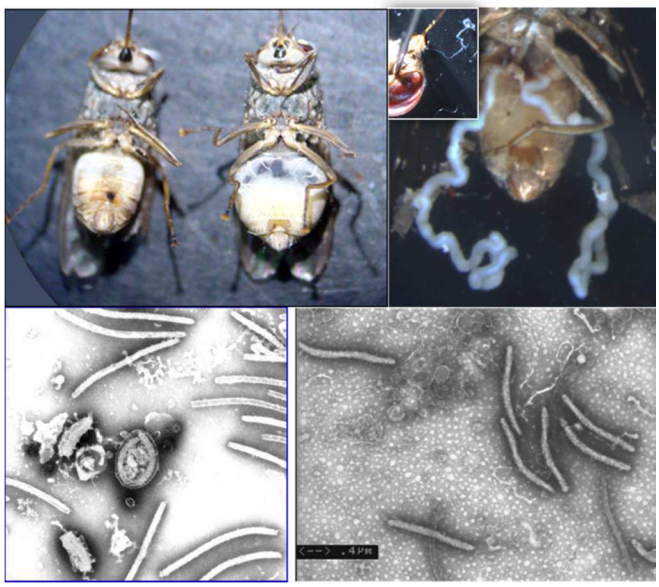
Standard Operational Procedures

To

Detect and Manage *Glossina pallidipes* Salivary Gland Hypertrophy Virus (GpSGHV) in Tsetse Fly 'Factories'



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Source

This procedure was developed by the Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. For more information please contact Adly Abd-Alla, a.m.m.abd-alla@iaea.org

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1. Introduction

Many species of tsetse flies are infected with a virus that causes salivary gland hypertrophy (SGH) symptoms associated with a reduced fecundity and fertility. A high prevalence of SGH has been correlated with the collapse of two laboratory colonies of *Glossina pallidipes* and colony maintenance problems in a mass rearing facility in Ethiopia. Mass-production of *Glossina species* is crucial for tsetse control programmes incorporating the sterile insect technique (SIT), and therefore requires a management strategy for this virus.

During the last decade the Joint FAO/IAEA Insect Pest Control Sub-programme investigated different strategies to manage the salivary gland hypertrophy virus (SGHV) in *G. pallidipes* mass production colonies, and developed an efficient virus management strategy, which succeeded in controlling and eliminating the virus from the tsetse mass production facility in Kality, Ethiopia. The virus management strategy that was developed is based on a combination of using antiviral drugs (Valacyclovir at a dose of 300 µg/ml of blood) and a clean feeding system. In addition to these methods, regular monitoring for the prevalence of SGH by dissection and the confirmation of SGHV by PCR is strongly recommended. In certain cases when attempting to start a new colony from field collected flies a new procedure to avoid using virus infected material is recommended.

To facilitate the monitoring for SGH or the SGHV infections and to properly implement the virus management strategy using a combination of the antiviral drug and the clean feeding system, this step-by-step standard operation procedure (SOP) has been developed, based on current knowledge and experiences from large-scale production of *G. pallidipes*. This document provides useful information for both large and small scale rearing facilities to maintain tsetse flies free from SGHV infection.

This SOP is addressed to Staff involved in tsetse rearing with sufficient education to recognize SGH symptoms, and with the ability to regularly monitor variations from normal “healthy” flies. Such variations include reproductive disturbances (reduced matings and egg production) and reduced longevity (premature mortalities and prolonged larviposition cycles). For some parts of this SOP, more sophisticated experience in molecular biology techniques is required to conduct virus diagnosis using the Polymerase Chain Reaction (PCR) method.

2. Standard Operating Procedure for detection of salivary gland hypertrophy (SGH) symptoms in tsetse colonies using a binocular microscope

2.1. Purpose

To detect and evaluate the prevalence of salivary gland hypertrophy (SGH) symptoms in a tsetse colony

2.2. Scope

This procedure helps institutions in Member States maintaining tsetse fly *G. pallidipes* species which suffer from low productivity.

2.3. Prerequisites

This procedure requires the following materials and equipment.

1. *Glossina pallidipes* established colony
2. Stereoscopic dissecting microscope
3. Dissection instruments (fine forceps, fine scissors)
4. Microscope slides
5. Normal saline
6. Refrigerator
7. Deep-freezer
8. Plastic petri-dishes
9. Ice box
10. Ice source

2.4. Responsibilities

Research or laboratory technician with the required skills for dissection and identifying normal tsetse salivary gland and hypertrophied salivary gland

2.5. Procedure

1. Select randomly tsetse flies (preferably 15 day old males)
2. Immobilize the flies by placing them in refrigerator for 10 min (or deep-freezer for 5 min) and then transfer them to petri-dish on ice
3. Place the microscope slide under the stereoscopic microscope and add a droplet of saline buffer
4. Place immobilized tsetse fly with its dorsal side downwards on the microscope slide **(Figure 1)**
5. Hold the thorax of the fly with fine forceps using left hand
6. Using the right hand, open the fly abdomen from the thorax-abdomen conjunction using fine forceps by gently tearing and pulling towards the end of the abdomen.
7. If the fly has salivary gland hypertrophy, large whitish tubes similar to those shown in **Figure 2** will be seen in the abdomen. This indicates that the fly has salivary gland hypertrophy
8. If no large whitish tube observed, pull the fly cuticle towards the tip of the abdomen to completely separate the abdominal organs and if small, transparent salivary glands are observed close to the thorax this indicates that the fly does not have salivary gland hypertrophy.

