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Great Lakes Forestry Centre
Insect Production Services

STANDARD OPERATING PROCEDURE

Number: IPS/010/003

Preparation of Artificial Diets

Effective Date: 9 February 2015

Canada

TITLE: Preparation of Artificial Diets

APPROVING OFFICIAL:

DD / MM / YY

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

SIGNIFICANT CHANGES FROM PREVIOUS VERSION:

-*Diet Ingredient Tracking Sheet* has been revised.
-*Diet Checklists* have been revised.
-method for making vitamin solution has been revised.

1.0 INTRODUCTION

1.1 Purpose

This Standard Operating Procedure (SOP) has been established to assure that artificial diets are prepared in a consistent manner, to reduce the incidence of microbial contaminants, to facilitate tracking of diet ingredients, and to track the distribution of prepared diet to end users.

1.2 Scope

This SOP shall be followed by all IPU personnel for the preparation and distribution of artificial diets.

1.3 Definitions

ALB – Asian long-horned beetle, *Anoplophora glabripennis*.

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.

EAB – Emerald ash borer, *Agrilus planipennis*.

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

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 (IPS) Manager – The individual who has overall responsibility for activities of the IPS team.

Insect Production Supervisor – A member of IPS having supervisory authority over the daily operation of the insectary.

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

Methods Development (MD) Lab – A research facility under the control of IPS used exclusively by the IPU for developing new rearing methods and for establishing new insect colonies.

NA – Not Applicable.

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.

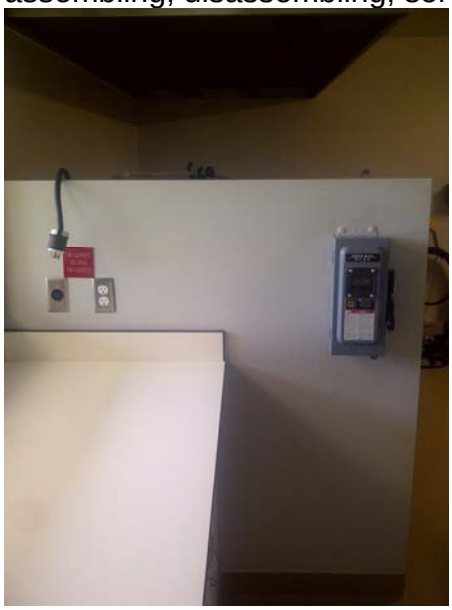
Standard Operating Procedures (SOPs) – Directives describing routine administrative or technical procedures conducted by IPS personnel or users of the IQ facility.

1.4 Safety

1.4.1 A minimum of two persons shall be present when operating the diet-making equipment.

1.4.2 Ensure that the power is turned off at the circuit breaker before assembling, disassembling, servicing or cleaning the Hobart blender or Cleveland steam kettle.

1.4.3 The Groen cooker/mixer is powered from two sources. Ensure that power is disconnected at both the wall receptacle and the circuit breaker before assembling, disassembling, servicing or cleaning.



1.4.4 A freshly laundered lab coat and disposable chemical protective polymer gloves shall be worn to perform weighing operations.

1.4.5 Weighing operations shall be conducted within the chemical fume hood with the exhaust fan turned on.

1.4.6 The ceiling-mounted exhaust hood shall be turned on prior to mixing any diet ingredients in the kettle or mixer.

1.4.7 A lab coat, rubber apron, disposable chemical protective polymer gloves and face shield shall be worn when adding diet ingredients to the kettle or mixer; once all ingredients have been combined in the mixer the face shield may be removed.

1.4.8 Rubber boots and heat resistant gauntlet style gloves shall be worn in addition to those items specified in 1.4.7 when pouring hot agar.

1.4.9 Personnel shall have access to, and be familiar with, the MSDS for all chemicals and diet ingredients used in the rearing facility.

1.5 Materials

1.5.1 Personal protective safety equipment:
- (a) lab coat dedicated to diet-making activities
- (b) plastic/rubber apron
- (c) disposable chemical protective polymer gloves
- (d) face shield
- (e) rubber boots
- (f) heat resistant gauntlet style gloves

1.5.2 Weighing and packaging materials and equipment:
- (a) top-loading balance
- (b) vacuum sealer
- (c) vacuum bags
- (d) screw top bottles (polyethylene, autoclavable)
- (e) chemical fume hood
- (f) weigh boats
- (g) scoops/spoons
- (h) measuring cylinders and beakers
- (i) labels

1.5.3 Storage materials for diet ingredients:
- (a) opaque totes for light sensitive ingredients
- (b) translucent totes for other ingredients
- (c) adhesive labels

1.5.4 Mixing equipment:
- (a) Cleveland steam kettle
- (b) Hobart mixer
- (c) Groen mixer/kettle
- (d) microwave
- (e) stainless steel pail
- (f) stainless steel funnel
- (g) thermometer
- (h) rubber spatulas
- (i) pyrex pitchers

1.5.5 Other equipment:
- (a) vacuum sealer
- (b) tortilla press

1.5.6 Cleaning supplies and equipment:
- (a) water hose
- (b) 6% Javex® solution
- (c) Windex®
- (d) scrub brushes
- (e) sterile paper towels
- (f) Diversol® detergent
- (g) Tide detergent

1.5.7 Dispensing materials:
- (a) stainless steel pitchers
- (b) plastic pitchers
- (c) perforated aluminum trays
- (d) disposable cardboard trays
- (e) labeling materials
- (f) plastic bags; ties

1.5.8 MSDS:
- (a) Javex® (or other sodium hypochlorite based substitutes)
- (b) Diversol®
- (c) Windex®
- (d) MSDS for all diet ingredients listed in Appendix 2.

1.5.9 *Diet Forms* binder including the following:
- (a) IPS Form Number 0071/007 *(Diet Ingredient Tracking Sheet)*
- (b) IPS Form Number 0016/004 (*Regular Diet Checklist*)
- (c) IPS Form Number 0017/004 (*Bell Diet Checklist*)
- (d) IPS Form Number 0144/003 (*Addy Diet Checklist*)
- (e) IPS Form Number 0145/003 (*ALB Diet Checklist*)
- (f) IPS Form Number 0163/002 (*EAB Diet Checklist*)
- (g) IPS Form Number 0164/001 (*Diet Orders*)
- (h) printed copies of diet requisitions from the on-line ordering system
- (i) spreadsheets for calculations of ingredients omitted from part of a batch of diet

1.5.10 Diet clipboard with current diet requisitions.

1.5.11 Equipment log books for:
- (a) vacuum sealer
- (b) Cleveland steam kettle
- (c) Hobart mixer
- (d) Groen mixer/kettle

1.5.11 Excel Spreadsheets:
- (a) Regular Special Order (attached to IPS Form Number 0016/004).

(b) Bell Special Order (attached to IPS Form Number 0017/004).
(c) *Diet Ingredient Inventory*

1.5.12 Diet ingredients (refer to Appendices 5-9)

2.0 PROCEDURES

2.1 Maintaining Inventories of Supplies and Ingredients

2.1.1 An inventory of non-perishable supplies (e.g., cups, trays, etc.) that are maintained in IPS storage rooms shall be updated as specified in the current version of SOP Number IPS/009 (*IPU Personnel Responsibilities*).

2.1.2 An inventory of diet ingredients (i.e., excel spreadsheet for *Diet Ingredient Inventory*) shall be updated at least monthly as follows:

(a) The binder labeled as *Diet Forms* shall be consulted to determine the number of batches made (including batch size and type of diet) during the previous month. This data shall be entered onto the spreadsheet page labeled 'Production'.

(b) The spreadsheet will automatically calculate and update the inventory for each diet ingredient in the 'Report' sheet. The user can input any number for "# months notice" (at the top of the 'Report' sheet) and determine whether or not sufficient quantities are currently in stock. When quantities are low the word "CHECK" will automatically appear on the spreadsheet next to the name of the ingredient. If an ingredient is about to expire, the word "EXPIRED" will automatically appear. Personnel shall then confirm the actual amount/expiry of material left in storage and inform the IPU supervisor. Expired ingredients shall be discarded and the spreadsheet updated.

