

Tsetse Collection Protocol

Core Procedures

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v0.2.1

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1 Deploying Traps

1.1 Materials

- Gum boots
- GPS device
- Panga
- Mallet/hammer
- Pencil(s)
- razor (keeping pencil(s) sharp)
- Paper for notes and trap-labels
- Trap materials (see below)

1.1.1 Per Trap

- 1 metal rod (~2 meters long)
- 1 biconical fabric trap
- 1 collection cage and net sleeve
- thick grease
- ~2 rubber bands

1.2 Team

Deployment teams generally consist of 2 to 5 people. Gear is divided among the team as works best but there are usually a few common classes of roles.

Record Keeper carries the GPS device, a pencil, a pad of paper.

Panga carries the panga and hammer.

General Purpose carries what is needed. Some combination of trap materials as needed including trap cones, collection cages/nets, grease, rubber bands.

1.3 Procedures

- As the team travels in the deployment area conducive sites are identified.
- The **Record Keeper** places the date and trap number into the collection cage; records the trap's GPS coordinates **in DECIMAL DEGREES** format to 5 decimal places; and makes notes about the type of surrounding habitat as well as human activity in sight of the trap.

- The site is cleared by the **Panga** and the rod is driven into the ground deep enough to provide robust “staying power”.
 - The fabric cone is slipped onto the rod and the bottom is secured with a rubber band.
 - the tagged cage is added to the top and secured with a rubber band.
 - a **generous** amount of thick grease is applied to the rod below the cone such that the safari ants will be denied access to the trap cone and collection cage.
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2 Processing Flies

2.1 Materials

Not exhaustive, as specific needs may vary.

- Buffer for dissection etc
- Microscope slides (with frosted labels)
- Cover-slips
- EtOH (100%)
- Cryo-tubes
 - ~2ml vol
 - screw-top are best
- Specialized storage solution (if needed)
- 50ml 'falcon' tubes for making personal solutions (washing forceps, etc)
- Pasteur-type pipets
- a couple p-1000 pipetmans
- 1 or more compound microscope (positive screening)
- 1 or more dissection scopes
- gloves
- permanent pens suitable for labeling the tubes
- fine forceps for multiple stations
- pencil(s)
- razor (keeping pencil(s) sharp)
- Parafilm M
- tube racks / cryo-boxes
- Kim-Wipes
- Paper towels
- Power generator and fuel (to run the scopes in the field if needed)
- Voltage regulator/stabilizer
- Field record data sheets
- Adhesive labels for the sides of the cryo-tubes (Figure 2.1)

2.2 Procedures

The fly processing is handled in an assembly-line format with stations responsible for specific parts of the task (Figure 2.2).



Figure 2.1: Labels typical of what is used at the **Label maker** station.

