	End Date	RESULT
Pre-irradiation microbial-screening				UFC:
Post-irradiation microbial-screening				UFC:
Bio-assay				QF:

Bottle irradiation sheet

Close

Blood collection date: **Friday, 01 May 2015**

Code:

Bottles Volume	Bottle Code	Bottle Number	Irradiation Date	Irradiation Responsible	Irradiation Operator	Irradiator	Exposure time (sec)	Storage after irradiation
2	LB: 2015-05-01 / 1-73 (2 l)	1						
	LB: 2015-05-01 / 2-73 (2 l)	2						
	LB: 2015-05-01 / 3-73 (2 l)	3						
	LB: 2015-05-01 / 4-73 (2 l)	4						
	LB: 2015-05-01 / 5-73 (2 l)	5						
	LB: 2015-05-01 / 6-73 (2 l)	6						
	LB: 2015-05-01 / 7-73 (2 l)	7						
	LB: 2015-05-01 / 8-73 (2 l)	8						
	LB: 2015-05-01 / 9-73 (2 l)	9						
	LB: 2015-05-01 / 10-73 (2 l)	10						
	LB: 2015-05-01 / 11-73 (2 l)	11						
	LB: 2015-05-01 / 12-73 (2 l)	12						
	LB: 2015-05-01 / 13-73 (2 l)	13						
	LB: 2015-05-01 / 14-73 (2 l)	14						
	LB: 2015-05-01 / 15-73 (2 l)	15						
	LB: 2015-05-01 / 16-73 (2 l)	16						
	LB: 2015-05-01 / 17-73 (2 l)	17						
	LB: 2015-05-01 / 18-73 (2 l)	18						
	LB: 2015-05-01 / 19-73 (2 l)	19						
	LB: 2015-05-01 / 20-73 (2 l)	20						
	LB: 2015-05-01 / 21-73 (2 l)	21						
	LB: 2015-05-01 / 22-73 (2 l)	22						
	LB: 2015-05-01 / 23-73 (2 l)	23						
	LB: 2015-05-01 / 24-73 (2 l)	24						
	LB: 2015-05-01 / 25-73 (2 l)	25						

Microbial screening of bottles

Close

Blood collection date: **Friday, 01 May 2015**

Code:

Bottles Volume	Bottle Code	Bottle Number	Start date	End date	Lab technichian	CFU
2	LB: 2015-05-01 / 1-73 (2 l)	1				
	LB: 2015-05-01 / 2-73 (2 l)	2				
	LB: 2015-05-01 / 3-73 (2 l)	3				
	LB: 2015-05-01 / 4-73 (2 l)	4				
	LB: 2015-05-01 / 5-73 (2 l)	5				
	LB: 2015-05-01 / 6-73 (2 l)	6				
	LB: 2015-05-01 / 7-73 (2 l)	7				
	LB: 2015-05-01 / 8-73 (2 l)	8				
	LB: 2015-05-01 / 9-73 (2 l)	9				
	LB: 2015-05-01 / 10-73 (2 l)	10				
	LB: 2015-05-01 / 11-73 (2 l)	11				
	LB: 2015-05-01 / 12-73 (2 l)	12				
	LB: 2015-05-01 / 13-73 (2 l)	13				
	LB: 2015-05-01 / 14-73 (2 l)	14				
	LB: 2015-05-01 / 15-73 (2 l)	15				
	LB: 2015-05-01 / 16-73 (2 l)	16				
	LB: 2015-05-01 / 17-73 (2 l)	17				
	LB: 2015-05-01 / 18-73 (2 l)	18				
	LB: 2015-05-01 / 19-73 (2 l)	19				
	LB: 2015-05-01 / 20-73 (2 l)	20				
	LB: 2015-05-01 / 21-73 (2 l)	21				
	LB: 2015-05-01 / 22-73 (2 l)	22				
	LB: 2015-05-01 / 23-73 (2 l)	23				
	LB: 2015-05-01 / 24-73 (2 l)	24				
	LB: 2015-05-01 / 25-73 (2 l)	25				
	LB: 2015-05-01 / 26-73 (2 l)	26				
	LB: 2015-05-01 / 27-73 (2 l)	27				
	LB: 2015-05-01 / 28-73 (2 l)	28				
	LB: 2015-05-01 / 29-73 (2 l)	29				
	LB: 2015-05-01 / 30-73 (2 l)	30				
	LB: 2015-05-01 / 31-73 (2 l)	31				
	LB: 2015-05-01 / 32-73 (2 l)	32				
	LB: 2015-05-01 / 33-73 (2 l)	33				
	LB: 2015-05-01 / 34-73 (2 l)	34				
	LB: 2015-05-01 / 35-73 (2 l)	35				
	LB: 2015-05-01 / 36-73 (2 l)	36				

