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Great Lakes Forestry Centre
Insect Production Services

STANDARD OPERATING PROCEDURE

Number: IPS/007/004

Quality Control for Trichoplusia ni

Effective Date: 12 November 2014

Canada

TITLE: Quality Control for Trichoplusia ni (T. ni)

APPROVING OFFICIALS:

DD / MM / YY

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

SIGNIFICANT CHANGES FROM PREVIOUS VERSION:

-New requirement to complete a *T. ni* multi-generational summary.
-Numerous editorial changes were made.

1.0 INTRODUCTION

1.1 Purpose

This Standard Operating Procedure (SOP) has been established to ensure that procedures are followed for the receipt and processing of *T. ni* samples for the detection of pathogens and to reduce the incidence and spread of pathogens and microbial contaminants in the Great Lakes Forestry Centre (GLFC) insect production facility.

1.2 Scope

This SOP shall be followed by all Quality Control Unit (QCU) personnel for the microbial screening and process control of *T. ni*.

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.

Great Lakes Forestry Centre (GLFC) – One of five Canadian Forest Service (CFS) research facilities in Canada.

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

Insect Production Services (IPS) – A 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 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.

Quality Control (QC) Lab – An analytical laboratory under the control of IPS used by the QCU for monitoring production, process and product control for all IPU insect colonies, and for developing new QC methods and procedures.

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.

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 and disposable chemical protective gloves) shall be worn to perform staining operations.
1.4.2 Naphthalene Black staining procedure shall be conducted within a functioning chemical fume hood.
1.4.3 Personnel shall have access to, and be familiar with, the MSDS for all chemicals used for the staining procedures.

1.5 Materials

Materials required include:

1.5.1 Personal protective safety equipment:
- (a) lab coat
- (b) disposable chemical protective gloves
- (c) chemical fume hood

1.5.2 Chemicals:
- (a) Naphthalene Black 10B stain (CAS#1064-48-8)
- (b) Bromophenol Blue stain
- (c) glacial acetic acid
- (d) methanol (99%)
- (e) MSDS

1.5.3 Tools:
- (a) hot plate with boiling water

(b) slide warmer
(c) ice-water bath
(d) staining racks/dishes
(e) glass microscope slides
(f) microscope with oil immersion capability

1.5.4 Forms:
(a) Microbial Screening of *T. ni* Samples (IPS Form Number 0045/001, Appendix 1)
(b) QC Report for *T. ni* Adults (IPS Form Number 0053/001, Appendix 2)
(c) Rearing Summary for *T. ni* - Colony Maintenance (IPS Form Number 0090/001, Appendix 3)
(d) QC Report for *T. ni* Non-adult Samples (IPS Form Number 0070/001, Appendix 5)
(e) *T. ni* Multi-generation Summary (IPS Form Number 0149/001, Appendix 6)

2.0 PROCEDURES

2.1 Types of Samples and Documentation from the IPU

2.1.1 Adults:
(a) Upon dismantling of mating chambers by the IPU, female adults will have been collected by IPU personnel and maintained separately by chamber number in screw cap vials (labeled with the ID code and mating chamber number).

2.1.2 Larvae:
(a) Cups of larvae found to have one or more cadavers at the time of thinning or at pupa checks will have been removed from the rearing process, labeled with the ID code and supplied to the QCU for examination.
(b) When batches of larvae are found to have poor development or symptoms of disease, representative samples will be labeled with the ID code and supplied to the QCU for immediate examination.
(c) Routine screening of apparently healthy larvae will not be conducted at this time.

2.1.3 Historical Samples:
(a) The QCU will periodically (i.e., every 10 generations) request the IPU to collect larvae for the maintenance of a historical DNA record (in order to determine genetic drift).
(b) Samples of each generation of *T. ni* will have been labeled with the ID code and supplied to the QCU.

2.1.4 Tracking sheets:
(a) The IPU will have documented the rearing process of each generation of *T. ni* on the current version of IPS Form Number

0015 (*T. ni* Tracking and Distribution Form) and will copy the completed form to the QCU.

2.1.5 Unhatched eggs:
When there is evidence of poor hatch, unhatched eggs will have been placed in a labeled (ID code) bag and supplied to the QCU for examination.

2.2 Receipt of Samples from the IPU

Any sample received from the IPU shall be documented and tracked as specified in the current version of the SOP IPS/029 (Tracking QC Samples).