(c) Each time the sheet for 'Production' is updated, the spreadsheet also automatically updates the individual pages for each diet ingredient. These individual pages shall be consulted when determining the number of pre-weighed packages to prepare of each batch size (i.e., 28.9, 16.8, 16.4, 14.4 8.4, 8.2, 3.7 or 3.6L) when new ingredients are received.

2.1.3 The *Diet Ingredient Inventory* shall be updated at least annually by counting the actual number of packages of each ingredient in every storage bin, since packages may have been removed for various reasons without sufficient documentation (e.g., for making custom orders or for methods development activities).

2.1.4 Each time new ingredients are received, the amount, lot #, date received and expiry date are inputted into the sheet labelled 'Inventory'. The ingredients are then packaged in aliquots (as specified in section 2.5). The spreadsheet will automatically update the *Amount Remaining* for each ingredient in the 'Report' sheet.

2.2 Ordering Ingredients and Supplies

2.2.1 Diet ingredients and supplies shall be bulk-purchased where possible to reduce costs but shall be ordered in sufficient quantities so that they are used up prior to their expiry date (refer to Appendix 1); expired materials shall not be used.

2.2.2 Whenever personnel notice that stocks of diet ingredients are low (as specified in 2.1.2), they shall immediately notify the IPU supervisor. Similarly, the IPU supervisor shall be notified when stocks of other diet-making supplies (e.g., cups, trays, etc.) are low. The supervisor shall check the monthly automated diet ingredient inventory report provided by IPU personnel and order ingredients that are low in stock or about to expire. Refer to the current version of SOP Number IPS/009 (IPU Personnel Responsibilities) regarding requirements for maintaining inventories of these other supplies.

2.2.3 Diet ingredients shall be ordered from the suppliers identified in Appendix 2. When ingredients are no longer available from the listed supplier, a new source will need to be located and the IPU supervisor will need to be contacted to determine testing requirements for the new material.

2.3 Access to Diet Kitchen

2.3.1 Access to the diet kitchen is restricted to IPS and facility maintenance personnel. Access procedures specified in the current version of SOP Number IPS/009 shall be followed.

2.3.2 Access to the diet kitchen shall be minimized to reduce the entry of microbials; the cold room for storage of prepared artificial diets (i.e., room AA115) shall normally be accessed via the hallway for retrieval of diet but may be entered via the diet kitchen for entry of diet.

2.3.3 QCU personnel may enter as required for the conduct of quality control testing.

2.3.4 Opening of kitchen doors to the hallway and to the prepared diets cold room shall be kept to a minimum, particularly during diet-making sessions.

2.4 Receipt and Storage of Diet Ingredients

2.4.1 Incoming diet ingredients shall be brought to the distribution window where the external packaging shall be removed (where applicable) before being brought into the main part of the facility. Cardboard is not permitted in the rearing facility. Contents of boxes shall be transferred to clear plastic bins prior to entry.

2.4.2 IPS Form Number 0071/007 (*Diet Ingredient Tracking Sheet*; Appendix 3) shall be initiated immediately upon receipt of an ingredient; a copy shall be forwarded to the QCU. The following information shall be documented:

(a) ingredient name
(b) lot number (provided by the supplier)
(c) date received
(d) expiry date (determined by consulting the shelf-life in Appendix 1).

(e) amount

2.4.3 The *Diet Ingredient Tracking Sheet* shall be used to input relevant information into the automated diet ingredient inventory and to make labels for aliquot preparation; it shall then be archived in the *Diet Production* binder.

2.4.4 Diet ingredients shall be stored under the conditions specified in Appendix 1 until sufficient time permits for aliquots to be made and vacuum sealed as specified in 2.5. Light sensitive items shall be stored in light-proof containers.

2.4.5 Bottles of ingredients for the vitamin solution (refer to Appendix 4) shall be labeled with the expiry dates specified in Appendix 1 (since these materials don't get aliquoted in entirety) then stored in a labeled (i.e., "Vitamin Solution Ingredients"), opaque (i.e., light proof) bin in the diet ingredient cold room until being partially aliquoted as specified in section 2.6.3. *Diet Ingredient Tracking Sheets* for each bottle shall be maintained within the bin.

2.5 Pre-weighing Diet Ingredients

2.5.1 Personal protective safety equipment specified in 1.4.4 shall be worn when weighing or measuring diet ingredients. All weighing and measuring shall be conducted in the diet ingredient preparation area (i.e., room AA118A).

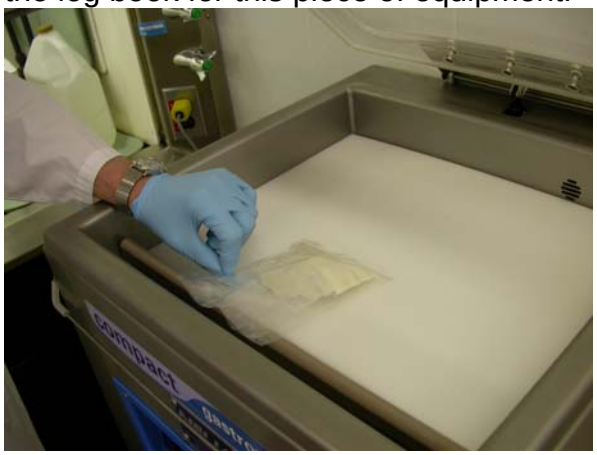
2.5.2 Ingredients requiring pre-treatment (e.g., toasting, mixing, etc.) shall be prepared as specified in 2.6.

2.5.3 The number of packages/bottles to prepare of each batch size shall be determined by consulting the *Diet Ingredient Inventory* to view the number currently in stock. The rule of thumb is to maintain twice as many full batches as half batches, and twice as many half batches as blender batches.

2.5.4 Diet ingredients shall be pre-weighed, pre-measured, pre-mixed and vacuum sealed (if applicable) in aliquots as soon as time permits. Materials shall not be aliquoted unless sufficient time is available to complete packaging of the entire lot (vitamin components need not be aliquoted in entirety).

2.5.5 Liquid ingredients shall be aliquoted into screw top plastic bottles.

2.5.6 Dry ingredients shall be vacuum sealed following the directions in the operator manual. This unit shall be maintained and serviced as specified in the manual. Any repair, maintenance or calibration shall be documented in the log book for this piece of equipment.



2.5.7 Each container/package of aliquoted material shall be labeled with:
(a) diet type
(b) ingredient name
(c) batch size

(d) mass or volume
(e) lot #

2.5.8 Aliquots shall be stored (refer to Appendix 1 for storage conditions) in separate bins for each ingredient type and for each batch size.

2.5.9 Additional storage bins (washed with Javex® and rinsed with water after each use) shall be assembled containing one package/container of each ingredient that is required to fulfill a complete diet recipe. Bottles of vitamin solution shall be excluded from the bins since they are not to be kept for more than two weeks after being mixed. New lots of ingredients shall not be started until previous ones have been depleted or discarded. Separate bins shall be prepared for full, half, quarter and blender batch recipes (as applicable) for each diet type. Each storage bin shall be labeled with the following:

(a) diet type
(b) batch size
(c) date that bin was prepared (written on tape used to seal bin)

2.5.10 Completed bins shall be rotated so that the oldest materials are used first (i.e., slide bins along shelf in the direction indicated by the arrow).

2.5.11 When two packages of an ingredient originally destined for small batches are combined to make a bin for a larger batch, a notation to this effect (including

the date of preparation of the smaller packages) must be documented on the applicable *Diet Checklist* (i.e., Appendices 10-14) during the diet-making session. Example: two 72g packages of Alphacel originally destined for half batches of regular diet may be combined (i.e., 144g total) when preparing a full batch.

2.5.12 After each diet-making session, bins shall be restocked as specified in 2.5.9 and 2.5.10.

2.6 Pre-treatment of Diet Ingredients

2.6.1 Wheat germ (for Regular and Bell diets) shall be toasted prior to making aliquots for storage as follows:

(a) Stainless steel baking pans shall be filled about ¾ full (i.e., 4 x 1L scoop).

(b) Wheat germ shall be toasted in the oven in room AA118A at 250°C for 45 minutes, stirring once half-way through the toasting process.

