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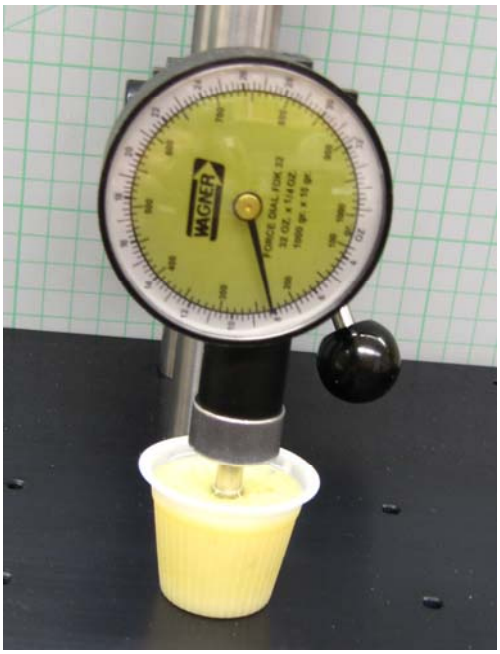
Great Lakes Forestry Centre
Insect Production Services

STANDARD OPERATING PROCEDURE

Number: IPS/011/006

Quality Control for Artificial Diets



Effective Date: 21 January 2015

Canada

TITLE: Quality Control for Artificial Diets

APPROVING OFFICIAL:

DD / MM / YY

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

SIGNIFICANT CHANGES FROM PREVIOUS VERSION:

-Targeted mean and upper/lower acceptable limits for the pH of regular diet has been revised.
-procedure for conducting iron analysis of ALB diet has been added.

1.0 INTRODUCTION

1.1 Purpose

This Standard Operating Procedure (SOP) has been established to assure the consistency of methodology used to test prepared artificial diet to ensure a high quality end product for the IPS and for distribution to clients.

1.2 Scope

This SOP shall be followed by all Quality Control (QC) Unit personnel for the testing of each batch of prepared artificial diet.

1.3 Definitions

Biological Safety Cabinet (BSC) – A class 2 containment cabinet designed for both worker and sample protection; room air is drawn into the front of the unit; the unit is designed in such a way that room air is HEPA filtered before blowing over the work area; air-borne hazardous particles coming off samples in the work area are pulled away from the worker and the air is vented back into the room after HEPA filtration; this type of unit is not suitable for worker protection from chemical fumes.

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 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.

Insectary – A multi-species rearing facility under the control of IPS used exclusively by the IPU for maintaining insect colonies and preparing artificial diets.

Quality Control Lab – An analytical laboratory under the control of IPS and 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 when handling and measuring all chemicals.
1.4.2 Agar plates containing microbial colonies shall only be opened within a class 2 BSC or chemical fume hood, and shall be autoclaved prior to disposal.
1.4.3 Ensure that the lid of the screw top bottle used for the preparation of agar is loose when heating on the stir plate, when microwaving or when autoclaving.
1.4.4 Personnel shall have access to, and be familiar with, the MSDS for all chemicals.

1.5 Materials

Materials required include:

1.5.1 Personal protective safety equipment:
- a) lab coat
- b) disposable chemical protective gloves
- c) BSC (class 2) or chemical fume hood

1.5.2 Chemicals:
- a) pH calibration buffers (4.0, 7.0)
- b) nutrient agar (Difco 213000)
- c) bleach working solution (refer to 2.9.1)

1.5.3 Tools:
- a) QC Sample Receipt Log
- b) pH meter equipped with a flat-bottomed probe
- c) gel strength tester (Wagner FDK Force dial)
- d) Petri dishes (10cm diameter; sterile, disposable)
- e) forceps
- f) balance
- g) dissection microscope
- h) pestels (micro-tube size)
- i) mechanical pipettors and sterile tips
- j) 46°C water bath
- k) diet QC kits

1.5.4 Forms:
- a) IPS Form Number 0097/005 (*Screening of Diet QC Samples,* Appendix 1)
- b) IPS Form Number 0092/005 (*Diet Screening-Regular with formaldehyde,* Appendix 2)
- c) IPS Form Number 0093/004 (*Diet Screening-Bell,* Appendix 3)
- d) IPS Form Number 0146/002 (*Diet Screening-MPB,* Appendix 4)
- e) IPS Form Number 0095/003 (*Diet Screening-Addy,* Appendix 5)
- f) IPS Form Number 0125/002 (*Diet Screening-ALB*, Appendix 6)
- g) IPS Form Number 0100/001 (*QC Report for Artificial Diet Samples,* Appendix 7)

2.0 PROCEDURES

2.1 Receipt of Diet Samples

2.1.1 Batches of diet produced by the IPU may have been divided into sub-batches having fewer ingredients, pending client requirements. Samples submitted to the QCU for analysis will be from the portion of the batch having the greatest number of ingredients (i.e., the QCU will not be requested to perform QC analysis for each sub-batch).