Delivery of bottles

Close

Blood collection date: **Friday, 01 May 2015**

Code:

Bottles Volume	Bottle Code	Bottle Number	Date out	Delivered by	Delivered to	For feeding	Discard blood
2	LB: 2015-05-01 / 1-73 (2 l)	1					No
	LB: 2015-05-01 / 2-73 (2 l)	2					No
	LB: 2015-05-01 / 3-73 (2 l)	3					No
	LB: 2015-05-01 / 4-73 (2 l)	4					No
	LB: 2015-05-01 / 5-73 (2 l)	5					No
	LB: 2015-05-01 / 6-73 (2 l)	6					No
	LB: 2015-05-01 / 7-73 (2 l)	7					No
	LB: 2015-05-01 / 8-73 (2 l)	8					No
	LB: 2015-05-01 / 9-73 (2 l)	9					No
	LB: 2015-05-01 / 10-73 (2 l)	10					No
	LB: 2015-05-01 / 11-73 (2 l)	11					No
	LB: 2015-05-01 / 12-73 (2 l)	12					No
	LB: 2015-05-01 / 13-73 (2 l)	13					No
	LB: 2015-05-01 / 14-73 (2 l)	14					No
	LB: 2015-05-01 / 15-73 (2 l)	15					No
	LB: 2015-05-01 / 16-73 (2 l)	16					No
	LB: 2015-05-01 / 17-73 (2 l)	17					No
	LB: 2015-05-01 / 18-73 (2 l)	18					No
	LB: 2015-05-01 / 19-73 (2 l)	19					No
	LB: 2015-05-01 / 20-73 (2 l)	20					No
	LB: 2015-05-01 / 21-73 (2 l)	21					No
	LB: 2015-05-01 / 22-73 (2 l)	22					No
	LB: 2015-05-01 / 23-73 (2 l)	23					No
	LB: 2015-05-01 / 24-73 (2 l)	24					No
	LB: 2015-05-01 / 25-73 (2 l)	25					No
	LB: 2015-05-01 / 26-73 (2 l)	26					No
	LB: 2015-05-01 / 27-73 (2 l)	27					No
	LB: 2015-05-01 / 28-73 (2 l)	28					No
	LB: 2015-05-01 / 29-73 (2 l)	29					No
	LB: 2015-05-01 / 30-73 (2 l)	30					No
	LB: 2015-05-01 / 31-73 (2 l)	31					No
	LB: 2015-05-01 / 32-73 (2 l)	32					No
	LB: 2015-05-01 / 33-73 (2 l)	33					No
	LB: 2015-05-01 / 34-73 (2 l)	34					No
	LB: 2015-05-01 / 35-73 (2 l)	35					No
	LB: 2015-05-01 / 36-73 (2 l)	36					No
	LB: 2015-05-01 / 37-73 (2 l)	37					No

3.6 Reports on current blood storage

Includes the information on the stored blood classified under:

1) Blood stored in abattoir cans: blood that has been collected but not yet thawed for proportioning
2) Blood currently thawing for proportion
3) Blood already proportioned
4) Blood ready for the microbial screening: blood that has already been proportioned, tested for bacterial contamination and for nutritional value and whose CFU and QF values are acceptable and irradiated. These bottles are ready to be tested individually for microbial screening.
5) Bioassay test failed: Blood that has already been proportioned and tested for bacterial contamination and nutritional value. The values obtained in the tests are not acceptable.

A sample of the form is reproduced below.

3.7 Reports on blood ready for the final microbial screening

Includes the information on the stored blood that has been tested for nutritional value with acceptable results. The volume of blood with acceptable QF, irradiated vs not irradiated, microbial screened vs not screened, acceptable CFU vs not acceptable CFU are displayed under a tree shaped diagramme. The pending work load is also summarized in the report.