(c) Upon completion of toasting, the wheat germ from the two pans shall poured into a plastic tub, mixed with a spatula, covered with a new paper tray and allowed to cool for 1-2 hours.

(d) Once cooled, the wheat germ shall be poured into plastic bags and stored in a plastic bin in the diet ingredient cold room (i.e., AAQ118C) until aliquoted as specified in section 2.5.

(e) IPS Form Number 0071/007 (*Diet Ingredient Tracking Sheet*; Appendix 3) shall accompany the wheat germ throughout the process.

2.6.2 Vitamin ingredients in quantities sufficient for making 1L batches (refer to Appendix 4) shall be weighed, combined in a vacuum sealed bag, labelled as "vitamin solution powder", dated and stored in an opaque bin in the walk-in cold room (i.e., AA118C) for not more than 3 months.

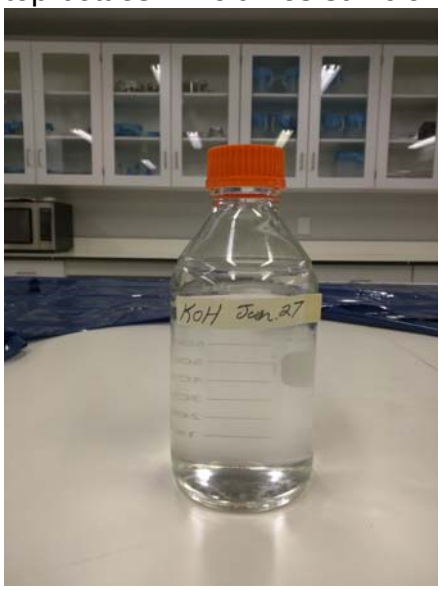
2.6.3 A 1L batch of vitamin solution shall be prepared as follows:

(a) Add the contents of one bag of vitamin solution powder (refer to 2.6.2) to a 2L screw-top bottle containing 1L sterile RO water.

(b) Using the solution, rinse the bag to ensure that nothing remains. Stir the solution vigorously to ensure complete dissolution of ingredients. Label the bottle with the date made and store (maximum two weeks) within a

light-proof container in the walk-in cold room (i.e., AA118C). On the day of diet preparation, aliquot into screw-top bottles in volumes sufficient for each batch of diet.

2.6.4 A 1L batch of 4M potassium hydroxide (KOH) solution shall be prepared as specified in Appendix 15. On the day of diet preparation, aliquot into screw-top bottles in volumes sufficient for each batch of diet.



2.6.5 A 4L batch of anti-fungal spray solution shall be prepared as specified in Appendix 16.

2.6.6 RO water shall be autoclaved prior to the day of diet preparation as follows:

(a) A sufficient number of 8L carboys (varies with the number of batches of diet that need to be prepared) shall be half-filled with RO water (i.e., 4L/carboy) and the lid shall be placed loosely on the top.

(b) The carboys shall be autoclaved for 90 minutes on the liquid cycle.

(c) Upon completion of autoclaving, the carboys shall be stacked on a lab cart and left to cool. Lids shall be tightened within 24h of autoclaving. Carboys shall be dated and maintained for a maximum of one month before use. If not used within that period of time, the water shall be discarded.

2.7 Requisition of Diet

2.7.1 Orders for diet shall only be accepted from internal and external clients through the IPS web-based storefront (i.e., requests submitted through standard email or telephone are not to be accepted).

2.7.2 The IPU email account shall be reviewed daily (excluding weekends and holidays) for the receipt of orders from the storefront. Orders shall be processed as per the current version of SOP Number IPS/009 (*IPU Personnel Responsibilities*). Diet requests shall be transcribed onto IPS Form Number 0164/001 (*Diet Orders*; Appendix 17) and maintained on the *Diet Clipboard*.

2.7.3 Prior to each diet-making session, IPU personnel shall determine their diet needs for maintaining GLFC insect colonies for the coming week and document them on IPS Form Number 0164/001 (*Diet Orders*).

2.7.4 Just prior to the diet-making session, the IPS email account shall be checked to ensure that all last minute requests have been appended to the *Diet Clipboard*.

2.7.5 At the start of each diet-making session, the number of full, half, quarter and blender batches of each diet type to be made shall be determined by consulting the *Diet Clipboard*. An applicable *Diet Checklist* (refer to Appendices 10-14) shall be initiated for each batch of diet to be prepared during the diet-making session.

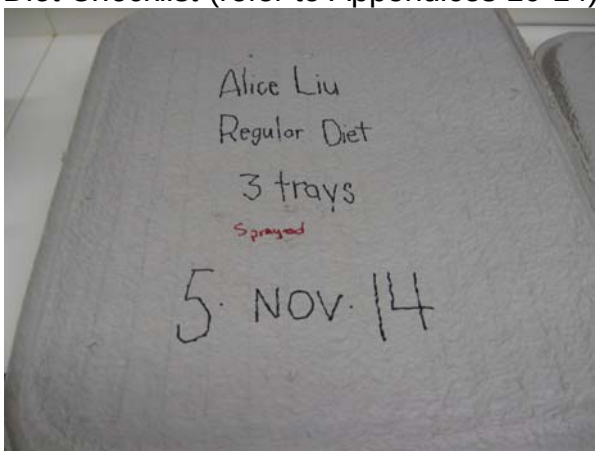
2.7.6 Diet shall normally be made on a weekly basis (typically on Wednesday). Clients who order diet on a regular basis shall be notified in advance by email whenever the routine diet-making schedule needs to be altered (e.g., due to short work week, scheduling of leave, etc.)

2.8 Cup set-up

2.8.1 Diet cups shall normally be set up on trays the day before a diet-making session. Cups may be set up a few days prior to the diet-making session only if they are stored in sealed plastic bags. The number of trays of cups, style of cup, style of tray and volume of diet varies with client orders and shall be

determined prior to the start of the diet-making session. Metal trays shall only be used to fulfill diet requests within the IPU.

2.8.2 At the start of the diet-making session, trays of empty cups shall be grouped by client order and covered with a cardboard tray that is labeled with the client name as well as a record of the type of diet, volume per cup, total order size and date made. When multiple batches of the same diet recipe are made during the same diet-making session, batches shall be identified as Batch A, Batch B, etc. The batch identifier shall also be designated on the applicable *Diet Checklist* (refer to Appendices 10-14).



2.9 Sanitization of Equipment and Facility Prior to Use

2.9.1 Aluminum diet trays shall be washed and autoclaved after each use. Trays shall be autoclaved (15 minutes sterilization and 15 minutes dry time) then stored in a closed cupboard in room AA118B. Trays shall be rotated within the cupboard each time more are added so that the oldest ones are used first.

2.9.2 Table tops and counters shall be cleaned at the start of each diet-making session by:

(a) Spray top surfaces with 6% Javex® and allow to stand for at least 10 minutes. Javex® solution shall be made fresh daily (refer to section 2.23.1).
(b) Wipe surfaces with sterile paper towel.

2.9.3 The mixer and kettle shall be cleaned at the start of each diet-making session as follows:
(a) Spray the interior surface of the bowl with 6% Javex® and allow to stand for at least 10 minutes. Javex® solution shall be made fresh daily (refer to section 2.23.1).
(b) Wipe surfaces with sterile paper towel.
(c) Rinse interior surface of the bowl with water to remove Javex® residue.

2.9.4 The heat resistant gauntlet style gloves shall be cleaned with 6% Javex® at the start of each diet-making session.

2.9.5 Prepare 2L of 6% Javex® in a 4L beaker and soak the spatulas that will be used during the current diet making session.