9. Record the number of dissected flies, the number of flies with SGH and the number of flies with normal salivary glands and calculate the percent of SGH in the colony as follows:

$$\boldsymbol{Percent\ (\%) of\ SGH\ in\ the\ colony} = \frac{\textbf{Number of flies with SGH}}{\textbf{Number of total dissected flies}}\ \boldsymbol{X\ 100}$$

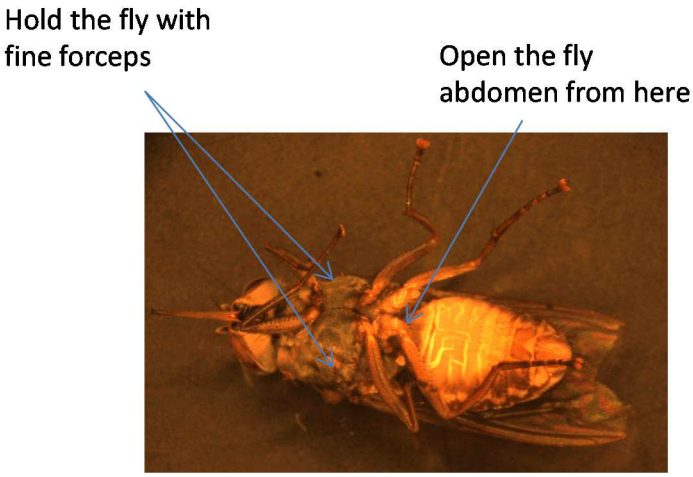

Figure 1. Tsetse fly abdomen containing SGH

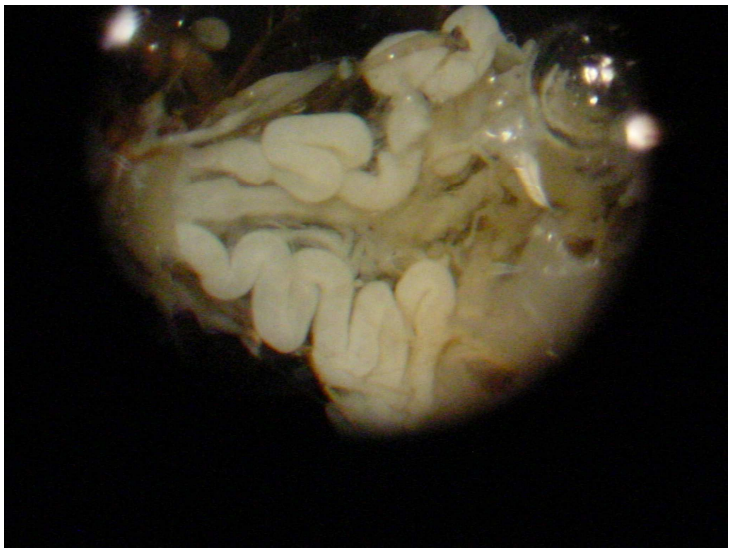

Figure 2. Tsetse fly abdomen containing SGH

2.6. References

Abd-Alla, A.M.M., Cousserans, F., Parker, A., Bergoin, M., Chiraz, J. and Robinson, A. 2009. Quantitative PCR analysis of the salivary gland hypertrophy virus (GpSGHV) in a laboratory colony of *Glossina pallidipes*. Virus Res. 139, 48-53.

Abd-Alla, A., Bossin, H., Cousserans, F., Parker, A., Bergoin, M. and Robinson, A. 2007. Development of a non-destructive PCR method for detection of the salivary gland hypertrophy virus (SGHV) in tsetse flies. J. Virol. Methods 139, 143-149.

2.7. Definitions

SGH: Salivary gland hypertrophy

SGHV: Salivary gland hypertrophy virus

3. Standard Operating Procedure for detection of SGHV infection in tsetse colonies using PCR

3.1. Screen for SGHV infection in whole flies

3.1.1. Purpose

This method is used to screen for infections of SGHV in tsetse colonies or in field collected flies where there is no need to keep the flies being tested alive.

3.1.2. Scope

This procedure is designed to screen tsetse flies of different species for the infection of SGHV in field collected or colony maintained tsetse flies.

3.1.3. Prerequisites

The following materials and equipment are needed.

1. Fresh or alcohol preserved tsetse flies
2. Micro tubes
3. Sterile micropestles
4. Liquid Nitrogen (N_2)
5. Autoclave
6. Clean room
7. Air conditioning
8. PCR work station
9. PCR thermocycler
10. Pipette (e.g. Pipetman set)
11. Pipette tips (e.g. Pipetman tips)
12. Water bath
13. Microcentrifuge i.e. Centrifuge 5424 / 5424 R, eppendorf.
14. Refrigerator
15. Deep-freezer
16. DNeasy kit
17. PCR master Mix reagent
18. Virus specific primers
19. Agarose
20. Gel electrophoresis apparatus
21. Ethidium bromide
22. DNA ladder
23. Nano-drop spectrophotometer
24. Sterile distilled water
25. Gloves

3.1.4. Responsibilities

Research or laboratory technician with the required skills for molecular biology work including DNA extraction and PCR technology and controlling PCR results on agarose gel.

3.1.5. Procedure

Warning: PCR detection method is very sensitive and precautions to avoid cross contamination between samples (from instruments, equipment etc.) should be taken.