A similar report specifying the same information by collection date can also be printed.
A sample of the form is reproduced below.

3.8 Reports on good quality blood, ready for delivery

This report lists all the good quality blood that is ready for delivery. The bottles are first ordered by decreasing value of the CFU. Within each CFU value, the bottles are ordered by increasing value of CFU.

A sample of the form is reproduced below.

Reports

3.9 Detailed information on each blood collection date

For a selected collection date, all the available information is shown in this report
A sample of the form is reproduced below.

close

Current storage of blood

stock:

Volume (l): **723**

Number of bottles: **277**

1-Blood stored in abattoir cans

Volume (l): **100**

Number of bottles:

Blood collection date **2015-06-08**

Available Volume: 100

Number of Bottles:

	pre-irradiation	post-irradiaton	QF bio-assay
Start date:			
Result:			

2-Blood currently thawing for proportion

Volume (l): **128**

Number of bottles:

Blood collection date **2015-05-02**

Available Volume: 128

Number of Bottles:

	pre-irradiation	post-irradiaton	QF bio-assay
Start date:			
Result:			

3-Blood proportioned

Volume (l): **120**

Number of bottles: **70**

Blood collection date **2015-05-09**

Available Volume: 120

Number of Bottles: 70

	pre-irradiation	post-irradiaton	QF bio-assay
Start date:			
Result:			

4-Blood ready for the final microbial screening

Volume (l): **247**

Number of bottles: **143**

Blood collection date **2015-04-18**

Available Volume: 122

Number of Bottles: 73

	pre-irradiation	post-irradiaton	QF bio-assay
Start date:	2015-04-26	2015-04-30	2015-05-05
Result:	15	3 ok	1.16 ok

Blood collection date **2015-05-01**

Available Volume: 125

Number of Bottles: 70

	pre-irradiation	post-irradiaton	QF bio-assay
Start date:	2015-05-11	2015-05-13	2015-05-18
Result:	15	2 ok	1.15 ok

5-Bio-assay test failed

Volume (l): **128**

Number of bottles: **64**

Blood collection date **2015-04-25**

Available Volume: 128

Number of Bottles: 64

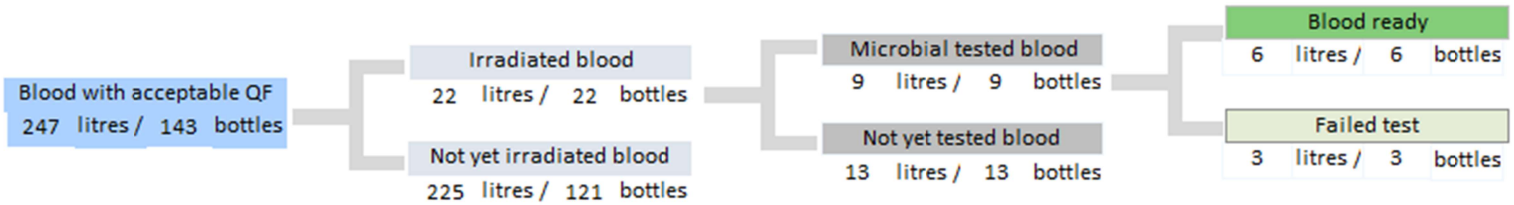
	pre-irradiation	post-irradiaton	QF bio-assay
Start date:	2015-05-11	2015-05-12	2015-05-16
Result:	9 ok	1 ok	0.85

close

Blood ready for the final microbial screening

Information of the data entered into the database until:

Wednesday, 10 June 2015



Pending work:

Total blood pending to be irradiated:	225	litres /	121	bottles
Total blood pending to be tested:	13	litres /	13	bottles
Total blood pending to be discarded:	3	litres /	3	bottles

Ready for delivery:

6 litres / 6 bottles

Blood quality ready to be delivered

close

Blood ready for delivery: 14 litres / 14 bottles

QF: 1.15 5 litres / 5 bottles

CFU: 0 3 litres / 3 bottles

collection date **2015-05-01** : 3 litres / 3 bottles

Bottle code	Number	Volume	Storage after irradiation
LB: 2015-05-01 / 60-73 (1 l)	60	1	Room2
LB: 2015-05-01 / 59-73 (1 l)	59	1	Room2
LB: 2015-05-01 / 58-73 (1 l)	58	1	Room2