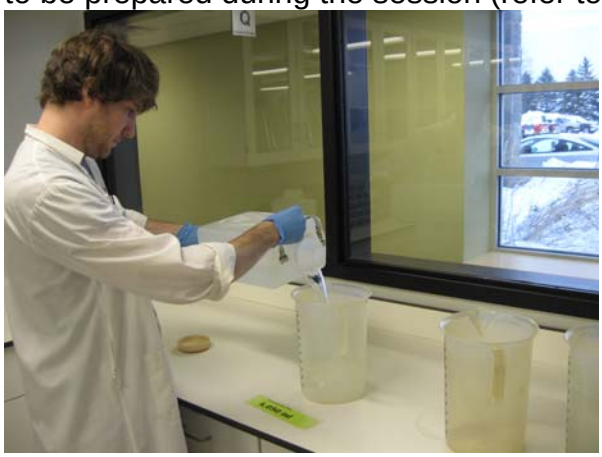
2.10 Assembly of Equipment

2.10.1 Ensure that the power to mixing bowl and the steam kettle is turned off at the electrical breaker switch.
2.10.2 Remove the cover from the steam kettle.
2.10.3 Ensure that the kettle and mixer are cleaned as specified in 2.9.3.
2.10.4 Install the mixing shaft into the kettle.
2.10.5 Assemble the mixer as required, taking care with sharp components.
2.10.6 After ensuring that all personnel are clear of the equipment, turn on all electrical breaker switches.
2.10.7 Turn on the overhead exhaust fan.

2.11 Retrieval of Diet Ingredients from Storage

2.11.1 Retrieve the required number of bins of ingredients for each diet type and batch size to be prepared during the diet-making session. Ensure that the oldest bins are used first and that the contents of each are kept separate.

2.11.2 Measure the required volumes of previously autoclaved water for all batches to be prepared during the session (refer to diet recipes in Appendices 5-9).



2.12 Mixing Full or Half Batches

2.12.1 Ensure that at least two people are present at all times when operating the diet-making machinery. Typically, one person shall conduct the mixing and monitoring of agar in the kettle while the other person adds ingredients to the mixer and ensures everyone is wearing the proper personal protection clothing.

2.12.2 Just prior to the mixing of each batch of diet, review each package of ingredients in the bin and check-off the applicable box on the *Diet Checklist* (refer to Appendices 10-14) to ensure that all components are present in the required amounts. The form shall be initialed by the individual marking the checklist. When an ingredient is to be eliminated from the recipe or portion of the recipe (refer to section 2.14), it shall be set aside at this time.

2.12.3 As each diet ingredient is added to either the mixer or kettle, mark it off on the *Diet Checklist* (refer to Appendices 10-14). The form shall be initialed by the individual adding the ingredient and shall be maintained in the binder labeled as *Diet Forms*.

2.12.4 Add the required amount of autoclaved water to the steam kettle.

2.12.5 Add required amount of agar to the cold water in the steam kettle. Stir with a spatula to break up clumps. EAB diet requires the addition of sodium bicarbonate at this stage. Lower the mixing paddle and turn it on.

2.12.6 Adjust the temperature control to about setting #5-7 until mixing of the ingredients in the mixing bowl is almost complete; then set the control to maximum.

2.12.7 The temperature of the agar shall be checked periodically until the target temperature has been attained (i.e., just beginning to boil).

2.12.8 The following procedures shall be performed for each specific diet type:

a) Regular, Bell and Addy diets:

i) While the agar is heating in the kettle, the remaining diet ingredients shall be combined in the mixer in the order specified on the applicable Diet Checklist (refer to Appendices 10-12).
ii) Add the required amount of water to the mixer before any other ingredient.

ii) Add the KOH (for Regular diet only) and casein to the water in the mixer.

iii) Using a sterile spatula, break up the casein and perform an initial mixing.

iv) Close the lid and run the motor on the "low" setting for about 1 minute to ensure complete mixing. While mixing, turn the handle manually to ensure that ingredients don't adhere to the mixing paddle.

v) Once the mixing of the casein and water is complete, turn off the motor, open the lid and ensure that materials have been sufficiently mixed (i.e., white and frothy) and are not stuck to the mixing paddle or blade. If mixing is insufficient, brake up lumps with a spatula and repeat the process.

vi) Add the remaining diet ingredients (with the exception of the vitamin solution) in the order that they appear on the recipe for that particular batch of diet. As each ingredient is added, mark it off on the *Diet Checklist* (refer to Appendices 10-12). Make sure that any applicable ingredients are eliminated when making custom diets (refer to 2.14).

vii) Once all ingredients have been added to the mixer, a minimum mixing time of 1 minute is required before the hot agar can be added.

viii) If the agar has not reached the target temperature by the time the mixing is complete, the mixer shall be left running on the “low” setting.

ix) Once the agar has attained the target temperature, turn off all steam kettle controls (i.e., switches for temperature controller and for mixer). Carefully, pour the hot agar into a clean stainless steel pail.

x) Turn off the mixer, open the lid and add all of the hot agar.

xi) Once all of the agar has been added, close the lid and mix on the "high" setting for 2 minutes. While mixing, turn the handle manually to ensure that ingredients don't adhere to the mixing paddle.

xii) Add the vitamin solution and mix for one additional minute on the "high" setting.

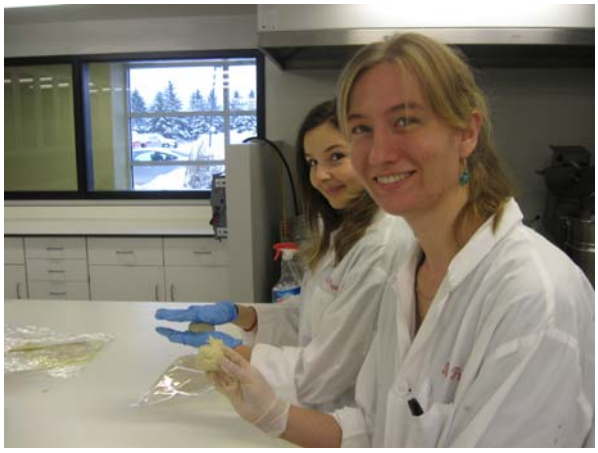
xiii) Turn off the mixer motor, open the lid and start dispensing diet immediately before it cools. When preparing custom diet (refer to 2.14) for a part batch, pour the required number of trays with the applicable ingredient eliminated, then close the mixer, add the required quantity of the ingredient (i.e., so that the remaining diet has the standard proportion of each ingredient), mix on high for 1 minute, then continue dispensing.

b) ALB diet (refer to the *ALB Diet Checklist*, Appendix 13; check off each ingredient as it is added):

i) Transfer the agar and water mixture from the kettle to the mixer once it has come to a full boil.
ii) Add *Group 1* diet ingredients and mix for 1 minute; turn off the motor.
iii) Add *Group 2* diet ingredients and mix for 1 minute; turn off the motor.
iv) Premix *Group 3* diet ingredients in a beaker, add to mixer and mix for 4 minutes, thus allowing the contents to cool somewhat; turn off the motor.
v) Add *Group 4* diet ingredients and mix for 1 minute; turn off the motor.
vi) Add half of the Alphacel (*Group 5*), mix for 1 minute, add the remainder of the Alphacel and mix for one final minute before turning off the motor and dispensing the diet.

c) EAB diet (refer to the *EAB Diet Checklist*, Appendix 14; check off each ingredient as it is added):
i) Transfer the agar, water and sodium bicarbonate mixture from the kettle to the mixer once it has come to a full boil.
ii) Premix *Group 1* diet ingredients in a bowl, add to mixer and mix for 1 minute; turn off the motor.
iii) Add the Soy Flour (*Group 2*) and mix for 1 minute; turn off the motor.
iv) Premix *Group 3* diet ingredients in a beaker, add to mixer and mix for 3 minutes thus allowing the contents to cool somewhat; turn off the motor.
v) Premix *Group 4* diet ingredients in a beaker, add to mixer and mix for 1 minute; turn off the motor.
vi) Add half of the Alphacel (*Group 5*), mix for 1 minute, add the remainder of the Alphacel and mix for one final minute; turn off the motor.
vii) Using gloved hands, break the clump of prepared diet into 5-10 smaller balls (about 10cm in diameter); place each ball into a clear plastic bag and seal to prevent drying; these balls shall be maintained on the bench-top for a maximum of 2 hours while proceeding through steps viii through xi.