2.1.2 Three creamer cups and three pre-weighed micro-tubes containing diet from the first pour of each diet type and batch (or sub-batch) prepared will be provided by the IPU after every diet making session.

2.1.3 Upon receipt of diet samples, QCU personnel shall ensure that sample cups have been filled to the very top, micro-tubes have not been filled

above the 0.5ml mark, and that each diet QC kit has been clearly labeled with:

a) date of diet preparation
b) type of diet
c) batch size (full, half, or blender)
d) batch number (if applicable)

2.1.4 The IPU shall be immediately contacted if clarification is required on any of the sample labels or when sample cups have not been filled appropriately. Notations of explanation may be added by QCU personnel to the sample label.

2.1.5 Any sample received from the IPU shall immediately be logged into QC records (refer to the current version of SOP Number IPS/029, *Tracking QC Samples*).

2.1.6 Diet samples shall normally be processed immediately after receipt. If this is not possible, diet cups shall be placed in the QC lab refrigerator until processing can occur, ensuring that lids have been properly placed.

2.1.7 Diet samples shall be tested within 24 hours of receipt. When diet samples can not be processed immediately, applicable notations shall be made in the *Comments* section of IPS Form Number 0097/005 (*Screening of Diet QC Samples*, Appendix 2) and in the *Comments* section of the following forms, depending on the type of diet screened:

a) IPS Form Number 0092/005 (*Diet Screening-Regular with formaldehyde,* Appendix 2)
b) IPS Form Number 0093/004 (*Diet Screening-Bell,* Appendix 3)
c) IPS Form Number 0146/002 (*Diet Screening-MPB,* Appendix 4)
d) IPS Form Number 0095/003 (*Diet Screening-Addy,* Appendix 5)
e) IPS Form Number 0125/002 (*Diet Screening*-ALB, Appendix 6).

2.1.8 Samples of raw diet ingredients may be provided by the IPU to the QCU when problems are suspected. Protocols for testing will be determined in consultation with the IPS manager.

2.2 QC Tests to be Performed

2.2.1 Three samples from each batch (or sub-batch) of each diet type shall first be tested qualitatively (refer to 2.4), then quantitatively for gel strength (refer to 2.5), pH (refer to 2.6), and microbial load (refer to 2.7).

2.2.2 A *Diet Ingredient Tracking Sheet* will be provided to the QCU each time a new lot of a diet ingredient is received by the insectary. QCU personnel shall maintain a file of these records to assist when determining the cause of problematic batches of diet. Each time the QCU receives samples of ALB diet for analysis, the *Diet Ingredient Tracking Sheets* shall be reviewed to determine whether or not a new lot of wheat germ, casein or Wesson salt mix was used in the preparation. If so, an analysis of the diet for iron content must be performed as per the procedure in Appendix 8.

2.3 Equipment Preparation

2.3.1 The 46°C water bath shall be turned on at the beginning of the day when diet QC is to be performed.

2.3.2 A minimum of six diet QC kits (see photo below) shall be prepared and supplied to the IPU prior to each diet making session as follows:

a) Maintain a supply of 1.5ml micro-tubes that are weighed, labeled and autoclaved (with lids open) within a storage container that is sealed immediately after sterilization.

b) Once the micro-tubes have cooled, snap the lids of the tubes shut (within a class 2 BSC and while wearing gloves) and maintain them within another sealed storage container.

c) Transfer three of the labeled/sealed micro-tubes to each of the diet QC kits, close the lid of the kit and apply a piece of steam indicator tape, then autoclave.

d) Transfer the prepared kits directly from the autoclave to the IPU.

Diet QC Kit

2.4 Qualitative Analysis

2.4.1 The three cups of diet within each QC kit shall first be visually inspected and notations regarding diet texture, colour, odour etc. shall be entered on IPS Form Number 0097/005 (*Screening of Diet QC Samples*, Appendix 1) under *Qualitative analysis.* The individual making the observations shall initial the applicable section of the form.