1. For fresh tsetse flies proceed to step 3
2. For ethanol (or other solutions for conserving specimens e.g. propylene glycol) conserved flies, pour-off the alcohol preserving the fly and replace with distilled water, then pour-off the water and dry the fly by placing it in on tissue paper.
3. Put each individual fly in a separate micro-tube, label each tube with a serial number
4. Insert one sterile micropestle in each micro-tube
5. Add liquid nitrogen to the microtube to freeze the fly and the micropestle and immediately grind the fly using the micropestle to obtain homogenized fly tissue as a fine powder.
6. Proceed with the DNA extraction using DNeasy® kit following the suppliers instructions http://mvz.berkeley.edu/egl/inserts/DNeasy_Blood_&_Tissue_Handbook.pdf
7. After eluting the DNA, quantify the DNA concentration using a Nano-drop spectrophotometer to ensure extraction of an equal DNA quantity from each fly or to use equal quantity of the DNA in PCR reaction (for more information see http://www.nanodrop.com/nucleicacid.aspx)
8. After collecting nano-drop spectrometry results, prepare a working solution of DNA containing an equal DNA concentration for PCR
9. Conduct PCR using virus-specific primers with negative and positive DNA samples as described in Abd-Alla et al., (2007).
10. Separate PCR products by electrophoresis on 1.5% agarose gel **(Figure 3)** as indicated in Sambrook et al., (1989). Ethidium bromide can be used to stain the PCR product, in such cases take precautions to avoid getting contaminated with this product as it is a powerful mutagenic.
11. Visualize the results of electrophoreses using Gel documentation system **(Figure 4)**.
12. Analyse the PCR results for each tested sample with the positive control and record the positive and negative samples based on the presence/absence of the expected PCR product. The size of the PCR product can be controlled using DNA ladder **(Figure 5)**.
13. Calculate the prevalence of SGHV infection in the tested sample as follows:

$$\textit{Percent}\ (\%)\ \textit{of SGHV in the colony} = \frac{\textbf{Number of flies with SGHV}}{\textbf{Number of total tested flies}}\ \textit{X}\ \mathbf{100}$$

3.1.6. References

Abd-Alla, A., Bossin, H., Cousserans, F., Parker, A., Bergoin, M. and Robinson, A. 2007. Development of a non-destructive PCR method for detection of the salivary gland hypertrophy virus (SGHV) in tsetse flies. J. Virol. Methods 139, 143-149.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