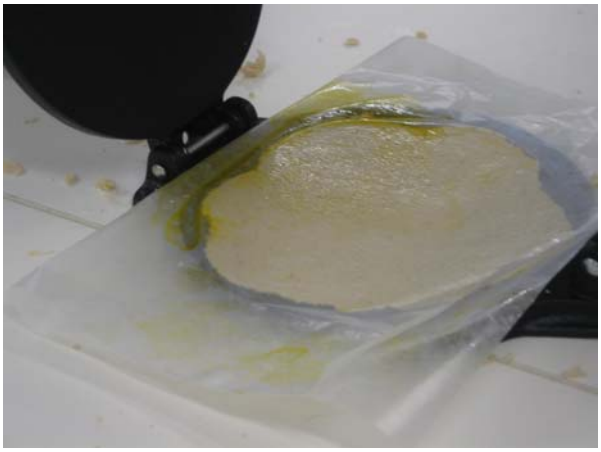
viii) Break each 10cm ball into smaller golf-ball-sized pieces.



ix) Spread 0.5ml wheat germ oil using a gloved hand over the surface of a 2-foot long piece of waxed paper.

x) Place one end of the wax paper (oil side up) on the tortilla press; place one golf-ball-sized piece of diet on top of the wax paper; fold the

remaining portion of wax paper on top of the ball of diet and flatten with the press.



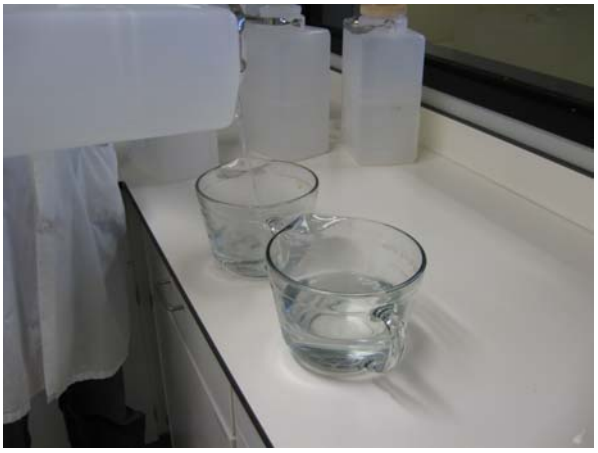
xi) Place 10 sets of flatted diet (with wax paper) in a sealable bag, vacuum seal, label with current date and store in a labelled bin in the cold room for prepared diet (i.e., AA115).

2.13 Mixing Blender Batches

2.13.1 Blender batches may be prepared when small volume and/or custom orders are required (e.g., when a client requests the omission of a particular ingredient such a formaldehyde; refer to 2.14).

2.13.2 Blender batches are prepared following procedures specified in 2.12 with the exceptions that:

(a) the agar is dissolved in water using two pyrex pitchers in a microwave; the agar and water are heated on the highest power setting (stirring frequently) until dissolved.



(b) mixing is conducted in 4L blender.

2.14 Custom Recipes (for Regular and Bell Diets only)

2.14.1 Custom recipes shall be prepared when a client requests the omission or addition of a particular ingredient (e.g., formaldehyde). Personnel preparing the diet have the discretion of making a separate batch of the custom recipe

or splitting a batch of the standard recipe and customizing a portion of it as follows:

(a) When an ingredient is to be eliminated from the recipe, a standard size batch shall be prepared with that item omitted. The requested quantity of custom diet shall be poured and the remaining diet shall have the missing ingredient added and mixed in the correct proportion. Calculations for the quantity of the omitted ingredient that is to be added shall be made using the excel spreadsheet for the applicable diet type (i.e., excel spreadsheet attached to either IPS Form Number 0016/004 or 0017/004).

Example: A 28.9L batch of regular diet is required to be split into 18.9L with formalin (i.e., standard recipe) and 10L without (i.e., custom recipe). This is achieved by mixing 28.9L of diet that has no formalin added and dispensing 10L as soon as it is prepared. The remaining 18.9L will have 9.4ml formalin added and mixed before pouring [Note: a 28.9L batch normally requires 14.4ml formalin, therefore 18.9L requires 9.4ml].

(b) When a custom ingredient is to be added to only a portion of a batch, the standard recipe shall first be prepared following routine procedures. After mixing the standard diet, dispensing shall proceed until only the requested quantity of custom diet remains in the mixer. At this time, the custom ingredient shall be added at the requested concentration, then the custom diet shall be mixed and poured in the requested cup size and fill volume.

Example: A 28.9L batch of regular diet is required to be split into 18.9L of the standard recipe and 10L with fumidil added (i.e., custom recipe). This is achieved by mixing 28.9L of the standard recipe and pouring 18.9L. The remaining 10L of is to have fumidil added (i.e., custom recipe) at the concentration requested by the client.

(c) When an ingredient is to be added to only a very small portion of diet (e.g., enough for one tray), a standard size recipe shall be prepared and

the requested volume of custom diet shall be removed and placed in an appropriate sized container. At this time, the custom ingredient shall be added at the requested concentration, then the custom diet mixed using a hand held mixer and poured into the requested cup size and fill volume.

2.14.2 Spreadsheets for calculations of quantities of ingredients to be omitted from part of a batch (i.e., for custom recipes) shall be printed, stapled to the applicable *Diet Checklist*, then filed in the binder labeled as *Diet Forms.*

2.15 Dispensing Diet

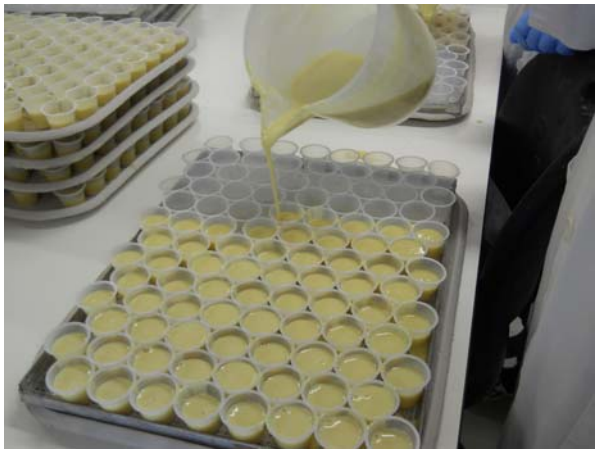
2.15.1 A face shield is not required when dispensing diet, although a lab coat, apron and chemical protective polymer gloves must be worn.

2.15.2 A diet QC sample (refer to section 2.20) shall be taken from each batch prior to dispensing into diet cups.

2.15.3 Sterile pitchers shall be used for dispensing diet. One pitcher shall be used to dip into the prepared diet and to obtain material to fill the pouring pitcher (this keeps the outside of the pouring pitcher clean and reduces spillage throughout the facility). The pitcher used for dipping shall be suspended on the mixer shaft or paddle between uses.

2.15.4 To reduce spillage when dispensing into cups on perforated metal trays, the tray may first be placed onto a disposable cardboard tray. Diet shall be dispensed (by visual estimate) to volume requested by the client.

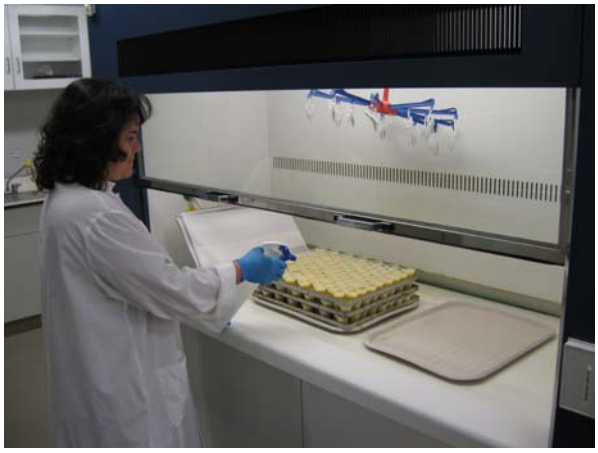
2.15.5 Trays may be stacked during dispensing so that trays of similar type and volume remain together. Trays of dispensed diet shall not be stacked more than 5 high in order to facilitate cooling and packaging (maximum 5 trays per package).



2.15.6 The mixer may be tipped during the dispensing process to facilitate removal of the prepared diet.

2.15.7 Each stack of prepared diet shall be covered with a disposable cardboard tray (labeled as specified in 2.8.2) after it has partially cooled.