2.5 Measuring Gel strength

2.5.1 The probe of the gel strength tester shall be wiped with bleach working solution (refer to 2.9.1) prior to use.

2.5.2 Place one of the three cups of diet from the diet QC kit on the flat surface beneath the gel strength tester. Ensure that the gauge of the instrument is set to "zero" before taking a measurement by pressing the button on the bottom left-hand corner of the dial.

2.5.3 Using the lever on the support stand of the device, slowly lower the probe of the tester onto a flat portion of the diet surface and continue to lower it until the diet surface is penetrated; return the lever to its starting position.

2.5.4 Determine the reading on the gauge of the device (using the scale for grams) and record the result on IPS Form Number 0097/005 *(Screening of Diet QC Samples,* Appendix 2*)* under *Gel Strength.*

Using the gel strength tester

2.5.5 Return the gauge of the instrument to the "zero" position by pressing the button on the bottom left-hand corner of the dial.

2.5.6 Clean the probe of the gel strength tester with the bleach working solution (refer to 2.9.1) between each diet type and/or batch.

2.5.7 Repeat steps 2.5.2 through 2.5.6 for the remaining two samples in the diet QC kit and for any other remaining diet QC kits to be tested.

2.5.8 The individual processing the sample shall initial the applicable portion of IPS Form Number 0097/005 *(Screening of Diet QC Samples,* Appendix 2*).*

2.6 Measuring pH

2.6.1 Calibrate the pH meter at the start of each session using two buffers with pH values (i.e., pH 4.0 and pH 7.0) bracketing the expected pH of the diet samples as follows:

a) Plug the pH meter into a power source, remove the flat-bottomed electrode from the storage buffer, rinse the electrode with distilled water and blot dry using a lint-free wipe.

b) Immerse the electrode in the pH 4.0 buffer solution and press the "Standardize" button.

c) Wait until the "S" icon on the screen stabilizes (i.e., stops flashing). The instrument recognizes the pH 4.0 buffer by showing the icon "4" on the lower left portion of the screen and provides a digital read-out of the actual pH reading that it made of the buffer.

d) Record the actual pH reading of the buffer on IPS Form Number 0097/005 (*Screening of Diet QC Samples,* Appendix 2*)*.

e) Remove the electrode from the pH 4.0 buffer, rinse with distilled water and blot dry using a lint-free wipe.

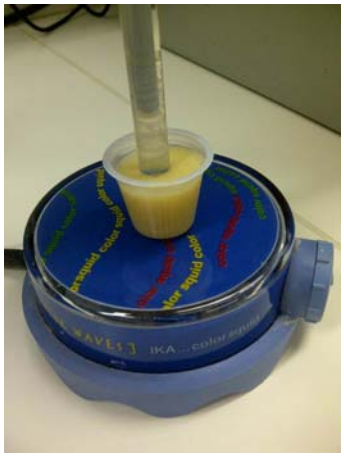
f) Repeat b) through e) using the pH 7.0 buffer.

g) Transcribe the actual pH readings obtained for the two buffers on all forms 0097/005 used during the QC session (i.e., the pH meter does not need to be re-calibrated between analysis of each batch of samples tested during the session)*.*

2.6.2 Measure the pH of each diet sample as follows:

a) Ensure that the pH meter is calibrated as per 2.6.1 and the electrode is rinsed with distilled water and blotted dry using a lint free wipe before measuring the pH of the diet sample.

b) Lower the electrode until it comes in contact with the diet surface. Wait until the read-out is stable (i.e., the "S" icon stops flashing) and record the reading in the applicable location on IPS Form Number 0097/005 (*Screening of Diet QC Samples,* Appendix 2).



Measuring pH

c) Rinse and blot the electrode, then proceed to measuring the pH of subsequent samples as per b) above.