CFU: 1 1 litres / 1 bottles

collection date **2015-05-01** : 1 litres / 1 bottles

Bottle code	Number	Volume	Storage after irradiation
LB: 2015-05-01 / 57-73 (1 l)	57	1	Room2

CFU: 8 1 litres / 1 bottles

collection date **2015-05-01** : 1 litres / 1 bottles

Bottle code	Number	Volume	Storage after irradiation
LB: 2015-05-01 / 56-73 (1 l)	56	1	Room2

QF: 1.16 **9 litres / 9 bottles**

CFU: 0 **5 litres / 5 bottles**

collection date 2015-04-18 : 5 litres / 5 bottles

Bottle code	Number	Volume	Storage after irradiation
LB: 2015-04-18 / 60-79 (1 l)	60	1	Room2
LB: 2015-04-18 / 59-79 (1 l)	59	1	Room2
LB: 2015-04-18 / 58-79 (1 l)	58	1	Room2
LB: 2015-04-18 / 56-79 (1 l)	56	1	Room2
LB: 2015-04-18 / 50-79 (1 l)	50	1	Room2

CFU: 1 **3 litres / 3 bottles**

collection date 2015-04-18 : 3 litres / 3 bottles

Bottle code	Number	Volume	Storage after irradiation
LB: 2015-04-18 / 53-79 (1 l)	53	1	Room2
LB: 2015-04-18 / 52-79 (1 l)	52	1	Room2
LB: 2015-04-18 / 51-79 (1 l)	51	1	Room2

CFU: 4 **1 litres / 1 bottles**

collection date 2015-04-18 : 1 litres / 1 bottles

Bottle code	Number	Volume	Storage after irradiation
LB: 2015-04-18 / 55-79 (1 l)	55	1	Room2

The following sheet shows detailed information for each bottle

		bottle irradiation				Lab bottle microbial screening					
	Bottle code	Irradiation Date	Responsible	Irradiator	Exposure time	Start Date	End Date	Responsible	CFU	Date out	colony
1	LB: 2015-04-18 / 1-79 (2 l)										
2	LB: 2015-04-18 / 2-79 (2 l)										
3	LB: 2015-04-18 / 3-79 (2 l)										
4	LB: 2015-04-18 / 4-79 (2 l)										
5	LB: 2015-04-18 / 5-79 (2 l)										

Detailed information of all the data entered for each blood collection date

close

Blood collection date: Saturday, 18 April 2015

Blood collection info:

Blood code:
Abattoir:
Team leader
Collection team: GF
Volume collected at the abattoir: 128
Number of abattoir cans: 32
Breakdown: 32 cans of 4 litres

Storage of the cans: Room2

Batch blood thawing:

Start Date: 2015-04-21
Responsible: Rafa

Blood proportioning:

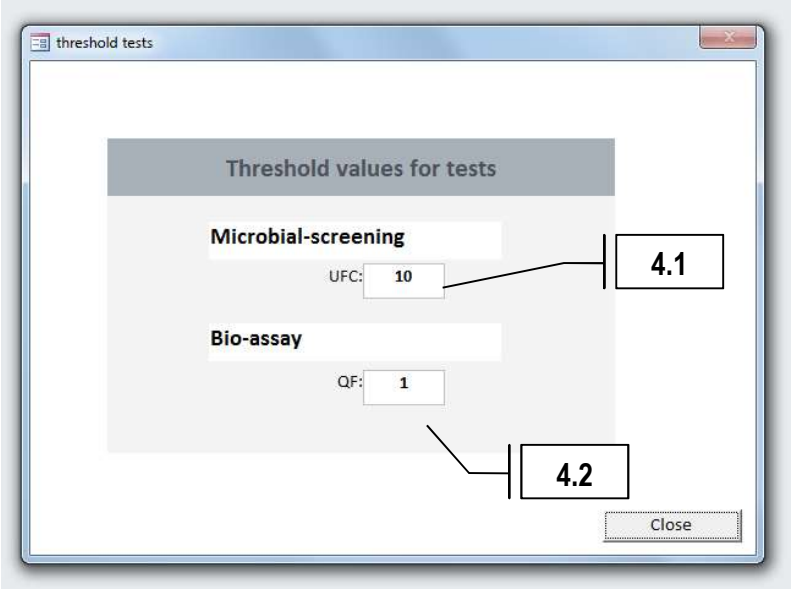
Date: 2015-04-25
Responsible: Rafa
Blood proportioned into: 79 laboratory bottles
30 bottles of 1 litres stored in Room2
49 bottles of 2 litres stored in Room 1