3.1.7. Definitions

PCR: Polymerase Chain Reaction

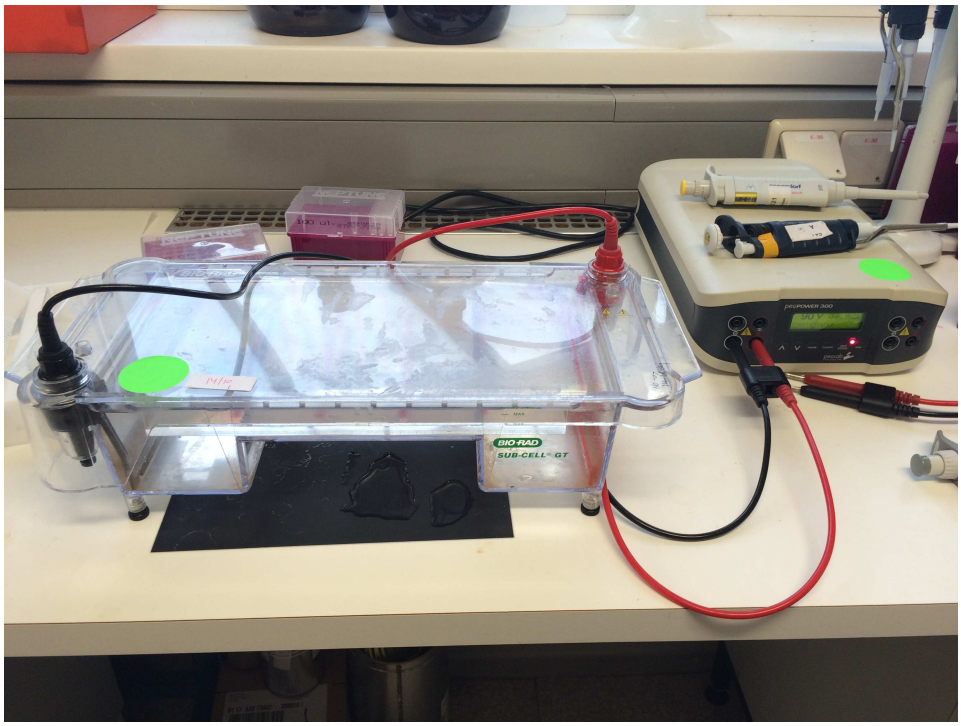

Figure 3. Gel electrophoresis system

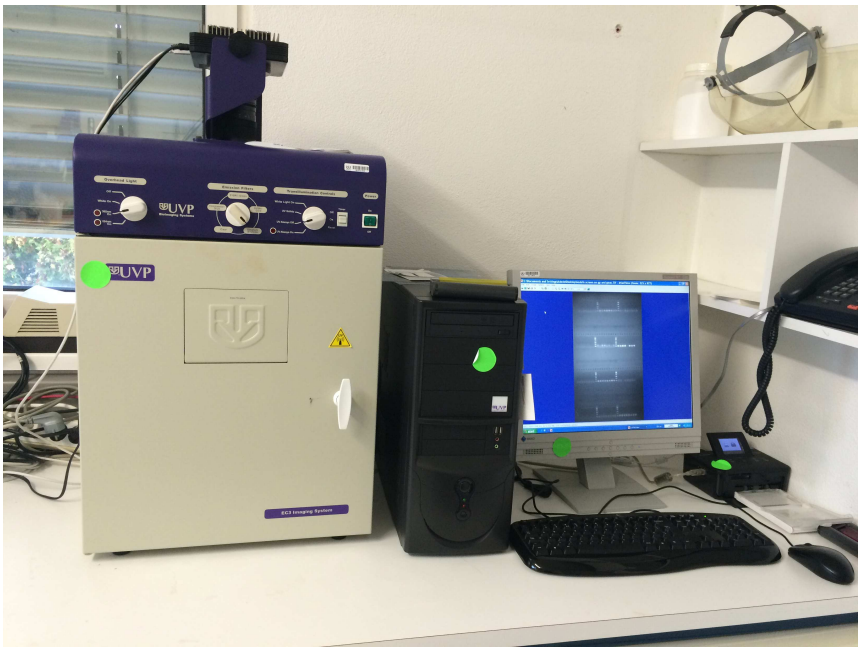

Figure 4. Gel documentation system

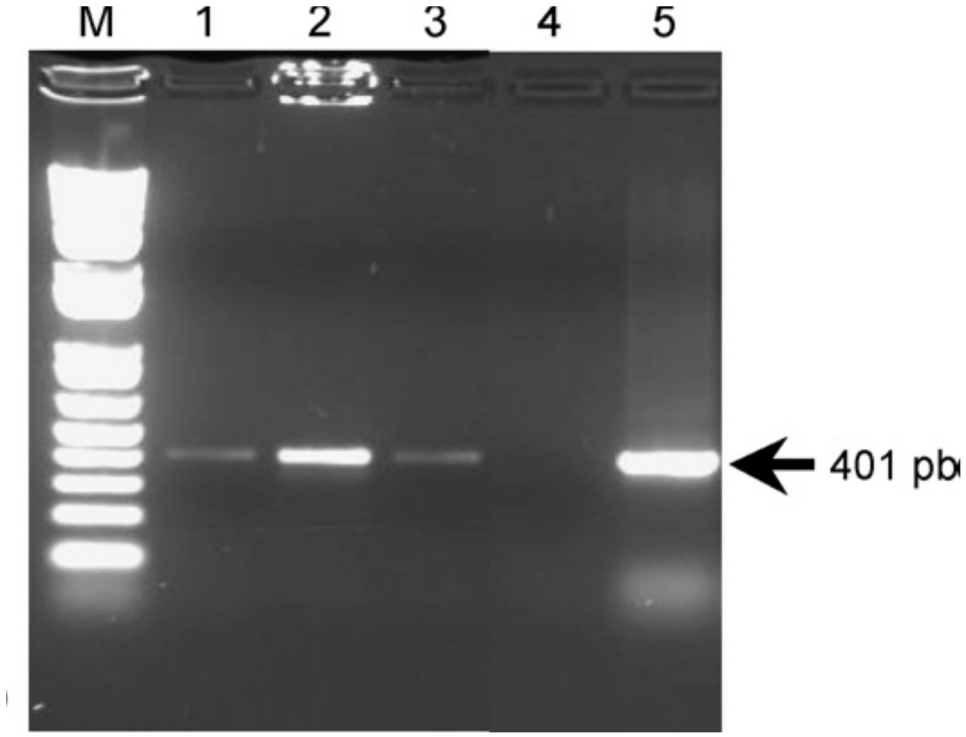

Figure 5. Detection of SGHV infection in tsetse colonies using PCR. Lane M: molecular weight marker; lanes 1, 2, and 3, DNA extracted from 3 tsetse flies infected with SGHV; lane 4, negative control (no template) and lane 5, positive control using salivary gland hypertrophy virus DNA as template.

3.2. Screening for SGHV infection in live flies using one leg

3.2.1. Purpose

This method is designed to screen for the presence of infection with SGHV in a tsetse colony in live flies that will be used to start a virus free colony.

3.2.2. Scope

This procedure to screen tsetse flies of different species for the infection of SGHV in live flies to start a virus free colony.

3.2.3. Prerequisites

The following materials and equipment are needed.

1. Teneral tsetse flies
2. Micro tubes
3. Sterile micropestles
4. Fine Scissors
5. Benzene flame
6. Ethanol 70%
7. Ice box
8. Plastic Petri-dishes
9. Alcohol proof marker
10. Fine forceps
11. Liquid Nitrogen (N2)
12. Autoclave
13. Clean room
14. Air conditioning
15. PCR work station
16. PCR thermocycler
17. Pipette (e.g. Pipetman set)
18. Pipette tips (e.g. Pipetman tips)
19. Water bath
20. Centrifuge
21. Refrigerator
22. Deep-freezer
23. DNeasy® kit
24. PCR master Mix reagent
25. Virus-specific primers
26. Agarose
27. Gel electrophoresis apparatus
28. Ethidium bromide
29. DNA ladder
30. Nano-drop spectrophotometer
31. Gloves
32. Cylindrical plastic Tubes (6 cm long with 4 cm in diameter with netting from both sides) for holding the live fly

3.2.4. Responsibilities

Research or laboratory technician with the required skills for molecular biology work including DNA extraction and PCR technology and controlling PCR results on agarose gel.