2.2.1 Adults: The QCU shall ensure that containers received are appropriately labeled and shall freeze samples immediately for microbial screening.

2.2.2 Larvae, pupae or unhatched eggs: The QCU shall ensure that samples received from the IPU are labeled with the ID code, collection date, and type of sample (i.e., dead at thinning, slow developers, suspected microbial infection, etc.) and shall freeze the samples immediately for subsequent microbial screening if deemed necessary by the QCU.

2.2.3 Historical samples: The QCU shall ensure that a larval sample (n=10) of each cohort is received periodically (i.e., every 10 generations) from the IPU and maintained at –70ºC for a historical DNA record.

2.3 Sample Preparation for Detection of Pathogens

2.3.1 Processing of vials of adults:
(a) Adults shall be maintained frozen in separate (labeled) containers until the time of processing.
(b) Adults from each mating chamber shall be macerated as described in the current version of SOP IPS/004 (Homogenizer).

2.3.2 Slide preparation of adults:
(a) Two preparations shall be made from each subsample for microscopic examination using different staining methods (i.e., Naphthalene Black and Bromophenol Blue).
(b) 5µl of each subsample shall be applied to each of two prelabeled glass slides (I.D. code, bag number, type of sample and staining method).
(c) Each 5µl subsample shall be spread over an area of approximately 1cm^2 using a new sterile pipette tip and shall be allowed to air dry before staining using one of the two methods; up to five samples can be applied to one slide.

2.3.3 Processing of samples of larvae or pupae:
(a) Samples of larvae, pupae or unhatched eggs shall be processed and examined for pathogens only if deemed necessary by the

QCU (i.e., when significant numbers of dead larvae are found at emergence, thinning or pupa checks).

(b) A representative number of larvae, pupae or unhatched eggs (to be determined by the IPU at the time of processing) from each group of samples shall be processed and examined in a timely manner (i.e., to reduce wasted effort in the rearing of contaminated batches). The number of insects examined shall be documented in the comments section on the screening form (IPS Form Number 0045/001, Appendix 1).

2.3.4 Slide preparation of samples of larvae or pupae:

(a) Two preparations shall be made from each insect smear for microscopic examination using two staining methods (i.e., Naphthalene Black and Bromophenol Blue).

(b) Each insect shall be smeared (using a new toothpick for each) onto a prelabeled glass slide (I.D. code, date of collection, type of sample and staining method) ensuring that sufficient midgut tissue is obtained; slides shall be allowed to air dry before staining.

2.4 Staining and Examination of Slides

2.4.1 Staining with Naphthalene Black:

(a) One of the two sets of slides shall be stained with Naphthalene Black 10B and examined for the presence of pathogens (i.e., viral occlusion bodies, microsporidia, other).

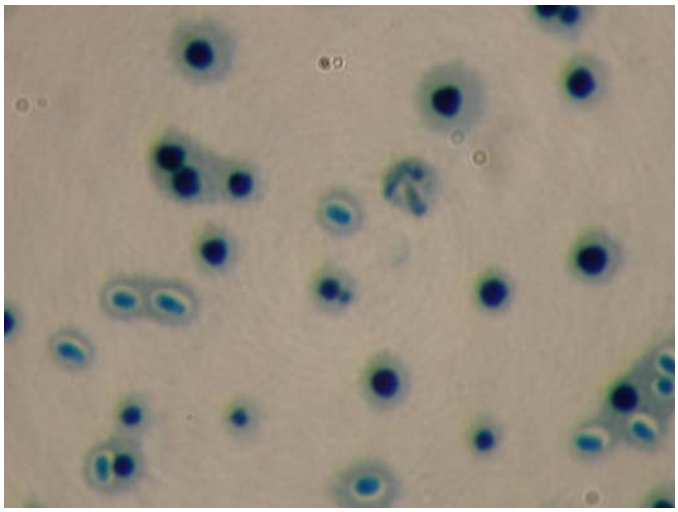
(b) The staining solution shall be prepared by dissolving 2.4g Naphthalene Black 10B in 130 ml distilled water and 70 ml glacial acetic acid (use a magnetic stirrer with gentle heat); replace the stain monthly or when the volume becomes depleted with use (i.e., slides are no longer covered); Naphthalene Black 12B may be substituted for 10B.

(c) Slides shall be immersed in the preheated stain (40-45ºC) for 10 minutes, removed and rinsed gently in tap water.

