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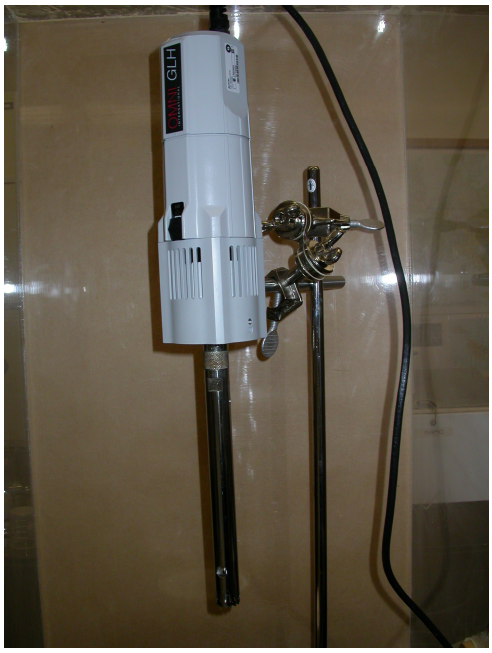
Great Lakes Forestry Centre
Insect Production Services

STANDARD OPERATING PROCEDURE

Number: IPS/004/002

Homogenizer



Effective Date: 7 November 2013

Canada

TITLE: Homogenizer

APPROVING OFFICIAL:

DD / MM / YY

*Manager, Insect Production Services (IPS)*_____ ___/___/___

SIGNIFICANT CHANGES FROM PREVIOUS VERSION:

This Standard Operating Procedure (SOP) has been edited and revised for consistency with other SOPs used by Insect Production Services. Technical procedures have not been revised.

1.0 INTRODUCTION

1.1 Purpose

This SOP describes procedures to be followed for the operation and sanitization of the OMNI General Lab Homogenizer to ensure sufficient maceration of samples and to eliminate and/or deactivate carryover between samples.

1.2 Scope

This SOP shall be followed by all Quality Control (QC) Unit personnel when homogenizing adult QC samples for the detection of microbial pathogens in IPS insect colonies.

1.3 Definitions

Controlled Copy – A copy of an SOP distributed to select GLFC personnel having a unique copy number and dated signature of the IPS manager. Controlled copies are intended to ensure that GLFC personnel follow the most recent version of the SOP.

Effective Date – The date from which the procedures given in an SOP are to be implemented.

Head Quality Control Technician – A member of IPS having authority over the daily operation of the QC lab and other QC personnel.

Insect Production Services (IPS) – A Great Lakes Forestry Centre (GLFC) work team consisting of the Insect Production Unit (IPU), the Quality Control Unit (QCU) and Insect Quarantine (IQ) personnel who perform insect rearing, quality control and quarantine activities in support of forest pest research activities internal and external to the Canadian Forest Service (CFS).

Insect Production Services Manager – The individual who has overall responsibility for activities of the IPS team.

Insect Production Unit (IPU) – A work unit of IPS consisting of personnel who perform insect rearing, diet making and methods development activities at GLFC.

Material Safety Data Sheet (MSDS) – A summary description of a chemical, reagent or substance prepared by the manufacturer or supplier and required by WHMIS legislation to inform workers about procedures required to safely work with the material.

Quality Control Unit (QCU) – A work unit of IPS consisting of personnel who conduct routine production, process and product control testing and develop new QC methodology in support of IPU activities.

Reverse Osmosis (RO) Water – Water that has been generated using the process of reverse osmosis.

Standard Operating Procedures (SOPs) – Directives describing routine administrative or technical procedures conducted by IPS personnel or users of the IQ facility.

1.4 Safety

1.4.1 Personal protective safety equipment (i.e., lab coat, disposable chemical protective gloves, safety goggles and hearing protection) shall be worn when operating the homogenizer.

1.4.2 Personnel shall have access to, and be familiar with, the MSDS for all chemicals used to clean and sterilize the homogenizer.