2.15.8 Unless specified otherwise by the client, each freshly prepared tray of diet shall be misted lightly with anti-fungal spray (prepared as specified in Appendix 14) within the chemical fume hood with the exhaust fan running and while wearing the personal protective safety equipment specified in 1.4.4. The spray may be applied while the diet is still warm.



2.15.9 Stacks of prepared diet shall be allowed to fully cool on the table or countertop before packaging.

2.15.10 No cleaning of the mixing equipment is required between batches of the same recipe prepared during the session. When multiple batches of different recipes are prepared, the mixer shall be hosed down until visible residue is gone. The kettle does not require cleaning between batches.

2.16 Packaging Diet

2.16.1 Bags to be utilized for packaging shall be stored in the plastic receptacle in room AA118A. Only new opaque bags shall be used.

2.16.2 Prepared diet shall be packaged in bundles (maximum 5 trays) of similar types and volumes using only new opaque plastic bags. Care shall be taken so that the bag is not punctured.

2.16.3 Each bundle shall be sealed with a twist tie and shall be labeled with the client name (if applicable), type of diet, volume of diet, date prepared and batch number (when multiple batches of the same recipe are made on the same day).

2.17 Storage of Diet

2.17.1 Bundles of diet shall be transferred to cold room AA115 where they are maintained at 2°C until distribution. Diet destined for maintaining IPU insect colonies or destined for distribution shall not be kept for more than two weeks.

2.17.2 Shelving shall be labeled with the type of diet that is to be stored there. Diet prepared for client orders shall be labeled in red ink.

2.18 Sanitation of Equipment and Facility After Use

2.18.1 Once the steam kettle is no longer required during the diet-making session, it shall be cleaned as follows:

(a) The kettle shall be filled with water; add one scoop (i.e.,10-20ml) of Diversol® detergent and one scoop (i.e., 10-20ml) of laundry detergent.

(b) The mixer motor shall be turned on and the heat turned on to setting 8-9 until the agar remnants begin to loosen.

(c) Heater/mixer switches and the electrical circuit breaker shall be turned off.
(d) Contents of the kettle can either be poured into the pail and discarded through the garburator in the sink, or poured directly onto the floor and washed into the drain as per 2.18.5.
(e) The mixer shaft shall be removed from the kettle and washed with other instruments (specified in 2.18.4) in the sink with soap and hot water.
(f) The kettle shall be scrubbed with soap and water using a bristle brush until all diet ingredients have been loosened.
(g) The interior and exterior surfaces of the kettle shall be hosed down with water and allowed to air dry. Care should be taken to avoid electrical components by covering them with a plastic bag.

2.18.2 At the end of the diet-making session, the mixer(s) shall be cleaned as follows:
(a) The electrical breaker shall be turned off.
(b) Disassemble the mixer as required and wash components in the sink with soap and hot water, taking care with sharp components.
(c) Large pieces of diet shall be scraped loose using a spatula and shall be discarded.
(d) The mixer shall be scrubbed with soap and water using a bristle brush until all diet ingredients have been loosened. Loose materials shall be poured into a pail and discarded through the garburator in the sink.
(e) The interior and exterior surfaces of the mixer shall be hosed down with water and allowed to air dry. Care should be taken to avoid electrical components by covering them with a plastic bag.
(f) Once the mixer is dry, the cover shall be closed to keep the bowl free from air borne contaminants.

2.18.3 At the end of the diet-making session, the table and counter tops shall be cleaned as follows:
(a) Spray with Windex® to assist with loosening dry diet.
(b) Diet shall be scraped loose using a sharp instrument and shall be discarded.

(c) Surfaces shall be sprayed with a 6% Javex® solution and allowed to soak for a minimum of 10 minutes before being wiped down with sterile paper towel.
(d) Residual Javex® shall be removed by spraying with Windex® and wiping with sterile paper towel.

2.18.4 At the end of the diet-making session, all instruments and equipment components shall be washed in the sink with soap and hot water. Instruments and equipment components that are autoclavable shall be wrapped in foil and sterilized on the gravity cycle for 15 minutes followed by 30 minutes dry time. Items that are not autoclavable shall be soaked in 6% Javex® for 10 minutes, rinsed with water, then air-dried. All instruments and components shall be stored in closed cupboards or shelving units until the next session (i.e., do not re-install components on equipment).

2.18.5 At the end of the diet-making session, the floor shall be cleaned as follows:
(a) The floor shall be hosed down so that diet pieces are washed into the floor drain.
(b) A squeegee shall be used to ensure that residual water and diet pieces are directed toward the drain.
(c) The entire floor of the diet kitchen shall be mopped with a 6% Javex® solution (refer to 2.23.1).

2.19 Distribution of Diet

2.19.1 Distribution of diet to clients shall be documented on the applicable *Diet Checklist* (refer to Appendices 10-14), including:
(a) name
(b) date made
(c) pick-up date
(d) number of trays
(e) cup type
(f) fill volume
(g) special order

2.20 Quality Control

2.20.1 IPU personnel shall provide samples of diet ingredients to the QCU upon request.

2.20.2 Prior to the start of each diet-making session, IPU personnel shall ensure that one sterile diet QC sample kit is readily available for each batch that is to be prepared. Contact the QCU for additional kits. Each kit will contain three pre-weighed micro-tubes; creamer cups will be supplied by the IPU.

2.20.3 Upon the preparation of each batch of diet, aliquots shall be kept as QC samples prior to dispensing. Three replicate samples shall be collected in creamer cups (half filled) and three replicate samples in the supplied micro-tubes (i.e., one drop shall be collected in each tube using a disposable pipette). Samples shall be placed within the box which shall be labelled with the batch identifier.

2.20.4 At the completion of the diet-making session, IPU personnel shall immediately notify QCU personnel to retrieve the boxes of QC samples from the IPU distribution window.

2.20.5 IPU personnel shall allow facility access by QCU personnel for the purpose of taking/retrieving samples, monitoring procedures or reviewing IPU records.

2.21 Modifications

2.21.1 Diet recipes, suppliers of ingredients or methods of production shall not be modified without consultation and approval of the IPU supervisor.

2.22 Shipping and Billing

Once diet orders are prepared and distributed/shipped, billing charges to clients external to GLFC shall be recorded and submitted to the GLFC finance department as specified in 2.7.2.

2.23 Calculations

2.23.1 Prepare 6% Javex® solution (i.e., 0.3% sodium hypochlorite) by combining 60ml Javex® (5.25% sodium hypochlorite) with 940ml water. If another brand is used, volumes may need to be adjusted to provide a 0.3% sodium hypochlorite working solution.

2.23.2 Proportions of ingredients in split batches of custom diet recipes are determined using methods specified in 2.14.

2.24 Power Interruption

Upon determination that a power failure has occurred in the cold rooms for diet materials or prepared diets (i.e., rooms AA114 or AA115), corrective actions and documentation of the occurrence shall be performed as specified in the current version of the SOP for Environmental Chambers and Rearing Rooms (SOP Number IPS/001).

2.25 Documentation and Reporting

2.25.1 Compliance to this SOP shall include the completion and maintenance of the following forms, logs and binders:
- (a) IPS Form Number 0071/007 (*Diet Ingredient Tracking Sheet*)
- (b) IPS Form Number 0016/004 (*Regular Diet Checklist*)
- (c) IPS Form Number 0017/004 (*Bell Diet Checklist*)
- (d) IPS Form Number 0144/003 (*Addy Diet Checklist*)
- (e) IPS Form Number 0145/003 (*ALB Diet Checklist*)
- (f) IPS Form Number 0163/002 (*EAB Diet Checklist*)
- (g) IPS Form Number 0164/001 (*Diet Orders*)
- (h) diet requisitions from the on-line ordering system
- (i) Excel spreadsheets for calculations of ingredients omitted from part of a batch of diet
- (j) binder labeled as *Diet Forms* including items (a)-(h) above
- (k) Excel spreadsheets for *Diet Ingredient Inventory*
- (l) equipment log book for mixer
- (m) equipment log book for kettle
- (n) equipment log book for vacuum sealer

2.25.2 IPU personnel shall record unusual diet-making occurrences on the applicable *Diet Checklist* (Appendices 10-14) and directly on bags of affected diet. Personnel shall ensure that this information is carried forward on tracking sheets for each cohort of insects for which the diet is used.