Laboratory tests:

Sample irradiation date: 2015-04-28
Responsible: Rafa

Test Type	technician	Start Date	End Date	UFC	QF
Pre-irradiation microbial-screening	Rafa	2015-04-26	2015-04-29	15	
Post-irradiation microbial-screening	Rafa	2015-04-30	2015-05-03	3	
Bio-assay	Rafa	2015-05-05	2015-06-03		1.16

Thresholds Menu



4.1 Microbial screening threshold: define the number of CFU above which the blood bottle will not be acceptable for feeding flies.

4. 2 Bio-assay: define the value of the QF below which the blood batch will not be acceptable for feeding flies.

ANNEX II: **Female Uterus dissection procedures**

Determining the uterus content and ovulation status is part of the procedures to complete the bio assay.
Females uterus dissection is a complex technique that requires skilled technicians. Below are the procedures for females dissection, as extracted from the "Training Manual for Tsetse Control Personnel: Tsetse biology, systematics and distribution" (J.N. Pollock) published by FAO.

Routine for the dissection of female Glossina:

Freshly caught flies, or flies caught and immediately placed in an ice box, are the best material to use for the following dissection. The routines require an appropriate note to be made on a data sheet (see later).

a. Remove wings and legs. Examine wings, if wing fray estimate is to be made.
b. Dissect open the abdomen to show the female reproductive system. To do this, place the insect dorsal side uppermost, on a slide on a black background. Cut the abdomen by a small nick on either side, between segments 6 and 7, using small scissors or a fine blade (scalpel or Borradaile needle). Then pull back the cut part of the abdomen slowly, to reveal the female reproductive system. Add a drop of 0.9% saline, or water. Note if the fly has fed or not.
c. Examine spermathecae. Estimate spermathecal index (0 = apparently empty; 1-10 increasing amounts of sperm, to apparent maximum capacity).
d. Which ovary is larger? The ovaries may either be left in place for later attention, or can be cut off close to the uterus and transferred to a drop of clean water for later dissection.
e. Open the uterus, to reveal the contents. Is the uterus
    1. empty
    2. with a spermatophore
    3. with an egg
    4. with a 1st instar larva
    5. with a 2nd instar larva
    6. with a 3rd instar larva?
f. Examine the larger ovary. Is the inner or the outer ovariole larger? (If this point is difficult to determine, examine the other ovary) •
g. Dissect the largest ovariole to see if it has a follicular relic. If this dissection is spoiled, examine the second largest ovariole for its follicular relic.
h. Determine the physiological age. (See interpretation of results).

Notes

(i) If it is a virgin fly then there is no need to dissect for follicular relics (except to gain practice).

(ii) If the inner right ovariole is the largest ovariole, and the uterus contains an egg or larva, do not bother to dissect for follicular relics, as all ovarioles will have them.

(iii) If the outer left ovariole is the largest, then your dissection for follicular relics should be carried out with every care, as the second largest ovariole (the inner right) is certain to show a follicular relic and therefore is of no help in identifying the ovarian category.

(iv) A data sheet can be drawn up to record the required information: Fly number, sex, wing fray category, fed/non-fed, spermathecal index, largest ovary (I/R), uterine content (empty/spermatophore/egg/ 1st/2nd/3rd instar larva), largest ovariole (yes/no/supplied), follicular relics in second largest ovariole (yes/no/spoiled), estimated physiological age, special notes.

(v) If the dissections are part of a survey following an aerosol spraying, then it can be very useful to measure the size of the largest (inner right) ovariole in flies of ovarian category O, to the nearest 0.1 mm. This can be used to distinguish very young flies from slightly older flies still in category 0.