(d) Slides shall be air dried or dried on a slide warmer before examination.

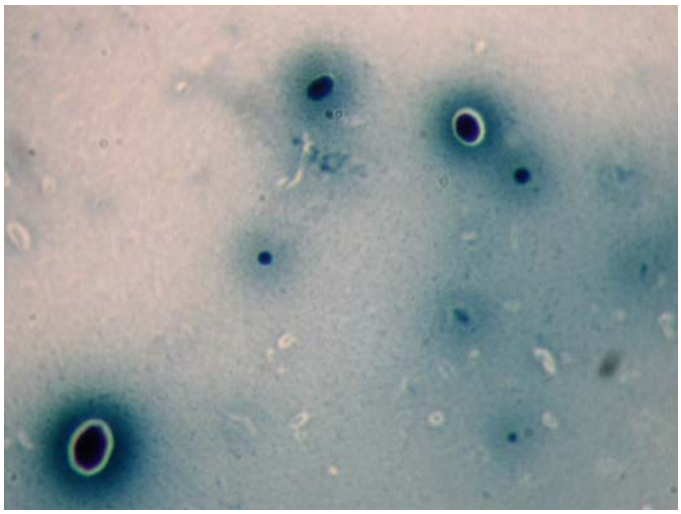
(e) A minimum of 20 fields of view of each sample shall be examined at 1000x magnification using oil immersion (bright field optics).

(f) Note that protein occlusion bodies (i.e., CPV and NPV) stain deep blue-black with a light blue background and cannot be differentiated using this stain (Bromophenol Blue staining will be used to distinguish between these two types of occlusions when detected using the Naphthalene Black stain); microsporidia stain a distinctive dark blue at one end and light blue at the other (no attempt will be made to distinguish between species of microsporidia):



CPV, NPV and microsporidia in 10B stain

EPV also stains a deep blue-black but can be differentiated from CPV and NPV by their oval shape and larger size:



EPV and CPV in 10B stain

(g) Observations and preliminary determination of microbial contamination shall be recorded on IPS Form Number 0045/001 (Microbial Screening of *T. ni* Samples, Appendix 1). Pathogens shall be quantified by class ranges defined as:

- high (+++) = more than 50 occlusions per field of view
- medium (++) = average of 5 to 50 occlusions per field of view
- low (+) = less than an average of 5 occlusions per field of view, but more than 3 in 20 fields

- trace (T) = total of 3 or fewer occlusions in 20 fields of view
- negative (-) = no pathogens observed in 20 fields of view

2.4.2 Staining with Bromophenol Blue:

(a) The second set of slides shall be stained with 0.1% Bromophenol Blue and shall be examined for the presence of CPV occlusion bodies.

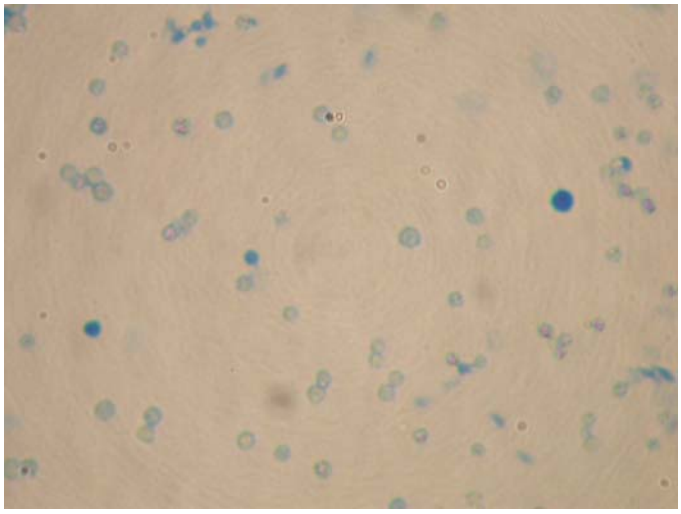
(b) The staining solution will be prepared by dissolving 0.2g Bromophenol Blue in 199.8ml distilled water and shall be stored in the dark; replace the stain monthly or when the volume becomes depleted with use (i.e., slides are no longer covered).

(c) Slides shall be fixed by immersion in 99% methanol for 90 sec., followed immediately by immersion in boiling water for 5 sec., ice-water (less than 5ºC) for 5 sec., 0.1% Bromophenol Blue stain for 15 min., then rinsed gently in tap water.