3.2.5. Procedure

1. Collect teneral tsetse flies from targeted colony
2. Expose the flies to cold air at 4°C for 2-3 min until falling immobilized; place the flies in plastic petri-dishes on ice
3. Sterilise the fine forceps using the 70% ethanol and benzene flame and cut off an intermediate leg; place the fly in a small tube and the leg in a micro-tube; label both the small tube and the microtube with the same number, and continue doing this for all flies to be tested.
4. Hold the flies in a rearing room with suitable conditions of temperature and humidity.
5. For the legs, insert one sterile micropestle in each micro-tube
6. Add liquid nitrogen in the microtube to freeze the fly leg and the micropestle and immediately grind the fly leg using the micropestle to obtain a fine powder.
7. Proceed with the DNA extraction using ZR-96 Quick-gDNA extraction kit following the suppliers 'instructions**,** http://www.zymoresearch.com/downloads/dl/file/id/19/d3010i.pdf**)**.
8. After eluting the DNA, quantify the DNA concentration using Nano-drop spectrophotometer to ensure extraction of an equal DNA quantity from each fly or to use equal quantity of the DNA in PCR reaction (for more information see http://www.nanodrop.com/nucleicacid.aspx)
9. After collecting nano-drop spectrometry results, prepare a working solution of DNA containing an equal DNA concentration for PCR Reaction
10. Conduct PCR reaction using virus specific primers with negative and positive DNA samples as described in Abd-Alla et al., (2007).
11. Control PCR results on 1.5% agarose electrophoresis gel as described in Sambrook et al., (1989). Ethidium bromide can be used to stain the PCR product, in such cases precautions should be taken to avoid getting contaminated with this product as it is powerful mutagenic**.**
12. Visualize the results of electrophoreses using Gel documentation system**.**
13. Control the PCR results for each tested sample with the positive control and record the positive and negative samples based on the presence/absence of the expected PCR product. The size of the PCR product can be controlled using DNA ladder.
14. Calculate the prevalence of SGHV infection in the tested sample as follows:

$$\boldsymbol{Percent\ (\%) of\ SGHV\ in\ the\ colony} = \frac{\textbf{Number of flies with SGHV}}{\textbf{Number of total tested flies}}\ \boldsymbol{X\ 100}$$

3.2.6. References

List resources that may be useful when performing the procedure; for example, Admin policies, Municipal Code, government standards and other SOPs.

Abd-Alla, A., Bossin, H., Cousserans, F., Parker, A., Bergoin, M. and Robinson, A. 2007. Development of a non-destructive PCR method for detection of the salivary gland hypertrophy virus (SGHV) in tsetse flies. J. Virol. Methods 139, 143-149.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

See:

http://www.nanodrop.com/nucleicacid.aspx for Nano-drop spectrophotometer instructions

http://www.zymoresearch.com/downloads/dl/file/id/19/d3010i.pdf for Quick-gDNA extraction kit

3.2.7. Definitions

PCR: Polymerase chain reaction

Teneral tsetse fly: a newly emerged fly that has not yet taken a feed with its first blood meal

DNA Ladder: This is a set of standard molecular-weight size markers that are used to identify the approximate size of a molecule run on a gel during electrophoresis

4. Standard Operating Procedure Managing SGHV infection in tsetse colonies

4.1. Using antiviral drug (valacyclovir)

4.1.1. Purpose

To manage the SGHV infection in tsetse colonies using antiviral drug (valacyclovir)

Valacyclovir is an antiviral drug originally produced to control *Herpes simplex* virus; its mode of action is based on its phosphorylation by the viral thymidine kinase into a monophosphate which then forms an active triphosphate (Aciclo-GTP) by further phosphorylation. This is a potent inhibitor for viral DNA polymerase (a gene responsible for the virus multiplication). Reducing the virus multiplication keeps the virus infection at a low titre and avoids the development of symptomatic infection.

2. Scope

This procedure is designed to help institutions in Member States to maintain tsetse fly production facilities for species suffering from SGHV infection

3. Prerequisites

The following materials and equipment are required:

1- Tsetse fly colony
2- Antiviral drug Valacyclovir hydrochloride
3- Distilled water
4- Bacteriology hood
5- Plastic syringes
6- 45µm filter
7- Magnetic stirrer plate
8- Magnetic stirrer bar
9- Sterile flask
10- Microbalance
11- Laminar-flow bench
12- Bunsen burner
13- A bottle of antiseptic (eg.70% ethanol)
14- Balance (1µg resolution)
15- 250ml Glass bottle (sterile)
16- 2000ml Glass bottle (sterile)
17- Autoclaved distilled water
18- Vortex stirrer
19- Aluminium foil
20- Quality Controlled blood (thawed)

4.1.4. Responsibilities

Research or laboratory technician with the required skills for preparing quality controlled blood for tsetse colony

4.1.5. Procedure

Prepare Valacyclovir stock solution (100 ml) at a concentration of 50mg/ml H_2O

1. Clean working bench and hands with antiseptic/70% ethanol.
2. Take a 250ml glass bottle (sterile)
3. Weigh 5 gm valacyclovir in a piece of aluminium foil
4. Put the valacyclovir in the 250ml glass bottle (sterile)

5. Add 100ml distilled water to the bottle
6. Mix well using a magnetic stirrer and ensure that the valacyclovir is completely dissolved

Prepare blood diet (1000 ml) with valacyclovir 300 µg/ml

7. Take 2000ml glass bottle (sterile)
8. Ignite the Bunsen burner in the laminar flow and wait to sterilize the working zone then conduct the following steps in aseptic conditions .
9. Add 6 ml of valacyclovir stock solution (50mg/ml) in the bottle
10. (300 µg/mlx1000ml=300 mg=300/50=6 ml of 50 mg/ml stock solution)
11. Add 994 ml blood to the bottle
12. Mix well using a magnetic stirrer to achieve a homogeneous distribution of the drug

4.1.6. References

Abd-Alla, A.M.M., Adun, H., Parker, A.G., Vreysen, M.J.B. and Bergoin, M. 2012. The antiviral drug valacyclovir successfully suppresses salivary gland hypertrophy virus (SGHV) in laboratory colonies of *Glossina pallidipes*. PLoS One 7, e38417.