1.5 Materials

Materials required include:

1.5.1 Personal protective safety equipment:
- (a) lab coat
- (b) disposable chemical protective gloves
- (c) safety goggles
- (d) hearing protection

1.5.2 Chemicals:
- (a) RO water
- (b) soap solution (one drop liquid dish detergent in 250ml RO water)
- (c) 6% bleach solution (refer to 2.3.1)
- (d) 50% bleach solution (refer to 2.3.2)

1.5.3 Tools:
- (a) power generator
- (b) 19mm diameter sawtooth generator probe
- (c) speed control

(d) squeeze bottle filled with RO water
(e) 250 ml graduated cylinders
(f) beaker for collecting waste

1.5.4 MSDS for sodium hypochlorite.

2.0 PROCEDURES

Abbreviated instructions for macerating adults and sanitizing the homogenizer are identified in Appendix 1 and shall be posted in the immediate vicinity of the instrument for quick reference.

2.1 Homogenization of Samples

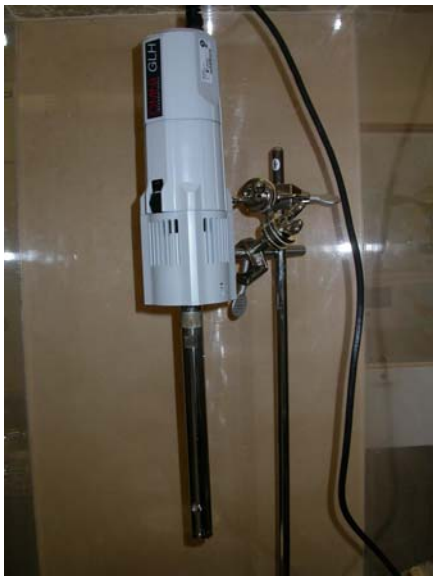
2.1.1 QCU personnel shall ensure that samples provided by the IPU have been placed in screw top bottles or tubes suitable for freezing, and have been labeled with an appropriate identifier.

2.1.2 RO water shall be added to the frozen sample. Unless specified in the QC SOP for the insect species, the volume of water added shall be sufficient to produce a viscous slurry once macerated. When the initial volume of adults is too large to accommodate the generator probe and the required volume of water, the sample shall first be transferred to a larger container.


Transferring sample to a larger container

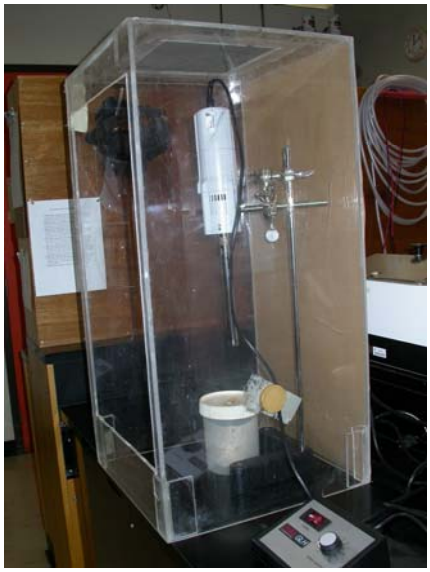

Adding water to sample

2.1.3 The power generator shall be fitted with a 19mm diameter sawtooth generator probe (sterilized as per section 2.2) and shall be adjusted to setting #6.

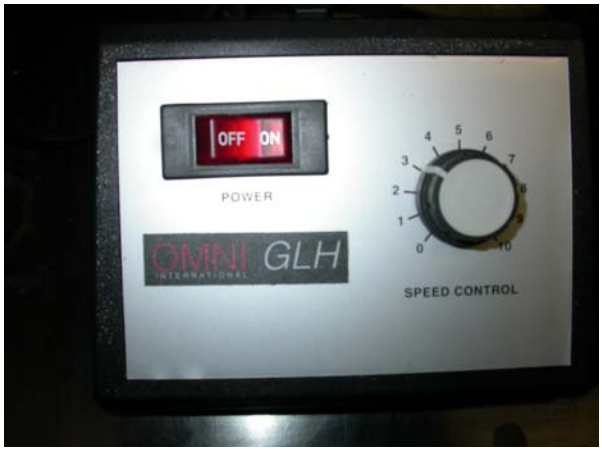


Generator fitted with sawtooth probe

2.1.4 To minimize potential contamination of the QC lab by splattering of samples during maceration, the homogenizer assembly shall be used within a confined chamber (e.g., plexiglass box, safely cabinet, or fume hood).