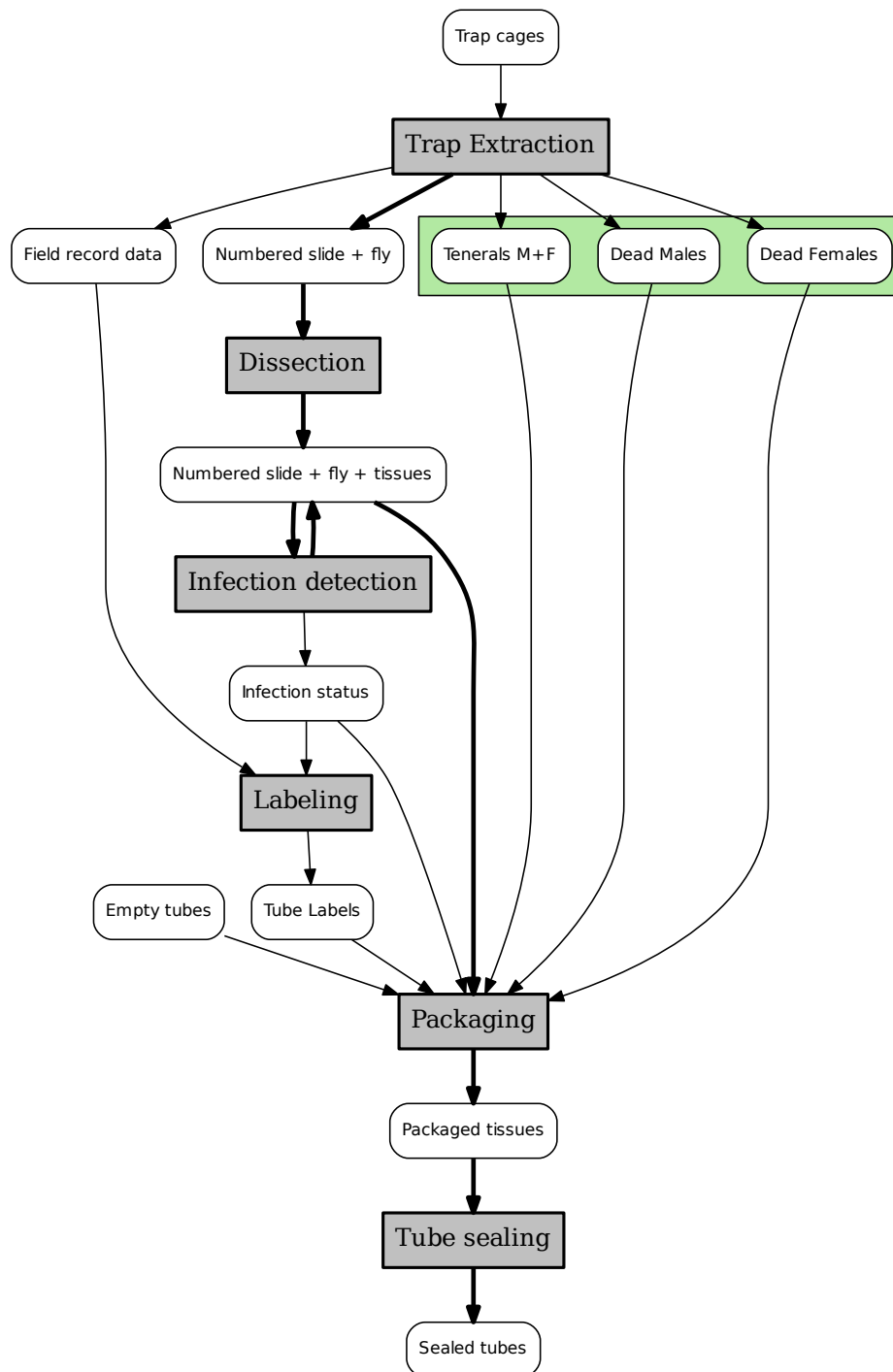


Figure 2.2: Basic Processing Flow – Grey boxes are processing stations. White rounded boxes are inputs/outputs of processing stations. Arrows show the flow of processing materials. BOLD arrows show the flow of fly materials. The green box represents materials set aside for processing after the live flies have been processed.

2.2.1 Trap extraction station

This station is responsible for removing the flies from the cage of the current trap and recording the initial information for this fly in the field collection sheet¹. This information includes (but may not be limited to):

- date (once per sheet)
- trap number
- the fly number
- sex
- hunger stage and/or wing fray
- collection date
- town name/ID symbol

For each fly:

1. Incapacitate fly by cracking its head or some other way of killing the fly without causing too much physical destruction to the fly.
2. Collect and record in the field record sheet the prescribed information (see above list).
3. Place fly on microscope slide with a frosted labeling end.
4. Write the number that corresponds to this fly in the field record sheet on the slide label with *pencil*.
5. Send slide to **Dissection**.

Remarks:

- **Don't get bitten.**
- **Close attention** must be paid to ensure the number on the slide matches the number for each fly in the field record. If *even ONE* numbered fly is mistakenly left out of the processing stream or an additional added to the stream it will cause **ALL SUBSEQUENT fly information** to be incorrect².

2.2.2 Labeling station

- This station does not strictly “follow” in the assembly-line but is more of a concurrent station with the **Trap extraction** station.
- The **Labeling** station writes the relevant information for each fly as it is being removed from the trap and incapacitated in preparation for dissection.
- The information is recorded in pencil on a pad of small labels that will be placed on each tube (by the **Packaging** station) that contains part of the fly with the corresponding number (Figure 2.1).
- The important information to be recorded is:
 - Location code
 - Date (MM-DD)
 - Fly number
 - Trap number

¹To obtain the sheets that we used in the field you will need to contact Richard Echodu (richardechodu2009@gmail.com), but the excel file templates we sent should give an idea of what is included.

²Similar to a frame-shift mutation: it shifts the register of identities out of sync with all remaining flies.

- Tissue code (Something like: M, S, C, H ~ Midgut, Salivary glands, Carcass, Head)
- A label must be written for each tube that a fly will be packed into.
 - If you are collecting carcass and midguts: two labels with the above information will be generated for each fly and given to **Packaging**.
- Periodically, the labels are sent to the **Packaging** station(s) for application to the tubes.

Remarks:

- The sooner that **Packaging** gets these labels, the faster the whole process will go and the less likely it will be that flies become mislabeled.

2.2.3 Dissection station

For each fly:

1. **Wash tools (forceps/scissors/etc) in 90% EtOH and wipe with Kim-Wipes to avoid contamination of current fly.**
2. Dissect according to the needs of the collection trip.
3. Organize tissues on the slide in PBS so as to best facilitate the inspection for positives in next station.
4. Send slide to **Infection status**.

Remarks:

- Avoid cross contaminating flies with Trypanosomes by frequent and thorough cleaning of your forceps etc.