2.7 Determining Microbial Load

2.7.1 Turn on the water bath and allow sufficient time for the temperature to stabilize at 46°C.

2.7.2 The quantity of nutrient agar to be prepared (i.e., one plate for each sample) is determined by the total number of diet QC samples received from the IPU after the diet making session. Prepare the required volume of agar as follows:

a) Add the applicable mass of nutrient agar to the designated volume of autoclaved RO water in a 1L screw top bottle:

# of plates	nutrient agar (g)	RO water (ml)
25-40	23.0	1000
13-24	13.8	600
1-12	6.9	300

b) Add a sterile stir-bar and mix gently using a magnetic stirrer/hot-plate on a low heat setting until the agar goes into suspension (i.e., avoid the addition of air bubbles).

c) Loosen the lid of the bottle and microwave for two-minute intervals with gentle agitation (i.e., swirl the liquid in the bottle to avoid the addition of air bubbles) between each interval until the agar goes into solution.

d) Place bottle of agar solution in an autoclavable pan/bin and autoclave for 15 minutes using the “liquid” cycle (ensure that the lid is loose).

e) Cool the agar to 46°C (to minimize subsequent condensation in the Petri dishes and to avoid damaging potential microbial contaminants in the sample) first by swirling the bottle in a stream of cold tap water for several minutes, then by immersion in a 46°C water bath for at least one hour.

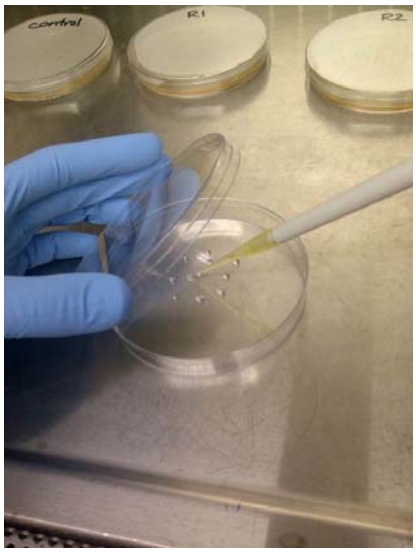
2.7.3 Prepare each sample as follows:

a) Ensure that the volume of diet in the 1.5ml micro-tube is less than 0.5ml. If a greater sample volume is present, remove some of the diet within a class 2 BSC using a sterile pointed spatula.

b) Determine the weight of diet sample (in grams) by weighing the micro-tube and subtracting the pre-weight value previously written on the tube. Enter this value into IPS Form Number 0097/005 (Screening of Diet QC Samples, Appendix 2) under *weight of diet sample*.

c) Within a class 2 BSC, add 1ml sterile RO water then macerate using a sterile pestle to make a uniform slurry.

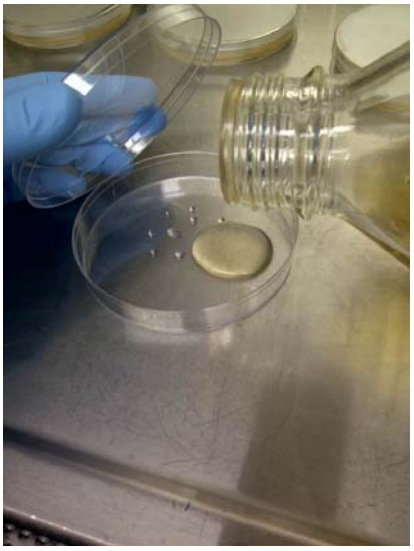
d) Within a class 2 BSC, partially lift the lid of a pre-labeled sterile 10cm diameter Petri dish (to minimize potential contamination from the air) and add 100ul of the sample to the middle of the dish using a mechanical pipettor. While adding the sample, distribute it over at least 10 smaller droplets to facilitate subsequent dispersion throughout the agar (refer to 2.7.4). Replace the lid on the Petri dish.



Addition of macerated sample to Petri dish

2.7.4 Add the nutrient agar from 2.7.2e to the Petri dish containing the sample as follows:

a) Within a class 2 BSC, partially lift the lid of the Petri dish and pour in just enough agar to cover the bottom surface.



Addition of agar to macerated sample

b) Replace the lid and allow the dish to cool completely before handling (i.e., do not swirl the sample as this causes agar to splash up the side of the dish, making analysis more difficult).
c) Tape the Petri dish shut with at least two pieces of masking tape and maintain it upside down in a dark incubator at 28°C (keeping the Petri dish upside down minimizes the growth of secondary fungal spores that become dislodged from hyphae and break the surface of the agar).