2.1.5 The probe shall be inserted into the sample and the speed increased slowly to setting #3 using the external speed control unit. The sample shall be macerated until whole insect abdomens are no longer visible.



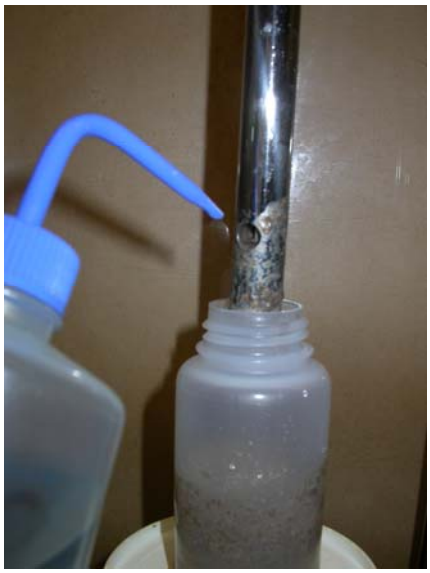
External speed controller


Macerating a sample

2.1.6 Turn off the homogenizer and use a clean toothpick, where required, to dislodge large pieces from the hole in the probe or from the cutter head. These pieces shall be added to the sample bottle and shall be remacerated.


Dislodging insect tissue

2.1.7 Using a squeeze bottle, rinse the probe with RO water and collect the rinsate in the sample container.

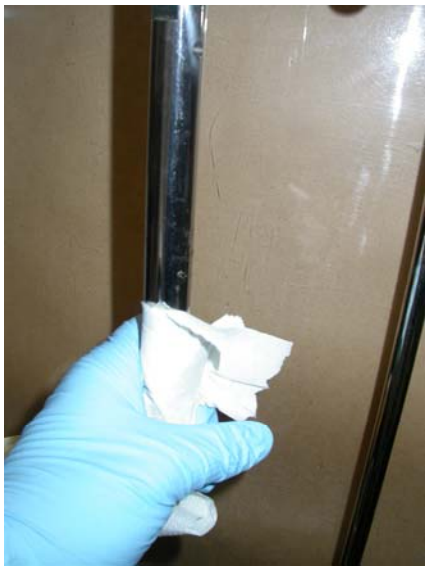


Rinsing probe with RO water

2.2 Sanitization of Homogenizer

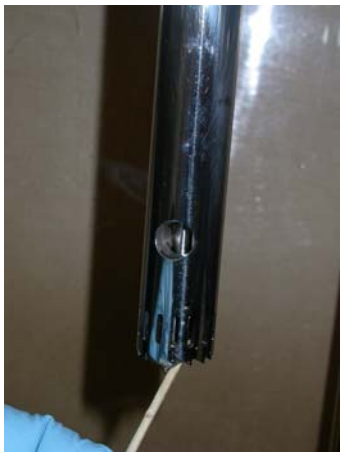
The following sanitization procedure will sufficiently eliminate and/or deactivate carry-over between samples that are to be analyzed microscopically.

2.2.1 After homogenizing the sampling as specified above, conduct a cursory cleaning of the probe first by wiping with paper towel to remove the majority of insect parts and lipids, then using a toothpick to dislodge particles from the cutters, followed by additional rinsing with RO water and collection of the water in a waste beaker.