Interpretation of results of ovary/uterus dissection:

Female flies can be age-graded according to the contents of the uterus, the relative development of the four ovarioles, and the presence or absence of follicular relics in these ovarioles.

Flies in the ovarian categories 0-3 (see Fig. 8.6) can be age-estimated with considerable accuracy and certainty. Unfortunately flies in the next four ovarian categories (4-7) cannot with certainty be distinguished from flies in the succeeding four ovarian categories (8-11) or the next four after that (12-15). The reason is that successive ovulations can make no difference to the number of follicular relic as all ovarioles have than at this stage.

The scheme summarised in Figure 8.6 shows how this age-grading may be done. Two examples will illustrate how this scheme may be used.

Example 1. Fly with inner left ovariole largest; this ovariole with no follicular relic; 3rd instar larva present in the uterus. By reference to the chart this fly would fall into ovarian category 1c, and could therefore have an estimated physiological age of 16-19 days.

Example 2. Fly with outer right ovariole largest; this ovariole with follicular relic; uterus empty. By reference to the chart this fly would fall into ovarian category 6c, 10c or 14c and could therefore have an estimated physiological age of 67-70 days, or 107-110 days, or 147-150 days. The youngest of these possibilities is the most likely to be the correct category, but the degree of wing fray may be a guide here.