2.7.5 Analyze the plates as follows:

a) Microbial colonies shall be quantified on each plate over a number of sampling times. This makes analysis easier since early-forming colonies become difficult to quantify as they become large and coalesce. The cumulative number of microbial colonies present on each plate shall be counted 1-, 2-, 5- and 6-days post set-up (assuming set-up on a Wednesday) using a dissection microscope with approximately 10x magnification. Sample times may be adjusted to facilitate holidays, periods of leave, or set-up days other than Wednesdays).
b) Each colony counted shall be marked by a dot on the surface of the Petri dish using a permanent marker.
c) Results shall be documented on IPS Form Number 0097/005 (*Screening of Diet QC Samples,* Appendix 2) under *Colony Counts* for the respective day and sample number.
d) After the 6^{th} day post-setup, the cumulative number of colony-forming units per gram of wet diet (cfu/g) shall be calculated for each sample using the formula below, and shall be recorded on the data sheet.

cfu/g = (# colonies on day 6) ÷ (wet weight of diet sample in grams)

e) The mean number of cfu/g shall then be calculated from the three samples comprising the batch of diet and shall be recorded on IPS Form Number 0097/005 (*Screening of Diet QC Samples,* Appendix 2).

2.8 Dissemination of Results to IPU

2.8.1 Results of diet sample analysis (pH, gel strength, and microbial load) shall be entered onto the corresponding form:

a) IPS Form Number 0092/005 (*Diet Screening-Regular with formaldehyde,* Appendix 2)
b) IPS Form Number 0093/004 (*Diet Screening-Bell*, Appendix 3)

c) IPS Form Number 0146/002 (*Diet Screening-MPB*, Appendix 4)
d) IPS Form Number 0095/003 (*Diet Screening-Addy*, Appendix 5)
e) IPS Form Number 0125/002 (*Diet Screening-ALB*, Appendix 6)

When values fall outside of the targeted upper and lower tolerance limits, these data are to be highlighted in red. Applicable comments shall be entered into the *comments* section.

2.8.2 The IPU shall be notified immediately via email when any tolerance limit has been exceeded by a considerable amount. QC personnel decide whether to have the IPU discard the entire batch of diet or to flag any associated tracking sheets in which the diet is used. The printed email shall be attached by QCU personnel to the QC report and a notation to this made in the *comments* section of the QC report. When tolerances are not exceeded, or exceeded by a minimal amount, the IPU will be notified as per 2.8.3.

2.8.3 A QC report (i.e., IPS Form Number 0100/001, *QC Report for Artificial Diet Samples,* Appendix 9) shall be sent to the IPU for each diet batch after all results have been entered into the required diet screening summaries.

2.9 Process Control Charting

2.9.1 Data for pH, gel strength and microbial screening from the *Diet Screening* form (i.e., current version of IPS Form Number 0092, 0093, 0095, 00125 or 00146) shall be transcribed onto the applicable table for *Process Control Charts* on the QC&MD Network Drive. Microsoft Excel will automatically generate/update process control charts for pH, gel strength, and microbial screening for each batch of each diet type.

2.9.2 Print the generated *Process Control Charts* on a colour printer and maintain them in a file along with the current version of IPS Form Number 0097 (*Screening of diet QC Samples*) and with the associated *Diet Screening* form.

2.9.3 Examine each *Process Control Chart* for data indicating that there is a problem with a process:

(a) data outside of the established upper or lower tolerance limits (i.e., more than two standard deviations from the mean).
(b) nine (or more) points in row are on the same side of the mean.
(c) six (or more) points in a row are continually increasing or decreasing.
(d) 14 (or more) points alternate in direction.
(e) four (or five) out of five points in row are more than one standard deviation from the mean in the same direction.
(f) eight points in a row exist with none within one standard deviation of the mean and the points are in both directions from the mean.
(g) line of regression for 9 (or more) points has a significant upward or downward slope.

2.9.4 Whenever tolerance limits are exceeded, as per (a) above, the Head QC Technician shall instruct IPU personnel to either discard the entire batch of diet, or to flag *Tracking Sheets* associated with that particular batch of diet. Decisions shall be documented on the applicable *QC Report for Artificial Diet Samples*.

2.9.5 Whenever processes are out of control or trending to go out of control, as per (b) through (g) above, the Head QC Technician shall meet with IPU Supervisor to determine cause(s) and to implement corrective action.