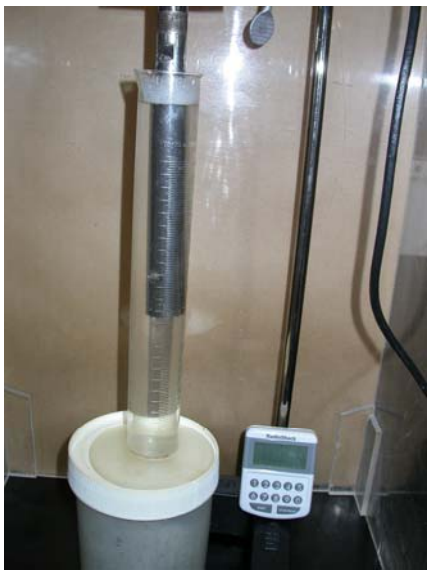


Cursory cleaning of probe using paper towel


Rinsing of probe with RO water


Using toothpick to dislodge particles from cutter

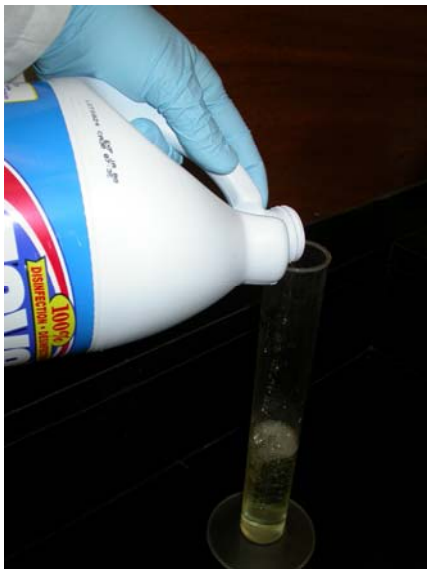
2.2.2 The probe shall then be inserted into a 250 ml graduated cylinder containing soap solution (use one drop of liquid dish detergent and 250 ml RO water; make fresh for each sample) and the homogenizer turned on for about 1 minute to flush the majority of remaining insect parts from the internal components of the shaft. The soap solution shall be discarded after it is used, however, the cylinder may be reused for additional samples from the same cohort if it is sufficiently rinsed between samples (max. 5 samples).


Cleaning probe with soap solution

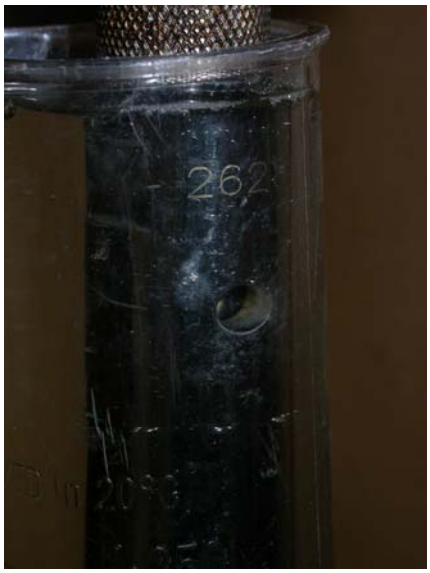
2.2.3 Rinse the probe with RO water and discard the rinsate in a waste beaker.

Rinsing soap off probe

2.2.4 Insert the probe into a cylinder containing 250ml 50% bleach solution (refer to 2.3.2) and run the homogenizer for 3 minutes. Ensure that the solution enters the top hole on the probe (for part of the 3-minute run time) in order to sanitize the internal components. Both the cylinder and the bleach solution may be reused for additional samples from the same cohort (max. 5 samples).