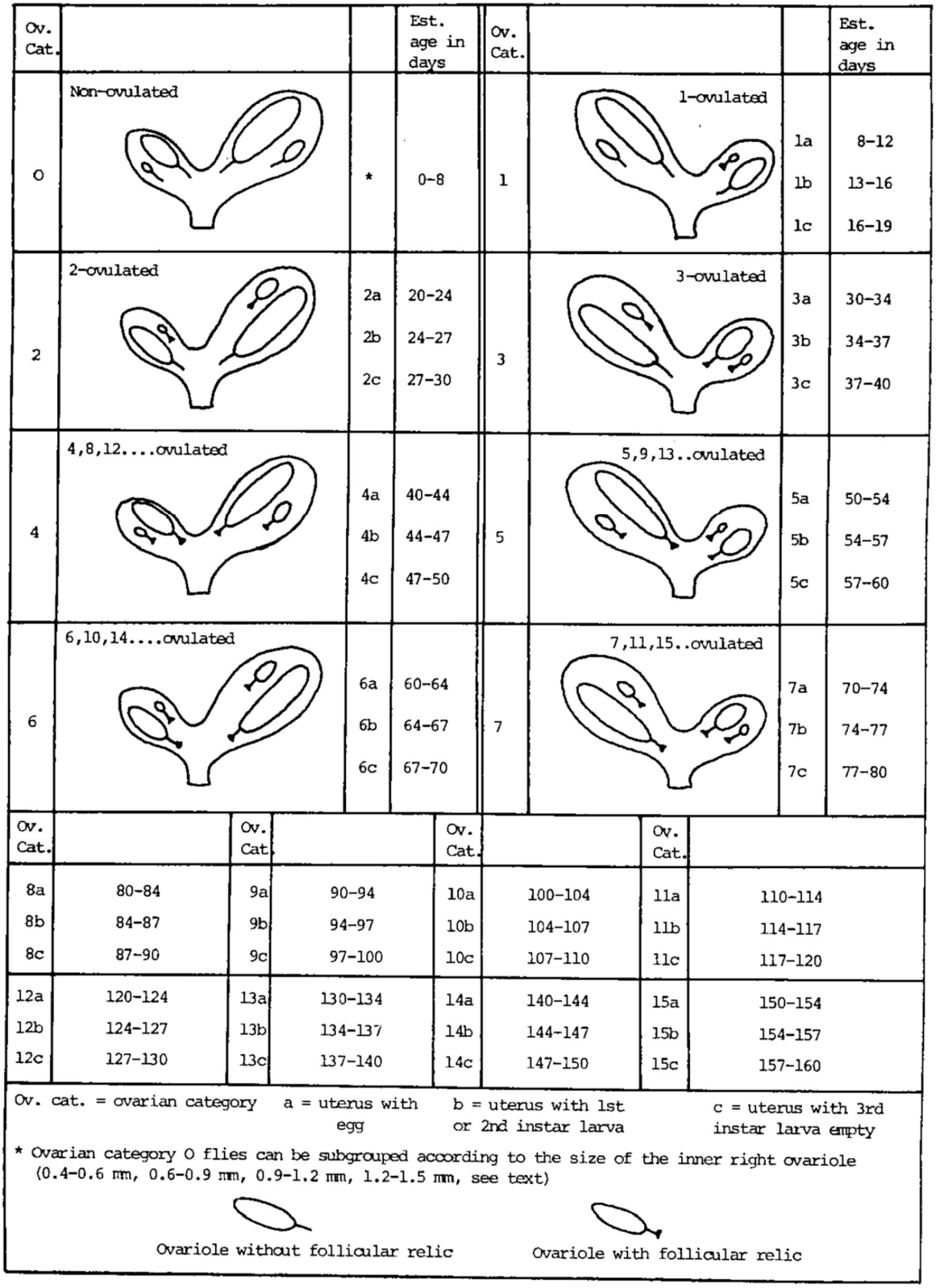


Figure 8.6. To show the successive changes in the ovaries with increasing age in Glossina (diagrammatic)

ANNEX III: Special Equipment and Materials with Specifications

Item	Specifications	Example of supplier and/or distributor
Nutrient agar	Dehydrated agar media	GranuCult™ Nutrient agar, Potential supplier VWR (catalogue number 1.00415.0500)
Autoclave	Bench top vertical autoclave, with integrated printer, VAPOUR- Line Lite P	Potential supplier VWR (catalogue number 1481-0666)
Bacterial colony-viewing/counting device	e.g. Colonicont, Schütt	Potential supplier VWR (catalogue number 710-0937)
Balance	Precision balance 120 g weighing capacity, precision: 0.1 mg, AC/DC input 230 V AC	Potential supplier VWR (catalogue number 611-3364)
Blood-collection equipment	Blood-collection defibrination set¸ consisting of screw-capped 25-l container (polyethylene), funnel (stainless steel or plastic, upper diameter 25–40 cm) and hose (transparent silicone, autoclavable, with strong walls), paddle (stainless steel rod of 1-cm diameter on which oar-blade is mounted), and a separate electric drill (stirrer) approx 800 W, double insulated (insulation protected "schutzisoliert"), electronic adjustable and self-maintainable speed, and with (to be fitted) IP44 rubber plug (IP44 2p+E), aluminium sieve (milk sieve) with upper diameter 30–40 cm and removable sieve	Potential supplier: Strack Claus 2443 Loretto, Lindengasse 29 Austria Tel.: 0699 10 84 89 84
Blood storage bottles	PE bottles to store blood with a capacity of 2L (or 4L, depending on local availability);	Potential supplier: Zhongshan Guoyu Plastic Products Factory Description: 1- Stackable 4.5L Bottle Designed to store animal blood at -20 to -25°c temperature Approx. as per approved technical drawing Specifications: Dimensions: 26.924cm H x 17.89 cm Material: HDPE plastic Construction: Sturdy handle for easy pouring with one hand Less than 280 mm high 2- Polypropylene lids for the above quoted bottle, constructed with a leak-proof polyethylene gasket
Blood transport container	PE container with a capacity of 200L (or 100L depending on the volume of blood to be collected per session); transparent or	