2.10 Calculations

2.10.1 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]

2.11 Documentation and Reporting

2.11.1 Compliance of this SOP shall include completion of the following forms:

a) IPS Form Number 0097/005 (*Screening of Diet QC Samples,* Appendix 1)
b) IPS Form Number 0092/005 (*Diet Screening-Regular with formaldehyde,* Appendix 2)
c) IPS Form Number 0093/004 (*Diet Screening-Bell,* Appendix 3)
d) IPS Form Number 0146/002 (*Diet Screening-MPB,* Appendix 4)
e) IPS Form Number 0095/003 (*Diet Screening-Addy,* Appendix 5)
f) IPS Form Number 0125/002 (*Diet Screening-ALB*, Appendix 6)
g) IPS Form Number 0100/001 (*QC Report for Artificial Diet Samples,* Appendix 7)

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 all forms specified in 2.10.1 are maintained for expedient retrieval.
3.2.3 Diet samples shall be discarded upon completion of processing and analysis.
3.2.4 Tracking forms shall be maintained in a *QC Sample Receipt Log*.
3.2.5 QCU personnel shall transfer the contents of the *QC Sample Receipt Log* to a historical file on an annual basis.

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 those persons have been appropriately trained in the use of this SOP.
4.1.3 IPU 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 SOP Number IPS/029 (*Tracking QC Samples*)

9.0 APPENDICES

Appendix 1: IPS Form Number 0097/005 (*Screening of Diet QC Samples*)
Appendix 2: IPS Form Number 0092/005 (*Diet Screening-Regular with formaldehyde*)
Appendix 3: IPS Form Number 0093/004 (*Diet Screening-Bell*)
Appendix 4: IPS Form Number 0146/002 (*Diet Screening- MPB*)
Appendix 5: IPS Form Number 0095/003 (*Diet Screening-Addy*)
Appendix 6: IPS Form Number 0125/002 (*Diet Screening-ALB*)
Appendix 7: IPS Form Number 0100/001 (*QC Report for Artificial Diet Samples*)
Appendix 8: Iron Analysis of ALB Artificial Diet

Appendix 1

Screening of Diet QC Samples

ID Code:

Qualitative and Quantitative Assessment

Sample Number	Date Examined (DD/MM/YY)	Qualitative analysis	Gel Strength (g)	pH	Comments
1					
2					
3					
Initials					
		Mean ($\bar{x}$)			

↓

pH meter calibration:
pH 4.0=
pH 7.0=

Microbial Screening

Sample Number	Date Samples plated (DD/MM/YY)	Weight of diet sample (g)	Nutrient agar Colony counts				cfu/g of wet diet	Comments
			Day 1	Day 2	Day 5	Day 6		
1								
2								
3								
Initials								
					Mean ($\bar{x}$)			

IPS Form Number 0097/005

Appendix 2

Diet Screening

Regular, with formaldehyde, first pour

Diet ID	pH	Gel strength (g)	Microbial counts (cfu/g of wet diet)	Comments

	pH	Gel strength (g)	Microbial counts
Targeted Mean	5.47	283.43	257.11
Targeted Lower Limit	5.15	187.03	0
Targeted Upper Limit	5.79	379.83	1807.05
Actual Mean			

IPS Form Number 0092/005

Appendix 3

Diet Screening
Bell

Diet ID	pH	Gel strength (g)	Microbial counts (cfu/g of wet diet)	Comments

	pH	Gel strength (g)	Microbial counts (cfu/g of wet diet)
Targeted Mean	5.23	181.51	123.57
Targeted Lower Limit	5.09	117.81	0
Targeted Upper Limit	5.37	245.21	670.47
Actual Mean			

IPS Form Number 0093/004

Appendix 4

Diet Screening
MPB

Diet ID	pH	Gel strength (g)	Microbial counts (cfu/g of wet diet)	Comments

Targeted Mean	5.00	582.53	27505.00	
Targeted Lower Limit	4.76	370.87	0.00	
Targeted Upper Limit	5.24	794.19	3025.18	
Actual Mean				

IPS Form Number 0146/002

Appendix 5

Diet Screening
Addy

Diet ID	pH	Gel strength (g)	Microbial counts (cfu/g of wet diet)	Comments

Targeted Mean	5.10	225.04	61.97
Targeted Lower Limit	4.82	119.45	0.00
Targeted Upper Limit	5.38	377.27	342.25
Actual Mean			

IPS Form Number 0095/003

Appendix 6

Diet Screening
ALB

Diet ID	pH	Gel strength (g)	Microbial counts (cfu/g of wet diet)	Comments

Targeted Mean	4.99	564.8	106.12
Targeted Lower Limit	4.89	377.88	0
Targeted Upper Limit	5.23	751.72	527.78
Actual Mean			