(d) Slides shall be air dried before examination.

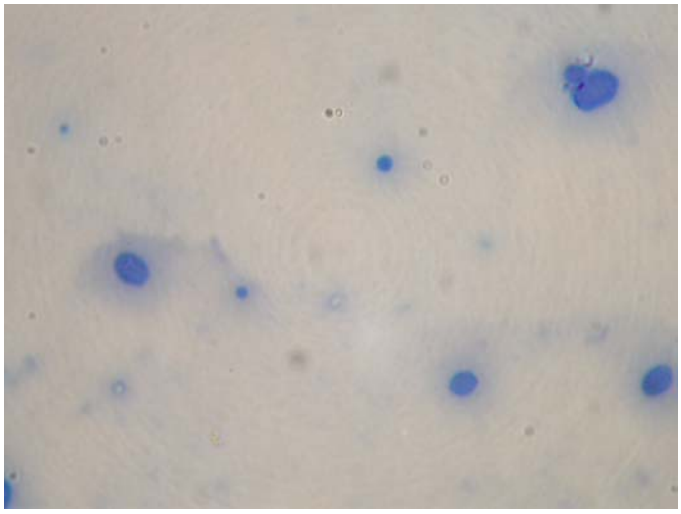
(e) A minimum of 20 fields of view of each sample shall be examined at 1000x magnification using oil immersion (bright field optics).

(f) Note that CPV stains a medium blue, whereas NPV, microsporidia and the background remain relatively unstained:



CPV, NPV and microsporidia in Bromophenol Blue stain

EPV stains medium to dark blue, is oval in shape and is considerably larger than CPV:



EPV and CPV in Bromophenol Blue stain

(g) Observations and preliminary determination of CPV contamination shall be recorded on IPS Form Number 0045/001 (Microbial Screening of *T. ni* Samples, Appendix 1). CPV contamination shall be quantified by class ranges defined as:

- high (+++) = more than 50 occlusions per field of view
- medium (++) = average of 5 to 50 occlusions per field of view
- low (+) = less than an average of 5 occlusions per field of view, but more than 3 in 20 fields
- trace (T) = total of 3 or fewer occlusions in 20 fields of view.
- negative (-) = no CPV occlusions observed in 20 fields of view

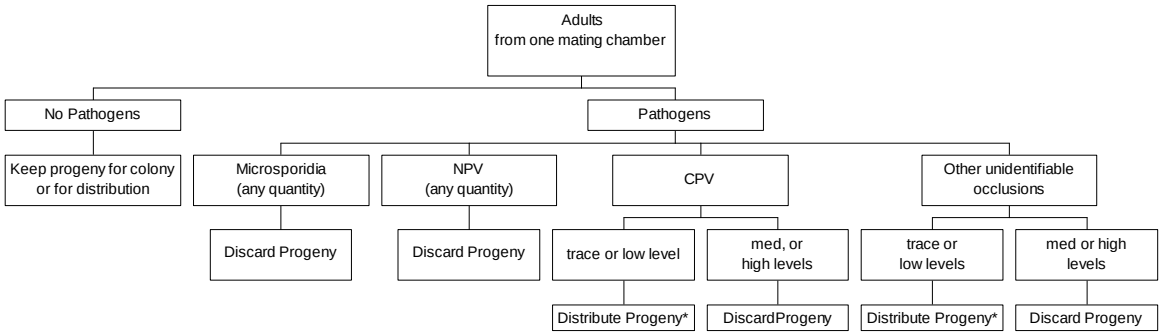
2.4.3 The QCU may solicit the assistance of other GLFC (or external) personnel in the determination of the presence of pathogens using whatever means are deemed necessary; results of screening by others shall be maintained with QC records for the applicable cohort.

2.4.4 Adults will not routinely be examined for the presence of NPV using Naphthalene Black, since adults contain numerous protein occlusions that stain similarly to NPV and cannot be distinguished; it is assumed that the presence of NPV in the insect population would cause high larval mortality and be easily recognized/detected at an earlier life stage.

2.4.5 The QCU shall retain all samples and derivative slides as specified in the current version of SOP IPS/029, Tracking of QC Samples.