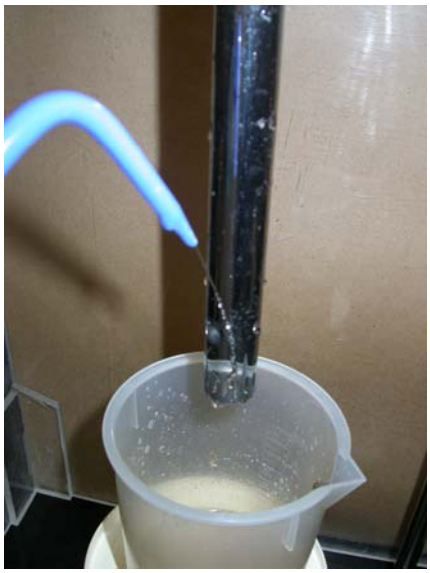


Preparing bleach solution



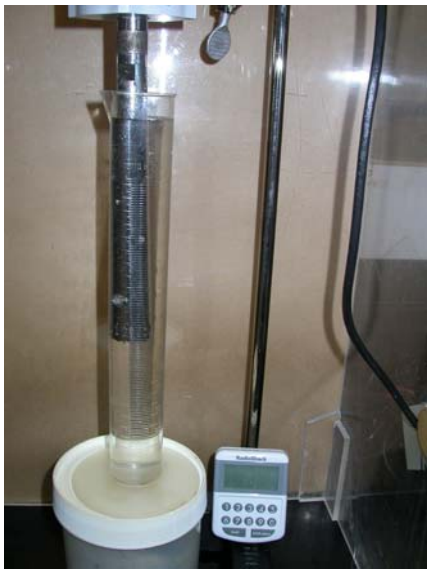
Bleach solution entering hole on probe

2.2.5 Rinse the probe with RO water and discard the rinsate in a waste beaker.



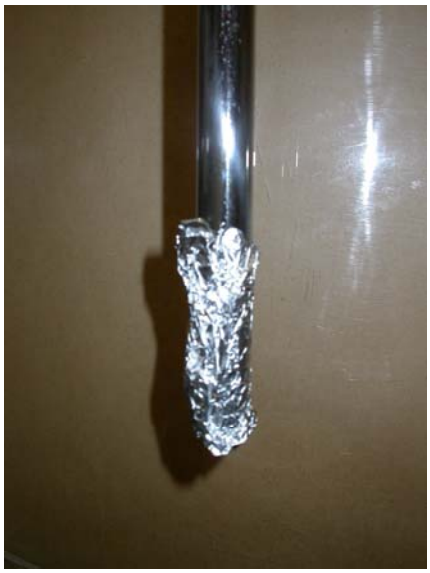
Rinsing bleach off probe

2.2.6 Insert the probe into a cylinder containing 250ml RO water and run the homogenizer for 1 minute. Ensure that the solution enters the top hole on the probe (for part of the 1-minute run time) in order to flush the bleach residue from the internal components. The water shall be discarded after it has been used, however, the cylinder may be reused for additional samples from the same cohort if it is sufficiently rinsed between samples (max. 5 samples).



Removing bleach solution from probe

2.2.7 When no additional samples are to be macerated during the session, the rotor shall be air dried and wrapped in foil until the next use.



Probe wrapped in foil for storage

2.2.8 Upon completion of the grinding session, the power generator, stand, benchtop and cabinet shall be sprayed with a 6% bleach solution (refer to 2.3.1) and allowed to sit for at least 10 minutes of contact time before wiping with paper towel.

2.2.9 All cylinders and waste beaker shall be washed and autoclaved before the next grinding session.

2.3 Calculations

2.3.1 The bleach working solution for general cleaning shall have a final sodium hypochlorite concentration of 0.3%. Bleach stock material with a 5.25% sodium hypochlorite concentration (e.g., Javex®) shall be diluted by combining 60ml bleach and 940ml water (i.e., 6% dilution). Bleach stock material with a 6.0% sodium hypochlorite concentration (e.g., Ultra Javex®) shall be diluted by adding 53ml bleach and 947ml water (i.e., 5.25% dilution). If another brand of bleach is used, volumes may need to be adjusted to provide a 0.3% sodium hypochlorite working solution.
[Note: minimum contact time of 10 minutes is required for effective sanitation]

2.3.2 The homogenizer probe cleaning solution shall have a final sodium hypochlorite concentration of 2.625%. Bleach stock material with a 5.25% sodium hypochlorite concentration (e.g., Javex®) shall be diluted by combining 125ml bleach and 125ml RO water (i.e., 50% dilution) in a 250ml graduated cylinder. If another brand or concentration (e.g., Ultra Javex®) of bleach is used, volumes may need to be adjusted provide a 0.3% sodium hypochlorite working solution.