2.25.3 When packages of ingredients originally destined for small batches are combined to make a larger batch, a notation to this effect (including the date of preparation of the smaller packages) must be documented on the applicable *Diet Checklist*.

2.25.4 *Diet Checklists* shall be archived in the "*Diet Production*" binder located in Room AA220.

3.0 DISTRIBUTION AND ARCHIVING

3.1 Distribution

This SOP shall be distributed by the IPS manager to all IPU 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 The IPU supervisor shall ensure that files of all documentation identified in 2.25.1 are maintained for expedient retrieval.

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 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 IPU supervisor is responsible for assuring that this SOP is valid.
4.1.2 The IPU supervisor is responsible for assuring that this SOP is followed by IPU personnel and that these persons have been appropriately trained in its use.
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 IPU supervisor is responsible for assuring that this SOP is current. If necessary, the IPU supervisor 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 IPU personnel find circumstances that do not permit compliance with this SOP, the IPU supervisor 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

(a) current version of SOP Number IPS/001 (*Environmental Chambers and Rearing Rooms*)
(b) current version of SOP Number IPS/009 (*IPU Personnel Responsibilities*)
(c) operator manual for vacuum sealer
(d) EAB diet recipe from: Keena MA 2015. Great Lakes Entomologist *48(1-2):9-33*

(e) Addy diet recipe from: Addy ND 1969. J. Econ. Ent. 62:270-271
(f) Regular diet recipe from: McMorran A 1965. Can. Ent. 97:58-62
(g) Bell diet recipe from: Bell RA, Own CD, Shapiro M, Tardif JR 1981. USDA Tech. Bull 1584
(h) ALB diet recipe from: Keena MA 2005, Entomol. Soc. Am. 98(4): 536-547
(i) procedure for flattening EAB diet using tortilla press from: Sharma *et al*. 2015 (*in press*)

9.0 APPENDICES

Appendix 1: Diet Ingredient Storage Conditions and Shelf-life
Appendix 2: Suppliers for Diet Ingredients
Appendix 3: IPS Form Number 0071/007 (*Diet Ingredient Tracking Sheet)*
Appendix 4: Vitamin Solution
Appendix 5: Regular Diet Recipe
Appendix 6: Bell Diet Recipe
Appendix 7: Addy Diet Recipe
Appendix 8: ALB Diet Recipe
Appendix 9: EAB Diet Recipe
Appendix 10: IPS Form Number 0016/004 (*Regular Diet Checklist*)
Appendix 11: IPS Form Number 0017/004 (*Bell Diet Checklist*)
Appendix 12: IPS Form Number 0144/003 (*Addy Diet Checklist*)
Appendix 13: IPS Form Number 0145/003 (*ALB Diet Checklist*)
Appendix 14: IPS Form Number 0163/002 (*EAB Diet Checklist*)
Appendix 15: 4M KOH Solution
Appendix 16: Anti-fungal Spray Solution
Appendix 17: IPS Form Number 0164/001 (*Diet Orders*)

Appendix 1

Diet Ingredient Storage Conditions and Shelf-life

Ingredient		Storage	Shelf-life	Light Sensitive
Agar (Bio-Serv)		Cold room	2 years	No
Agar (Gracilaria)		Cold room	2 years	No
Alphacel (cellulose)		Cold room	1 year	No
Ascorbic Acid		Cold room	3 years	Yes
Aureomycin		Cold room	4 years	No
Biotin		Cold room	1 year	No
Calcium Pantothenate		Cold room	1 year	No
Calcium Proprionate		Room temperature	5 years	No
Casein		Cold room	2 years	No
Choline Chloride		Cold room	2 years	No
Cholesterol		Room temperature	10 years	No
Ethyl Alcohol		Room temperature	1 year	No
Ferric Phosphate		Room temperature	5 years	No
Folic Acid		Room temperature	5 years	No
Formaldehyde		Room temperature	1 year	No
KOH (pellets)		Room temperature	2 years	No
Linseed Oil		Cold room	2 years	No
Methyl Paraben		Cold room	2 years	No
Nicotinic Acid		Room temperature	5 years	No
Potato Starch		Room temperature	1 year	No
Pyridoxine Hydrochloride		Room temperature	5 years	No
Riboflavin		Room temperature	5 years	No
Sodium Alginate		Freezer	3 years	No
Sodium Bicarbonate		Room temperature	5 years	No
Sodium Proprionate		Room temperature	5 years	No
Sorbic Acid		Cold room	3 years	No
Soy Flour		Cold room	1 year	No
Sucrose		Room temperature	2 years	No
Sugar		Room temperature	2 years	No
Thiamine Hydrochloride		Room temperature	5 years	No
Torula Yeast		Room temperature	18 months	No
Vitamin A Beadlets		Freezer	1 year	Yes
Vitamin B12		Cold room	1 year	No
Vitamin Mix for ALB		Cold room	1 year	Yes
Vitamin Mix (Vanderzant)		Cold room	9 months	Yes
Wesson Salt	Salt Mix W	Cold room	5 years	No
	Without Ferric Phosphate	Cold room	5 years	No
	Modified	Cold room	5 years	No
Wheat Germ	Raw	Cold room	1 year	No
	Toasted	Cold room	1 year	No
Wheat Germ Oil		Cold room	8 months	Yes

Appendix 2 (Part 1 of 3)

Suppliers for Diet Ingredients

Ingredient Description	Product Number (order size)	Supplier
Agar, USP 100 mesh	#7060 (25 kg)	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Agar (Gracilaria)	#41005 (25 lbs)	Moorhead & Company Inc 6923 Woodley Ave. Van Nuys, CA 91406 (916) 624-6056
Alphacel (Cellulose – Fiber) Non-nutritive Fiber Powdered	#3425 (25 kg)	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Ascorbic Acid USP Fine Granular	#0408093 (25 kg)	DSM Nutritional Products Canada Inc 395 Waydom Dr AYR, ON N0B 1E0 1-800-263-0867
Aureomycin Soluble 14% Active	#7135 (1 kg)	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Biotin (D-Biotin)	#101023 (5 g)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Calcium Proprionate	0210122880	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Calcium Pantothenate (D-Pantothenic Acid Calcium Salt USP Grade)	#101228 (100 g)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Casein High Nitrogen. FC	#1100V (25 kg)	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Cholesterol	#5180 (5 kg)	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Choline Chloride	#101386 (5 kg)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH,USA 44202 1-800-854-0530

Appendix 2 (Part 2 of 3) **Suppliers for Diet Ingredients**

Ethyl Alcohol (95% Ethanol)	#3013 (25L)	ACP Chemicals 4601 Boulevard des Grandes Prairies, Saint-Leonard, Québec H1R 1A5 (514) 327-0323
Ferric Phosphate	#F1523 (500g)	Sigma-Aldrich 2149 Winston Park Dr. Oakville, ON L6H 6JB 1-800-565-1400
Folic Acid	#101725 (25 g)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Formaldehyde 37%	#3048 (4L)	VWR 1-800-932-5000
KOH (Potassium hydroxide)	#3083 (3kg)	Fisher Scientific 1-800-234-7437
Linseed Oil (Raw)	#1675921 (6 x 4 L)	Home Hardware 594 Second Line E Sault Ste Marie (705) 759-5101
Methyl Paraben USP	#7685 (10 kg)	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Nicotinic Acid	#102446 (100 g)	MP Biomedicals 1263 S. Chillicothe Rd. Aurora, OH USA 44202 1-800-854-0530
Pyridoxine Hydrochloride	#102777 (25 g)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Riboflavin (USP Grade)	#102813 (100 g)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Sodium Alginate	6840	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Sodium Bicarbonate	box	NG Grocers 173 Trelawne Ave. Sault Ste. Marie, ON (705) 946-5462
Sodium Proprionate	0210292480	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Sorbic Acid NF, FCC	#6967 (10 kg)	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908