2.2.4 Infection detection station

For each fly:

1. **Wash tools (forceps/scissors/etc) in 90% EtOH and wipe with Kim-Wipes to avoid contamination of current fly.**
2. Examine tissues for signs of Trypanosome infection.
3. Call out the infection status for **Labeling** and **Packaging**.
 - in practice calling out only the status of flies that are positive is sufficient.
 - if you are able to distinguish between low, medium, and high levels of infection, it is good to do so.
4. Pass slide to **Packaging**.

Remarks:

- Avoid cross contaminating flies with Trypanosomes by frequent and thorough cleaning of your forceps etc.

2.2.5 Packaging station

For each fly:

1. **Wash tools (forceps/scissors/etc) in 90% EtOH and wipe with Kim-Wipes to avoid contamination of current fly.**
2. Assemble enough tubes (*pre-filled with ~250ul of 90% EtOH or appropriate storage solution*) to receive the number of separate tissues dictated by the needs of this collection.
3. If possible, use the labels generated by **Labeling** to pre-label the tubes to reduce confusion.
4. Package the tissues in correctly labeled tubes as swiftly as possible *while maintaining control of quality*.
5. **Double-check** that each tube has the correct side-label, then label the tube top with *only* the Site-ID code and fly number using lab marker/sharpie.
6. If the fly has a positive status, add a code to the side-label:
 - ' +' = low to normal infection level
 - ' ++ ' = mid to high infection
 - ' +++ ' = very high infection

After the live flies have been processed:

1. All teneral³ flies: live or dead are placed into communal falcon tubes by Village (or approximately synonymous site level).
 - males and females can be combined
2. Dead non-teneral flies are separated by sex and placed into communal falcon tubes by Village.
3. Communal tubes are labeled with information analogous to individual flies except that there is no fly number and the number of flies contained in each falcon tube is included.
4. Add enough 90% EtOH to cover the flies sufficiently, allowing for some extra.
5. This information should match the info recorded in the field data sheets.

Remarks:

- Avoid cross contaminating flies with Trypanosomes by frequent and thorough cleaning of your forceps etc.
- Pre-filling hundreds of tubes with ~250ul of EtOH the night before will save you **enormous** time and pain during the processing sessions and reduce the likelihood of mistakes.

2.2.6 Tube sealing station

1. Get a strip of parafilm with dimensions approximately 2 inches long/ ~0.5 inches wide (~5cm Long / ~1.5cm Wide).
2. Stretch it about 1.5 times around the tube where the label is affixed.
3. Break it off.
4. Stretch the rest around the seam where the top meets the tube: make sure that it covers the writing on the tube top AND seals the seam.
5. Organize the tubes in boxes or bags with a system similar to the following:

³An adult too young to have bloodfed.

- tubes of the same tissue type are stored together with other tubes with the same site-code (~Village level)
- Bags/boxes above can be placed into larger bags that group a particular ~village etc.

Remarks:

- *Use this opportunity to double-check that the side-labels of each tube match the tube-tops.*
 - This step is best done **by everyone** following the completion of the main processing queue.
 - Doing this on the same day as the processing of the tubes is best.
 - If the tubes are to be transported by plane/bus/over-seas, the quality of this step may be considered directly proportional to the ability of the tubes to be used correctly in the lab and therefore to the quality of results possible.
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3 Milking Traps

3.1 Materials

- See materials list for Trap Deploying.

3.2 Procedures

- The teams may be 2-3 people.
 - Multiple teams per location may operate simultaneously to speed recovery of trap cages.
 - Each team should have pencil and paper to place a labeled piece of paper¹ in the new trap cage that they use to replace the retrieved trap cage for each trap.
 - Retrieved trap cages should be handled in such a way to reduce the death rate before processing can be begun.
 - shade
 - avoid excessive temps
 - out of access from ants etc.
-

¹Should contain the new date and same trap number.

4 Recovering Traps

4.1 Materials

- See materials list for Trap Deploying.

4.2 Procedures

- Teams may be smaller for trap recovery.
 - Multiple teams per location may operate simultaneously to speed recovery of traps.
 - Traps are disassembled and cages retrieved if flies are to be collected.
 - Rods are cleaned on the grass etc to remove the grease.
-

5 Appendices

5.1 Collection Documentation Files

5.1.1 Collection Data Spreadsheet

5.1.1.1 Purpose:

- Provides an electronic version of the data recorded for each **fly** during the field collection efforts.
- Allows data to easily shared with collaborators.
- Allows data to be imported into computer-based sample tracking database in an automated fashion.

5.1.1.2 Column descriptions:

Below are the definitions for the type and format of data recorded in each column of this file type. Fields denoted with an asterisk are required if the data exist. The rest are technically optional but are still *very* important to include if you have the information.