IPS Form Number 0125/002

Appendix 7

QC Report for Artificial Diet Samples

Date Examined:
DD / MM / YY

Date of Diet Preparation:
DD / MM / YY

Diet Type:

Diagnostic Results:

Comments:

Completed by:

IPS Form Number 0100/001

Appendix 8

21 January 2015

Iron Analysis of ALB Artificial Diet

Background

- Too much iron in the diet can be toxic to ALB larvae.
- The diet ingredient *Wesson salt mixture* has been purchased without $FeSO_4$ to minimize the amount of iron in the diet.
- However, iron is present in other diet ingredients such as *wheat germ* and *casein* and there may still be trace amounts of iron in the *Wesson salt mix*.
- An iron analysis test will be performed on batches of ALB diet whenever a new lot of any one of the above diet ingredients (i.e., *wheat germ, casein*, or *Wesson salt mix*) is first used.
- Results will conclude whether iron levels are below the established acceptable threshold. Batches exceeding the threshold will be discarded. Any insects already on that diet will immediately be transferred to a new batch diet.

Solutions Required

1) 0.2 M Citrate (1L)
   - Dissolve 38.42g citric acid (anhydrous) in 900ml autoclaved RO H_20
   - Add 1.0g methyl paraben (0.1%) (added as a preservative) and dissolve with heat
   - Adjust to pH 4.0 by adding ammonium hydroxide (50% $NH_4OH{\cdot}HCl$) (~44.75g)
   - Bring final volume to 1000ml

2) Hydroxylamine (10% NH_2OH)
   - Dissolve 50g of anhydrous hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) in autoclaved RO H_20
   - Bring final volume to 500ml

3) 0.25% 1,10-Phenanthroline
   - Dissolve 1.0g of 1,10-phenanthroline monohydrate in ~100ml autoclaved RO H_20
   - Heat and stir solution until dissolved
   - Bring final volume to 400ml

4) 2% Sodium Acetate (NaAc)
   - Dissolve 20g NaAc and 1.0g methyl paraben in ~900ml autoclaved RO H_20
   - Heat and stir until dissolved
   - Bring final volume to 1000ml

5) Working Solution
   - Prepare a "working solution" by combining the following:

     2.5ml of 10% hydroxylamine (NH_2OH)
     2.5ml of 0.25% 1,10-phenanthroline
     5ml of 2% sodium acetate
     10ml total

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6) Calibration Standards
   - Prepare 3 replicates of 7 calibration standards (0, 2, 4, 6, 8, 10, and 12ppm) by combining a commercially supplied iron standard solution (1000µg/ml) with 0.2M citrate as per the following table. Add 100µl of each preparation to the designated wells of a 96-well place as specified in "Plate Setup" below.

Calibration Standard		Volume of Iron Standard (µl)	Volume of 0.2M Citrate (µl)
Standard #	Concentration of Iron		
1	0 µg/ml (0 ppm)	0	1000
2	2 µg/ml (2 ppm)	2	998
3	4 µg/ml (4 ppm)	4	996
4	6 µg/ml (6 ppm)	6	994
5	8 µg/ml (8 ppm)	8	992
6	10 µg/ml (10 ppm)	10	990
7	12 µg/ml (12 ppm)	12	988

Diet Sample Preparation

1) Ensure that three cups are obtained from the batch of ALB diet for iron analysis; three replicates will be derived from each cup for a total of nine samples.
2) Place 0.4±0.02g of each sample in separate, tared 15ml screw-top centrifuge tubes; label accordingly; record the net weight of each sample; use a plastic knife when collecting the sample, since metal may leave traces of iron.
3) Add 5ml of 0.2M citrate (pH 4.0) to each sample and vortex until completely macerated.
4) Place samples in a 75°C water bath for approximately 15 minutes (time can vary from 10-30 minutes); shake samples every 3-4 minutes.
5) Remove from heat and refrigerate for at least 30 minutes; samples may be left for up to two days in the refrigerator.
6) Centrifuge at 2400g for 10 minutes at room temperature.
7) Remove 100ul of the supernatant (clear citrate extract) from each sample and add to the applicable wells of a 96-well plate (as specified in "Plate Setup" below); each of the nine samples will be loaded three times on the 96-well plate.