2.5 Dissemination of Results to IPU

2.5.1 Upon examination of female adults obtained from mating chambers, the QCU shall use the following flow chart to determine instructions to be given to the IPU with regard to the fate of the progeny from each chamber:

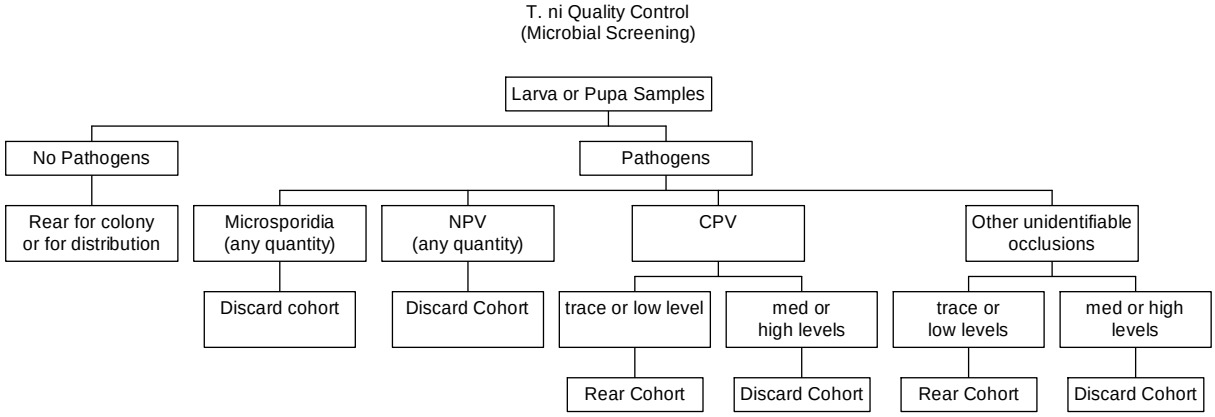


*Do not keep for colony unless there are insufficient numbers of insects from the other mating chambers.

2.5.2 Results of QC analysis of adults and a decision on of the fate of the progeny from each mating chamber shall be documented on IPS Form Number 0045/001 (Microbial Screening of *T. ni* Samples, Appendix 1) as well as on IPS Form 0053/001 (QC Report for *T. ni* Adults, Appendix 2).

2.5.3 IPS Form 0053/001 shall be sent electronically to the IPU and the dissemination of results documented as specified in the current version of SOP IPS/029 (Tracking QC Samples).

2.5.4 Upon examination of larva or pupa samples obtained during the rearing process, the QCU shall use the following flow chart to determine instructions to be given to the IPU with regard to the fate of the cohort:



2.5.5 Results of QC analysis of samples of larvae, pupae or unhatched eggs and a decision on of the fate of the cohort shall be documented on IPS Form Number 0045/001 (Microbial Screening of T. ni Samples; Appendix 1) as well as on IPS Form 0070/001 (QC Report for *T. ni* Non-adult Samples, Appendix 5).

2.5.6 IPS Form 0070/001 shall be sent electronically to the IPU and the dissemination of results documented as specified in the current version of SOP IPS/029 (Tracking QC Samples).

2.6 Statistical Process Control

2.6.1 Statistical Process Control monitoring shall be documented by the QCU as specified in Appendix 4 (Procedure for Maintaining *T. ni* Process Control Records) upon each receipt of a cohort tracking form from the IPU.

2.6.2 The QCU shall maintain IPS Form Number 0090/001 (Rearing Summary for *T. ni*, Appendix 3) which is part of the Procedure for Maintaining *T. ni* Process Control Records.

2.6.3 The QCU shall maintain a *T. ni* Multi-generation Summary (IPS Form Number 0149/001, Appendix 6).

2.7 Calculations

NA

2.8 Documentation and Reporting

2.8.1 Compliance to this SOP shall include the completion of the following forms:

(a) *T. ni* Tracking and Distribution Form (current version of IPS Form Number 0015).
(b) Microbial Screening of *T. ni* Samples (IPS Form Number 0045/001, Appendix 1).
(c) QC Report for *T. ni* Adults (IPS Form Number 0053/001, Appendix 2).
(d) Rearing Summary for *T. ni* (IPS Form Number 0090/001, Appendix 3).
(e) *T. ni* Multi-generation Summary (IPS Form Number 0148/001, Appendix 6).

2.8.2 Compliance to this SOP shall include completion of IPS Form Number 0070/001 only when non-adult samples are provided by the IPU.