	white colour with a volume scale to estimate the filling volume; 3/4" plug with EPDM seal in the bottom connected to a LDPE tap; threaded cover diameter 160mm with EPDM seal; external dimensions for the 200L container: height 1030 mm x 550mmx550mm; external dimensions for the 100L container: height 1030 mm x 550mmx550mm	
Cage (production, self-stocking production and holding, PVC, 20-cm diameter, with netting and rubber stopper)	200 x 50 x 4 mm, with 26-mm hole in side for rubber stopper. Cage material foam core PVC water pipe. 100% polyester netting (mesh size depends on tsetse species and type of cage). Glue for cage netting, SABA S 3, (not bigger than 1 lit./tin)	Potential supplier: Schranz Walter – Tischlerei American Scientific & Industrial Supplies
Chiller (modified chest deep-freeze)	Tsetse fly chiller, working temperature +3°C, +/- 3 °C, modified chill-food display unit, modified to include a work platform in clear plastic, two air circulation fans and an electromechanical thermostat to replace the original digital thermostat. External dimensions length 1250 mm, width 790 mm, height 1200 mm.	Potential supplier: MORO Vancurova ul 12 83101 Bratislava Slovakia Ing. Ivan Moravek Email: ivan.moravek@stuba.sk
Microtube	Microtube with screw cap with silicone seal, volume 1.5mL, graduated up to 1 mL, self-standing, PP	Potential supplier: Sigma-Aldrich, catalogue number Z637416
Gloves for oven	Heat proof oven gloves. Heat and cut resistant gloves 240 Size M Acc. to EN 420, EN 388 (3531), EN 407 (42212X) Seamless fine knit made of Kevlar®, modacrylic, glass fibre with foam neoprene palm. Flame resistant materials Kevlar backing fabric offers high cutting resistance: Level 5 Flat dipped neoprene coating ensures extra-secure grip The ergonomic design matches the natural shape of the hand and thus reduces operator fatigue and improves fit and wearing comfort. Protection against arcing: arcing protection class 2- 240 mm long. Range of uses: Work requiring a high level of protection against cutting and heat. Building trade, metallurgy, oily working conditions.	Potential supplier: Showa

Incubator	Incubator for bacterial culture, chamber volume 73 litres, electronic temp. control, 5–70°C.	Potential supplier: American Scientific & Industrial Supplies LABSCO - Laboratory Supply Company GmbH & Co. KG
Lab freezer	Laboratory freezer, upright, min. 500 litre, freestanding, manual defrost, min 59 x 55 x 150 cm internal, 6 shelves with two baskets each, electronic control of temperature with external display; temperature range -14C to -28C	Potential supplier: LABSCO Laboratory Supply Company GmbH&Co
Label printer	Small printer to print label for the stored blood, bioassay test, and microbiology samples	
Laminar flow bench	Device to provide a septic area for the bacteriology work for conducting the blood proportion, and bacteriology test.	
Microscope, stereoscopic (for insect dissection and identification of marked individuals)	Microscope, stereoscopic (for insect dissection and identification of marked individuals)	Potential supplier: SFSI (Service for Science and Industry, Inc.) Labsco (Laboratory Supply Company)
Oven (small size in case of using 100L blood containers)	Oven for membranes sterilization Stainless steel oven with forced circulation. Interior dimensions:w(A) x h(B) x d(C): 1040 x 720 x 600 mm. +80 - + 150 ºC; precision better than ±0.5º C; uniformity better than ±3.0º C; constructed in stainless steel, rigid, crevice-free with temperature insulation. Door with gasket seal and safety lock, thermostat with digital display. Adjustable parameters: temperature (Celsius or Fahrenheit), fan speed, air flap position, programme time. Voltage: 400V(3-Ph) V, 50/60 Hz.	
Petri dish	Plastic sterile disposable Petri dish (60 x 15 mm)	Potential supplier: LABSCO - Laboratory Supply Company GmbH & Co. KG
Pipette	Pipettes sterile individually packed with different volume 5, 10, 20 and 50 ml	Potential supplier VWR (catalogue numbers 612-5504, 612-5507, 612-5523, 612-5826, 612-5827, 612-5541, 612-5828, 612-5546)
Pipettor	Pipette controller	Potential supplier VWR (catalogue number 613-4442)
Pupal size-sorting machine	Pupal size and separating machine with 5 chutes. Main frame in aluminium construction, vibration bolt with control unit, 2 polished sorting wheels (distance adjustable: 0,5 - 10,0 mm), 1 gear motor, 5 channels, 5 collecting hoppers.	Potential supplier: PAKATECH Paral & Kalchhauser GmbH

Radiation source (gamma ray, industrial)	SVST Co-60 Industrial Scale Gamma Irradiator with 100 kCi Co-60 sources.	
Scintillation vials	50mL	Potential supplier VWR (catalogue number 252-0155)
Stereomicroscope	Richter Optica S850 Stereo Zoom Trinocular LED Microscope 8x-50x	Potential supplier Microscope World (catalogue number SKU:S850T-VLED)
Tips	Sterile tips 1mL	Potential supplier VWR (catalogue number 732-0532)
Pipettes	3 Pipettes with different volumes from 2-1000 uL	Potential supplier VWR (catalogue number 613-1144)