Abd-Alla, A.M.M., Marin, C., Parker, A. and Vreysen, M. 2014. Antiviral drug valacyclovir treatment combined with a clean feeding system enhances the suppression of salivary gland hypertrophy in laboratory colonies of *Glossina pallidipes*. Parasites & Vectors 7, 214.

4.1.7. Definitions

Valacyclovir: a commercially available antiviral drug

4.2. Using Clean Feeding System (CFS)

4.2.1. Purpose

To manage the SGHV infection in tsetse colonies using clean feeding system (CFS)

4.1.2. Scope

This procedure is designed to help institutions in Member States to maintain a tsetse fly production facility for species suffering from SGHV infection

4.1.3. Prerequisites

The required materials and equipment are the following:

1. Tsetse fly colony
2. Coloured tape
3. Bacteriology hood
4. Plastic syringes
5. 45µm filter
6. Magnetic stirrer plate
7. Magnetic stirrer bar
8. Sterile flask
9. Microbalance

4.1.4. Responsibilities

Researcher or laboratory technician has the required skills for tsetse rearing and colony management.

4.1.5. Procedure

The clean feeding protocol is used to prevent the flies from picking up the virus from the blood already used for feeding previous cages of tsetse flies. To implement the clean feeding protocol in the large-scale colony:

1. Calculate the number of feeding trays and from this calculate the number of fly cages that can be fed on the blood membrane at any one time; based on that, calculate the maximum number of fly cages that can used to establish a clean feeding colony.
2. Collect newly-emerged (teneral) *G. pallidipes* flies from the regular colony production according to the weekly productivity of that colony.
3. Feed the collected teneral flies with a clean blood meal (blood that has not previously been used for feeding) and feed the remaining colony on the same blood later.
4. Feed and maintain these flies and their progeny on fresh, clean blood; they should always be the first to be fed on this blood.
5. Continue adding cages of teneral flies each week until the maximum number of cages that could be fed first (during one round of feeding) on the available feeding trays has been attained.
6. Name this colony as “Clean Feeding Colony 1″ (CFC-1).
7. Subsequently, when the maximum number of cages for the CFC-1 colony has been attained, the excess flies from the CFC-1 progeny can be fed on the same membrane as a second feeding round after feeding the CFC-1 colony.
8. The second-round feeding group of flies is denoted as “clean feeding colony 2″ (CFC-2), and should always be maintained on a second feeding round after feeding the CFC-1 colony.

9. During the establishment of the CFC-1 and CFC-2, the regular colony will always be fed on the same membranes used to feed CFC-1 and -2 (at the third and subsequent feeding rounds), and is denoted as the "normal feeding colony" (NFC).
10. Keep the colony records (productivity and mortality) for each colony (CFC-1, CFC-2 and NFC) separately **(Figure 6)**.
11. Assess the prevalence of SGH in each colony by fly dissection at regular intervals (3-6 months).

4.1.6. References

Abd-Alla, A.M.M., Kariithi, H.M., Mohamed, A.H., Lapiz, E., Parker, A.G. and Vreysen, M.J.B. 2013. Managing hytrosavirus infections in *Glossina pallidipes* colonies: Feeding regime affects the prevalence of salivary gland hypertrophy syndrome. PLoS One 8, e61875.

Abd-Alla, A.M.M., Marin, C., Parker, A. and Vreysen, M. 2014. Antiviral drug valacyclovir treatment combined with a clean feeding system enhances the suppression of salivary gland hypertrophy in laboratory colonies of *Glossina pallidipes*. Parasites & Vectors 7, 214.

4.1.7. Definitions

CFC: Clean feeding colony

NFC: Normal feeding colony.