2.4 Documentation and Reporting

NA

3.0 DISTRIBUTION AND ARCHIVING

3.1 Distribution

This SOP shall be distributed by the IPS Manager to all QCU personnel.

3.2 Archiving

3.2.1 The IPS manager shall maintain a historical copy of this SOP when it is replaced by a new version.
3.2.2 QCU personnel shall ensure that macerated samples are retained as specified in the QC SOP for the insect species

3.3 Destruction of Outdated SOPs

When new versions of this SOP are available for distribution, all persons in possession of a controlled copy shall ensure that the retired version is returned to the IPS Manager upon request.

4.0 ASSURING SOP VALIDATION AND COMPLIANCE

4.1 Responsible Individual

4.1.1 The head QC technician is responsible for assuring that this SOP is valid.
4.1.2 The head QC technician is responsible for assuring that this SOP is followed by QCU personnel and that these persons have been appropriately trained in the use of this SOP.
4.1.3 QCU personnel are responsible for complying with procedures specified on a *Controlled Copy* of this SOP and shall never use non-controlled copies (which could be outdated).

5.0 REVISION OF THE SOP

5.1 Responsible Individual

The head QC technician is responsible for assuring that this SOP is current. If necessary, the head QC technician shall initiate the revision process.

5.2 Revision Schedule

This SOP shall be revised when its provisions no longer agree with current practices or GLFC policies, and shall be approved by the IPS manager.

6.0 CONTINGENCIES

When QCU personnel find circumstances that do not permit compliance with this SOP, the head QC technician shall be consulted.

7.0 CONFIDENTIALITY

IPS SOPs are not considered to be confidential documents and may be distributed to outside parties. *Controlled Copies* shall not be reproduced.

7.0 REFERENCES

NA

9.0 APPENDICES

Appendix 1: Abbreviated Instructions for Macerating Adults in Tissue Homogenizer

Appendix 1:

Abbreviated Instructions for
Macerating Adults in Tissue Homogenizer

1. **Add RO water to sample**; add a sufficient volume that will generate a viscous slurry after maceration; when the initial volume of adults is too large to accommodate the probe and required volume of water, the sample shall be transferred to a larger container.
2. **Macerate until no large pieces are visible**; use toothpick when required to dislodge large pieces from hole in probe or from the cutter head; add pieces to sample bottle and remacerate.
3. **Rinse probe with RO water** (collect rinsate in sample bottle).
4. **Conduct cursory cleaning of probe;** first by wiping with paper towel to remove the majority of insect parts and lipids, then using a toothpick to dislodge particles from the cutters, followed by additional rinsing with RO water and collection of the water in the waste beaker.
5. **Run probe in soapy water for 1 minute** (use one drop dish detergent and 250ml RO water in a 250ml cylinder); discard soapy water after use; cylinder may be reused for additional samples from the same cohort if it is sufficiently rinsed between samples (max. 5 samples).
6. **Rinse probe with RO water** (discard rinsate in waste beaker).
7. **Run probe in 50% bleach for 3 minutes** (use 125ml Javex® and 125ml RO water in a 250ml cylinder) ensuring that the solution enters the top hole on the probe shaft; Javex® solution and cylinder may be reused for additional samples from the same cohort (max. 5 samples).
8. **Rinse probe with RO water** (collect rinsate in waste beaker).
9. **Run probe in RO water for one minute** (use 250ml water in a 250ml cylinder); discard water after use; cylinder may be reused for additional samples from the same cohort if it is sufficiently rinsed between samples.
10. **Macerate next sample** or wrap probe in foil after air-drying.

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