2.8.3 The QCU shall maintain files of all forms identified above.

2.8.4 The QCU shall provide the IPU with electronic copies of QC Reports as specified in section 2.5.

2.8.5 The QCU shall make all records available to the Insect Rearing Advisory Group.

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 file of this SOP when it is replaced by a new version.
3.2.2 The QCU shall ensure that files of all documentation identified in section 2.8 are maintained for expedient retrieval.
3.2.3 The QCU shall retain all samples and derivative slides as specified in the current version of SOP IPS/029, Tracking QC Samples.

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 its use.
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.

8.0 REFERENCES

Current version of the following SOPs:
a) SOP IPS/004 (Homogenizer)
b) SOP IPS/029 (Tracking QC Samples)
Current version of the following form:
a) IPS Form Number 0015 (*T. ni* Tracking and Distribution Form)

9.0 APPENDICES

Appendix 1: IPS Form Number 0045/001 (Microbial Screening of *T. ni* Samples)
Appendix 2: IPS Form Number 0053/001 (QC Report for *T. ni* Adults)
Appendix 3: IPS Form Number 0090/001 (Rearing Summary for *T. ni*)
Appendix 4: Procedure for Maintaining *T. ni* Process Control Records
Appendix 5: IPS Form Number 0070/001 (QC Report for *T. ni* Non-adult Samples)
Appendix 6: IPS Form Number 0149/001 (*T. ni* Multi-generation Summary)

Appendix 1

Microbial Screening of *T. ni* Samples

ID Code:

Adult Screening

Cage No.	10B Stain			BPB Stain			Comments
	Date examined (DD/MM/YY)	Results		Date examined (DD/MM/YY)	Results		
		Micro-spordia	Other		CPV	Other	
	Conducted by:			Conducted by:			

Screening of Other Stages

Sample Date (DD/MM/YY)	Stage	Date Examined (DD/MM/YY)	CPV	Micro-sporidia	Other	Comments	Initials

IPS Form Number 0045/001

Appendix 2

QC Report for *T. ni* Adults

ID Code:

Report Date: DD / MM / YY

Cage No.	Microbial Contaminants (adult screening)			Instructions to IPU
	CPV	Microsporidia	Other	

Additional instructions for IPU:

Completed by:

IPS Form Number 0053/001

Appendix 3

Rearing Summary for *T. ni*
(Colony Maintenance)

ID code	total # of larvae at thin	total # of pupae	total # of adults	% loss thin to pupae	# of days egg set-up to first egg harvest	Comments

Sum
Targeted Mean
Targeted Limits
Actual Mean

IPS Form Number 0090/002

Appendix 4

12 November 2014

Procedure for Maintaining *T. ni* Process Control Records

Upon receipt of the *Tracking Sheet for T. ni* from the IPU:

1. Ensure that the form is complete, legible and is sequential to the last one that was received.
2. Stamp, date and initial the form.
3. Use a coloured marker to highlight data that is unusual or significant (e.g., larvae thinned late).
4. Calculate the following records and document results directly on the *Tracking Sheet for T. ni*:
   a) Total number of larvae at thinning
   b) Total number of pupae at pupae check
   c) Total number of adults at mating
   d) Development time in days (i.e., number of days from egg setup to the first egg harvest); Development time in days can be calculated using the following website: http://www.timeanddate.com/date/duration.html
5. Transcribe the following data onto the Excel *Rearing Summary for T. ni*:
   a) Total number of larvae at thinning (from *Tracking Sheet for T. ni*)
   b) Total number of pupae at pupae check (from *Tracking Sheet for T. ni*)
   c) Total number of adults at mating (from *Tracking Sheet for T. ni*)
   d) Development time in days (from *Tracking Sheet for T. ni*)
6. Enter comments onto the Excel *Rearing Summary for T. ni* for the current generation when production or process control abnormalities occur (e.g., rearing room breakdown).
7. The Excel *Rearing Summary for T. ni* spread sheet will automatically calculate % loss thin to pupation.
8. The Excel program will automatically mark data (with **red** text) that are outside of the targeted limits. Attempt to identify the source of the problem by discussion with IPU personnel, from notations on the tracking form, or by examination of production control records; document the source of the problem in the comments section of the rearing summary as well as on the tracking sheet for each affected cohort; take corrective action where possible to avoid future occurrences.
9. The Excel program will automatically record today's date on the spread sheet. Print the *Rearing Summary for T. ni* on a colour printer and maintain it in the file along with the *Tracking Sheet for T. ni* for the current generation.