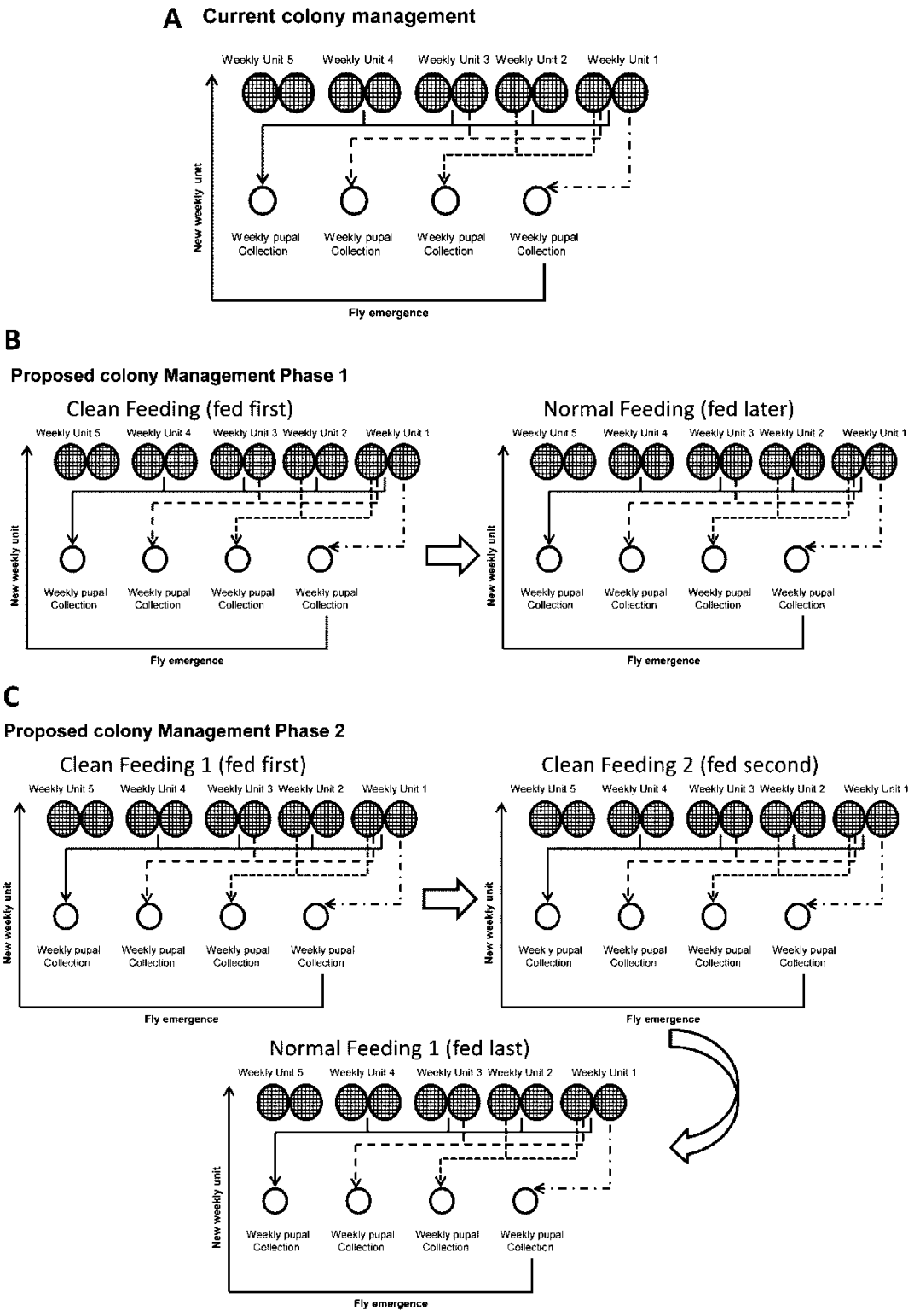

Figure 6. Handling, feeding and management of a tsetse colony. (A) Regular *in vitro* membrane feeding system, (B) intermediate phase, and (C) final phase of the clean feeding system

5. Standard Operating Procedure for establishing virus free colonies from natural populations or laboratory maintained colonies that show salivary gland hypertrophy virus infection

4.1. Purpose

To establish a new virus-free tsetse colony from flies collected from natural (wild) populations

4.2. Scope

This SOP is intended to help Member States institution working on tsetse fly with the objective of embarking on a SIT project for the suppression/eradication of *Glossina* species

4.3. Prerequisites

The following materials and equipment are needed:

4.4. Responsibilities

This process requires staff with sufficient knowledge in (i) collecting tsetse flies from natural populations using traps, (ii) rearing and maintaining tsetse flies in the laboratory and detecting SGHV infection using PCR method.

4.5. Procedure

1. Collect tsetse flies from a natural population as described in the SOP (http://www-naweb.iaea.org/nafa/ipc/public/Tsetse_Rearing_SOP_web.pdf)
2. Keep each female in an individual tube and collect the maximum number of pupae from each fly separately.
3. After collecting the maximum number of pupae from each female, freeze the female fly and proceed for total DNA extraction using DNeasy® kit following the suppliers 'instructions http://mvz.berkeley.edu/egl/inserts/DNeasy_Blood_&_Tissue_Handbook.pdf.
4. After eluting the DNA, quantify the DNA concentration using a Nano-drop spectrophotometer to ensure extraction of equal DNA quantity from each fly or to use equal quantity of the DNA in PCR reaction (for more information see http://www.nanodrop.com/nucleicacid.aspx)
5. After collecting nano-drop spectrometry results, prepare a working solution of DNA containing an equal DNA concentration for PCR.
6. Conduct PCR reaction using virus specific primers with negative and positive DNA samples.
7. Separate the PCR products on 1.5% agarose gel electrophoresis**.**
8. Visualize the electrophoresis result using Gel documentation system**.**
9. Analyse the PCR results for each tested sample with the positive control and record the positive and negative sample results based on the presence/absence of the expected PCR product
10. Determine the virus infection status of each individual female fly
11. Collect the pupae produced by the female flies showing negative results for the virus infection and consider these flies as a virus free colony.
12. Maintain the colony using clean feeding system as described in 4.2 combined with use of the antiviral drug as described in section D.
13. For each generation some flies should be randomly selected and processed for DNA extraction and tested for the virus infection using the PCR technique.

4.6. References

Abd-Alla, A., Bossin, H., Cousserans, F., Parker, A., Bergoin, M. and Robinson, A. 2007. Development of a non-destructive PCR method for detection of the salivary gland hypertrophy virus (SGHV) in tsetse flies. J. Virol. Methods 139, 143-149.

Abd-Alla, A.M.M., Kariithi, H.M., Mohamed, A.H., Lapiz, E., Parker, A.G. and Vreysen, M.J.B. 2013. Managing hytrosavirus infections in *Glossina pallidipes* colonies: Feeding regime affects the prevalence of salivary gland hypertrophy syndrome. PLoS One 8, e61875.

Abd-Alla, A.M.M., Marin, C., Parker, A. and Vreysen, M. 2014. Antiviral drug valacyclovir treatment combined with a clean feeding system enhances the suppression of salivary gland hypertrophy in laboratory colonies of *Glossina pallidipes*. Parasites & Vectors 7, 214.

Abd-Alla, A.M.M., Adun, H., Parker, A.G., Vreysen, M.J.B. and Bergoin, M. 2012. The antiviral drug valacyclovir successfully suppresses salivary gland hypertrophy virus (SGHV) in laboratory colonies of *Glossina pallidipes*. PLoS One 7, e38417.

4.7. Definitions

Nano-drop technology: A technology developed for micro-volume quantitation and analysis.