A. **Village***:

- Please list an appropriate name for the location common to a set of traps. This should be a village name or something at a similar level of resolution.

B. **Trap_No***:

- A number or other ID specific to a single trap.

C. **Date***:

- The date these flies were collected from the trap.
- The date should be formatted as YYYY-MM-DD to avoid ambiguity and ensure correct computer sorting.

D. **Species***:

- Use either the full species name of the fly or the first letter of each word. Examples: *Glossina fuscipes fuscipes* or Gff.

E. **Sex***:

- Use the letter **M** or **F** for male or female, respectively.

F. **Teneral***:

- Use the codes **T** or **NT** for teneral or non-teneral, respectively.

G. **Dead***:

- Use the letter **D** or **L** if the fly was dead or alive, respectively, at the time that the trap contents were being processed.

H. Fly_Number*:

- A unique number assigned to each fly collected during a collection trip.

I. Hunger_stage:

- Hunger stage as defined in:
 1. Jackson, C. H. N. The Causes and Implications of Hunger in Tsetse-flies. Bull. Entomol. Res. 24, 443–482 (1933).
 2. Leak, S. G. A. Tsetse biology and ecology: their role in the epidemiology and control of trypanosomosis. (ILRI (aka ILCA and ILRAD), 1999).
- Value range: 1 to 4
- If not collected: enter “NA”.

J. Wing_fray:

- If this measure is appropriate and you normally record it, you can place it here; if not, enter “NA”.

K. prob:

- If this tissue (proboscis) was checked for trypanosome infection, enter “0” for negative and “1” for positive results, “NA” otherwise.

L. midgut:

- If this tissue (midgut) was checked for trypanosome infection, enter “0” for negative and “1” for positive results, “NA” otherwise.

M. sal_gland:

- If this tissue (salivary gland) was checked for trypanosome infection, enter “0” for negative and “1” for positive results, “NA” otherwise.

N. Kept_in:

- Please list the solution that the sample is stored in.

O. Comment:

- Any comments that you think should be recorded about this sample.

P. Tube_or_box:

- Is this a box of existing samples of DNA or other extracted materials? Then choose “box”. Otherwise, choose “tube”.

Q. Tissue:

- When the sample is a material like DNA that has been derived from a part of or a whole fly, please list the tissue that was used to extract the DNA. A list of common tissues has been provided for your convenience, but non-listed values are allowed.
 - carcass

- head
- antennae
- midgut
- proventriculus
- midgut + proventriculus
- salivary glands
- reproductive parts
- legs
- whole

R. Method_of_prep:

- If the sample is DNA or another derived material, please include a brief comment about what method/kit was used to generate the material.

5.1.2 Collection Summary Spreadsheet

5.1.2.1 Purpose:

- Provides an electronic version of the data recorded for each **trap** during the field collection efforts.
- Allows data to easily shared with collaborators.
- Allows data to be imported into computer-based sample tracking database in an automated fashion.

5.1.2.2 Column descriptions:

Below are the definitions for the type and format of data recorded in each column of this file type. Fields denoted with an asterisk are required if the data exist. The rest are technically optional but are still *very* important to include if you have the information.

A. District:

- District or similar level of administrative hierarchy.

B. County:

- County or similar level of administrative hierarchy.

C. Subcounty:

- Subcounty or similar level of administrative hierarchy.

D. Parish:

- Parish or similar level of administrative hierarchy.

E. Village*:

- Village or similar level of administrative hierarchy.

F. Trap_No*:

- A number or other ID specific to a single trap.

G. Latitude*:

- Latitude GPS coordinates for the trap **in DECIMAL DEGREES** format to 5 decimal places.

H. Longitude*:

- Longitude GPS coordinates for the trap **in DECIMAL DEGREES** format to 5 decimal places.

I. Elevation_in_m*:

- Elevation at the trap's location in meters (m).

J. Human_Activity:

- A short (10 words or less if possible) description of the type of human activity within sight of the trap's location.
- farm plots, livestock, home, road, etc.

K. Vegtype:

- A short classification of the type of vegetation around the trap location.
- riverine, savannah, etc.

L. Deploy_date*:

- The date this trap was deployed.
- The date should be formatted as YYYY-MM-DD to avoid ambiguity and ensure correct computer sorting.

M. Harvest_date*:

- The date this trap was removed.
- The date should be formatted as YYYY-MM-DD to avoid ambiguity and ensure correct computer sorting.

N. Male*:

- the number of males collected from this trap.

O. Female*:

- the number of females collected from this trap.

P. Total*:

- the number of flies total collected from this trap.

Q. FTD:

- (F)lies per (T)rap per (D)ay
- Mean number of flies caught in this trap per day.
- Divide 'Total' number by the number of days the trap was deployed.

R. Other_info:

- Other information pertaining to a trap that you feel would be useful.