Appendix 5

QC Report for
***T. ni* Non-adult Samples**

Report Date:
DD / MM / YY

Type of Sample:

Sample Description or ID:

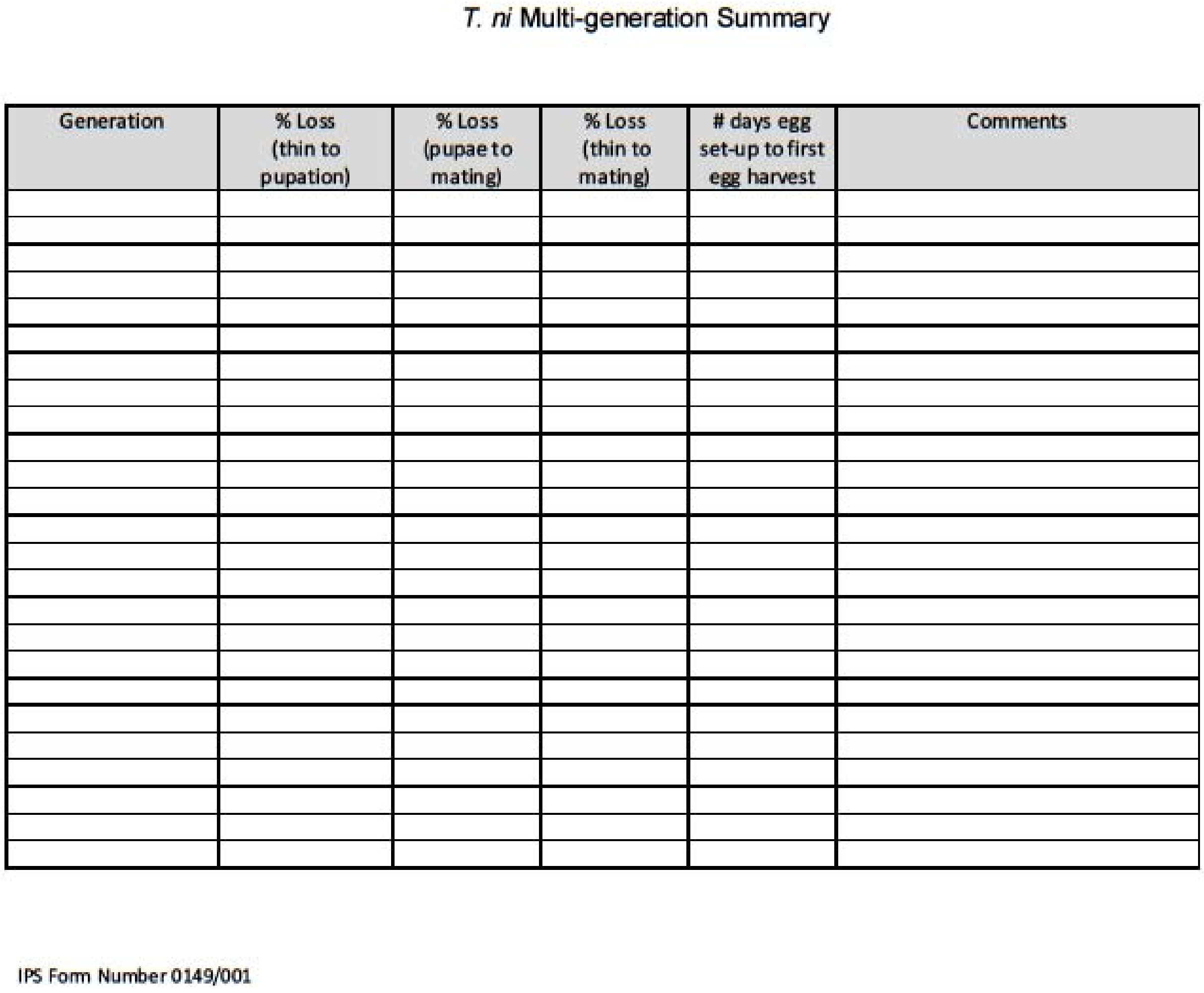
Diagnostic Results:

Instructions to IPU:

Completed by:

IPS Form Number 0070/001

Appendix 6



T. ni Multi-generation Summary

Generation	% Loss (thin to pupation)	% Loss (pupae to mating)	% Loss (thin to mating)	# days egg set-up to first egg harvest	Comments

IPS Form Number 0149/001

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