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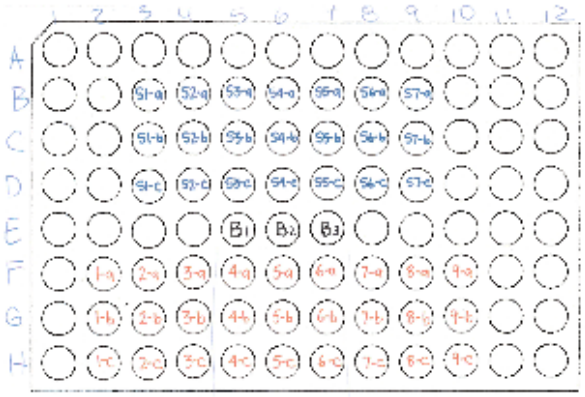
Plate Setup



Location of Calibration Standards in 96-well plate:

µg/ml	Description		ID #	Well #	Absorbance @ 510nm
0	Standard #1	Rep 1	S1-a	B3	
		Rep 2	S1-b	C3	
		Rep 3	S1-c	D3	
2	Standard #2	Rep 1	S2-a	B4	
		Rep 2	S2-b	C4	
		Rep 3	S2-c	D4	
4	Standard #3	Rep 1	S3-a	B5	
		Rep 2	S3-b	C5	
		Rep 3	S3-c	D5	
6	Standard #4	Rep 1	S4-a	B6	
		Rep 2	S4-b	C6	
		Rep 3	S4-c	D6	
8	Standard #5	Rep 1	S5-a	B7	
		Rep 2	S5-b	C7	
		Rep 3	S5-c	D7	
10	Standard #6	Rep 1	S6-a	B8	
		Rep 2	S6-b	C8	
		Rep 3	S6-c	D8	
12	Standard #7	Rep 1	S7-a	B9	
		Rep 2	S7-b	C9	
		Rep 3	S7-c	D9	

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Location of diet samples in 96-well plate:

Diet Cup #	Replicate Sample #	Weight (g)	ID#	Well #	Absorbance
1	1		1-a	F2	
			2-a	F3	
			3-a	F4	
	2		1-b	G2	
			2-b	G3	
			3-b	G4	
	3		1-c	H2	
			2-c	H3	
			3-c	H4	
2	1		4-a	F5	
			5-a	F6	
			6-a	F7	
	2		4-b	G5	
			5-b	G6	
			6-b	G7	
	3		4-c	H5	
			5-c	H6	
			6-c	H7	
3	1		7-a	F8	
			8-a	F9	
			9-a	F10	
	2		7-b	G8	
			8-b	G9	
			9-b	G10	
	3		7-c	H8	
			8-c	H9	
			9-c	H10	

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Colorimetry and Data Analysis

1) Add 200µl of the "working solution" to each well containing a diet sample or a standard; add 300µl of the "working solution" to the wells designated as blanks (i.e., B1, B2 and B3, located in wells E5, E6 and E7, respectively)
2) Wait at least 5 minutes before determining absorbance using a spectrophotometer (absorbance remains constant for at least 2.5 hours).
3) Follow the user's manual for the spectrophotometer used and measure absorbance at 510nm (492nm is acceptable if the spectrophotometer is not capable of 510nm). A standard curve will be generated using the calibration standards; diet sample preparations will be analyzed against this curve.
4) Record the absorbance reading for each diet sample preparation.
5) Determine the amount of amorphous iron in each sample by:

$$FePO4 = 216 \times \frac{\text{absorbance reading}}{\text{weight of diet sample (g)}}$$

6) The batch of ALB diet will be deemed to be acceptable for use when the average FePO4 content is less than or equal to 80 mg/L.
7) Prepare a QC Report for Artificial Diet Samples and submit to the IPU.

Sample Calculations:

Diet ID	Date	Weight of Diet Sample (g) A	Absorbance (492nm) B	FePO4 (mg/L) C	Average FePO4 (mg/L) D	Use this diet? (yes/no) E
ALB (Half)	16Nov2011	0.3983	0.151	81.89	68.55	Yes
		0.4021	0.099	53.18		
		0.3920	0.145	79.90		
		0.4057	0.121	64.42		
		0.4041	0.100	53.45		
		0.4046	0.123	65.66		
		0.4084	0.110	58.18		
		0.4101	0.142	74.79		
		0.4168	0.165	85.51		

C = 216 x B/A; D = average of C values; Discard when D>80

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