Gel documentation system: A **gel documentation system** (**gel** image **system** or **gel** imager), is equipment used in molecular biology for the imaging and **documentation** of nucleic acid and protein suspended within polyacrylamide or agarose **gels**.

DNeasy®: This is the registered trade mark for a kit used for DNA extraction

Annex 1 Special equipment and materials with specification

Item	**Specification**	**Example of supplier and/or distributor**
Stereoscopic dissecting microscope	Stereo-zoom microscope, binocular, 7-47 X, upper variable quartz halogen lamp, lower fluorescent lamp, complete. Eyepiece micrometer.	Labsco (laboratory Supply Company).
Dissection instruments (fine forceps, fine scissors)	Dissecting set in case, satinized, stainless steel, with incision scissors, fine pointed scissors, splinter, forceps, 2 rounded scalpels, cartilage knife, with scraper, double-bottom probes 1+2 mm, pointed dissecting needle, lancet	Labsco (laboratory Supply Company).
Sterile micropestles	Hand-operated or motor-driven grinders for disrupting tissue in microcentrifuge tubes. Pestle ends should be specially designed to mate with 0.5 mL or 1.5 mL microtubes.	Labsco or Sigma
Autoclave	An autoclave is a pressure chamber used to sterilize equipment and supplies by subjecting them to high pressure saturated steam at 121 °C for around 15–20 minutes depending on the size of the load and the contents.	Labsco or Sigma
PCR work station	A self-contained work area that will help protect PCR runs against contamination. It should have HEPA H14 filter, stainless steel work surface, and a UV light with a timer. Dual UV bulbs irradiate the work area prior to use, reducing the possibility that contaminating DNA will be amplified. Containment features reduce the chance of airborne contamination.	Labsco, Erlab or Sigma
PCR thermocycler	A device to conduct DNA	Bio-Rad, Eppendorf, Labsco

	amplification, it should be compact 96-well thermal cycler with thermal gradient optimization and reliable performance	or VWR
Pipette (e.g. Pipetman set)	A set of pipette includes 6 adjustable-volume pipettes (0.1-2.5 µL, 0.5–10 µL, 2–20 µL, 10–100 µL, 20–200 µL, 100–1,000 µL),	Eppendorf or Gilson
Pipette tips (e.g. Pipetman tips)	Sterile tips with reload or filtered which fits the different pipettes size.	Labsco or VWR
Water bath	Water bath with microprocessor controller for temperature selection, rapid heat-up and excellent stability. It should be equipped with an adjustable over temperature safety cutout which sounds an audible alarm and shuts the heater off to protect samples and recessed control panel to protect against spills. Also it should has a seamless passivated tank to prevent against rust and corrosion.	Labsco or VWR
Microcentrifuge. i.e. Centrifuge 5424 / 5424 R, eppendorf.	Micro-centrifuge with the following specification as example, Max. rcf: 20,238 x g, Max. speed: 14,680 rpm, Max. rotor capacity: 24x 1.5/2.0 mL, No. of rotors: 4, Acceleration time to max. speed: 16 s, Braking time from max. speed: 18 s, SOFT ramp: adjustable, Noise level with rotor FA-45-24-11: <51 dB(A), Dimensions in cm (W x D x H):24 x 32 x 23, Weight without rotor:13.4 kg, Power supply: 230 V/50–60 Hz, Power requirement max.:250 W	Eppedorf, Labsco or VWR
DNeasy kit	Kit for DNA extraction	Qiagen or labsco
PCR master Mix reagent	Ready-made mix for PCR reaction, It contains all the required components	Qiagen or Labsco

	(including $MgCl_2$, buffer, dNTPs, DNA polymerase) for PCR reaction except primers and DNA template	
Gel electrophoresis apparatus	A horizontal electrophoresis chambers with a wide (15 cm) platform to provide higher capacity It should include a buffer tank, safety lid with cables, and leveling bubble.	Bio-Rad, Or Labsco
DNA ladder	A mixture of DNA fragments with known length which helps to determine the size of unknown DNA fragments using gel electrophoresis.	Qiagen or Invitrogen
Nano-drop spectrophotometer	A spectrophotometer measures 1 µl nucleic acid samples with high accuracy and reproducibility. It should have a full spectrum (220nm-750nm) spectrophotometer. It requires a PC or laptop with suitable software to analyse and calculate the nucleic acid concentration.	Thermo Scientific Or labsco
Antiviral drug Valacyclovir hydrochloride	Valacyclovir hydrochloride hydrate, ≥98% (HPLC), solid 2-((2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy)ethyl ester monohydrochloride, L-Valine	Sigma or other suppliers
Bacteriology hood	Bacteriological cabinet with suitable dimension and shape with the following specification Exhaust airflow (m3/h): 400, Heat emission (in work mode) (W) (J/s): 805, Heat emission (in stand-by mode) (W) (J/s): 533, Light Intensity (lux): 1000, Sound Level dB(A): <60, Electrical connection (V)/(Hz): 230/50, Power consumption (in work mode) (W) (J/s): 805, Power consumption (in stand-by	

	mode) (W) (J/s): 533, Maximum power consumption (W) (J/s): 1613, Safety glass (8 mm laminated): Yes, Leak-tight: Yes, Pre-filter (EN 779) : G3, Downflow HEPA-filter (EN 1822): H14, Exhaust HEPA-filter (EN 1822): H14, 1st HEPA-filter (V-shaped): H14 .	
Quality Controlled blood (thawed)	Cow blood, defibrinated, irradiated with 1 KGry and controlled for bacterial contamination.	Collected locally from slaughterhouse or purchased from a local supplier