Appendix 2 (Part 3 of 3) **Suppliers for Diet Ingredients**

Sucrose	#3105 (2.5 kg)	NG Grocers 173 Trelawne Ave. Sault Ste. Marie, ON (705) 946-5462
Sugar	#6010 (2kg)	NG Grocers 173 Trelawne Ave. Sault Ste. Marie, ON (705) 946-5462
Thiamine Hydrochloride	#103028 (25 g)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Torula Yeast	# Torula Yeast (10kg)	Country Way Store 79 Brock St. Sault Ste. Marie (705) 949-1898
Vitamin A Beadlets	325CWS/GFB	ZMC-USA 1776 Woodstead Court, Suite 215 The Woodlands, TX 77380 (281) 419-6050
Vitamin B12	#103277 (10 g)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Vitamin Mix for ALB (Vitamin A deficient)	Special Order #8128	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Vitamin Mix (Vanderzant)	#F8045 (5kg)	Bio-Serv One 8th Street Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Wesson Salt (Salt Mixture W)	#902851 (10 kg)	MP Biomedicals 1263 S. Chillicothe Rd Aurora, OH, USA 44202 1-800-854-0530
Wesson Salt (without ferric phosphate)	#F8681-M (10 kg)	Bio-Serv One 8th Street, Suite 1 Frenchtown, NJ, USA 1-800-996-9908
Wesson Salt (Modification for Gypsy Moth) Amorphous FePO4	Test Diet 7434 21240 (25 lb)	Ren's Feed & Supplies Ltd 4002 Trafalgar Rd. Oakville, ON L6J 4Z2 1-800-610-7367
Wheat Germ	#Raw Wheat Germ (25 kg)	Country Way Store 79 Brock St. Sault Ste. Marie (705) 949-1898
Wheat Germ Oil	Wheat Germ Oil (250ml)	Country Way 79 Brock St. Sault Ste. Marie 705-949-1898

Appendix 3

Diet Ingredient Tracking Sheet

Ingredient: _____

Lot Number: _____

Date Received: _____
(DD/MM/YY)

Expiry Date: _____
(DD/MM/YY)

Amount: _____

IPS Form Number 0071/007

Appendix 4

Vitamin Solution
(1 Litre)

Ingredients:

- RO Water (autoclaved) 1 L
- Nicotinic Acid 1 g
- Calcium Pantothenate 1 g
- Riboflavin 1 g
- Thiamine Hydrochloride 0.25 g
- Pyridoxine Hydrochloride 0.25 g
- Folic Acid 0.25 g
- Biotin 0.02 g
- Vitamin B_{12} 0.002 g

Procedure:

1. Pour water into a blender.
2. Add ingredients in order ensuring containers are well rinsed in solution so no material is left over.
3. Mix solution vigorously and store in a light resistant container in the cold room.

Prepared vitamin solution shall not be kept more than two weeks.

Appendix 5

Regular Diet Recipe
(McMorran Diet)

Ingredient		Batch Size		
Kettle	Mixer	Blender = 3.6 L	Half = 14.4 L	Full = 28.9 L
Water*	-	2,232 ml	8,928 ml	17,856 ml
Agar (Bio-Serv)	-	62.5 g	250 g	500 g
-	Water*	792 ml	3,318 ml	6,336 ml
-	Casein	126 g	504 g	1008 g
-	KOH	18 ml	72 ml	144 ml
-	Alphacel	18 g	72 g	144 g
-	Wesson Salt	36 g	144 g	288 g
-	Sugar	126 g	504 g	1008 g
-	Wheat Germ (toasted)	110.5 g	442 g	884 g
-	Choline Chloride	3.6 g	14.4 g	28.8 g
-	Ascorbic Acid	14.4 g	57.6 g	115.2 g
-	Formaldehyde 37%	1.8 ml	7.2 ml	14.4 ml
-	Methyl Paraben	5.4 g	21.6 g	43.2 g
-	Aureomycin	7.8 g	31.3 g	62.5 g
-	Linseed Oil	18 ml	72 ml	144 ml
-	Vitamin Solution	36 ml	144 ml	288 ml

(Diet recipe from: McMorran A 1965. Can. Ent. 97:58-62)

*autoclaved RO water

Appendix 6

Bell Diet Recipe

Ingredient		Batch Size		
Kettle	Mixer	Blender = 3.7 L	Quarter = 8.2 L	Half = 16.4 L
Water*	-	2,100 ml	4,725 ml	9,450 ml
Agar (Bio-Serv)	-	50 g	112.5 g	225 g
-	Water*	900 ml	2,025 ml	4,050 ml
-	Casein	83.3 g	187.5 g	375 g
-	Wesson Salt	26.7 g	60 g	120 g
-	Sorbic Acid	6.7 g	15 g	30 g
-	Methyl Paraben	3.3 g	7.5 g	15 g
-	Ascorbic Acid	12.8 g	28.8 g	57.6 g
-	Choline Chloride	3.2 g	7.2 g	14.4 g
-	Wheat germ (toasted)	400 g	900 g	1,800 g
-	Linseed Oil	32 ml	72 ml	90 ml
-	Vitamin Solution	32 ml	72 ml	144 ml

(Diet recipe from: Bell RA, Owens CD, Shapiro M, Tardif JR 1981. USDA Tech. Bull 1584)

*autoclaved RO water

Appendix 7

Addy Diet Recipe

Ingredient		Batch Size		
Kettle	Mixer	Blender = 3.7 L	Quarter = 8.4 L	Half = 16.8 L
Water*	-	2,100 ml	4,725 ml	9,450 ml
Agar (Bio-Serv)	-	55.6 g	125 g	250 g
-	Water*	900 ml	2,025 ml	4,050 ml
-	Casein	130.7 g	294 g	588 g
-	Dextrose	130.7 g	294 g	588 g
-	Wesson Salt	37.3 g	84 g	168 g
-	Cholesterol	6.7 g	15 g	30 g
-	Sorbic Acid	4.5 g	10.2 g	20.4 g
-	Methyl Paraben	2.3 g	5.1 g	10.2 g
-	Choline Chloride	3.7 g	8.4 g	16.8 g
-	Alphacel	66.7 g	150 g	300 g
-	Linseed Oil	16 ml	36 ml	72 ml
-	Sodium Alginate	16.2 g	36.5 g	73 g
-	Ascorbic Acid	16.2 g	36.5 g	73 g
-	Formaldehyde	1.7 ml	3.8 ml	7.6 ml
-	Aureomycin	5.2 g	11.7 g	23.5 g
-	Ferric Phosphate	0.3 g	0.8 g	1.5 g
-	Wheat Germ (toasted)	186.7 g	420 g	840 g
-	Vitamin Solution	53.3 ml	120 ml	240 ml

(Diet recipe from: Addy ND 1969. J. Econ. Entomol. 62:270-271)

*autoclaved RO water

Appendix 8

ALB Diet Recipe

Ingredient		Batch Size				
Kettle	Mixer	Full = 22.9 L				
Water*	-	16,500 ml				
Agar (Gracilaria)	-	525 g				
		Group				
		1	2	3	4	5
-	Wheat Germ (Raw)	1,275 g	-	-	-	-
-	Torula Yeast	675 g	-	-	-	-
-	Wesson Salt	-	67.5 g	-	-	-
-	Sorbic Acid	-	45.6 g	-	-	-
-	Methyl Paraben	-	46.5 g	-	-	-
-	Sugar	-	375 g	-	-	-
-	Casein	-	225 g	-	-	-
-	Sodium Propionate	-	28.5 g	-	-	-
-	Wheat Germ Oil	-	-	105 ml	-	-
-	Cholesterol	-	-	22.5 g	-	-
-	Choline Chloride	-	-	-	6 g	-
-	Vitamin Mix (Bio-Serv)	-	-	-	99 g	-
-	Vitamin A Beadlets	-	-	-	7.5 g	-
-	Alphacel	-	-	-	-	2,925g

[Diet recipe from: Keena MA 2005, Entomol. Soc. Am. 98(4): 536-547]

*autoclaved RO water

Appendix 9

EAB Diet Recipe

Ingredient		Batch Size				
Kettle	Mixer	Full = 8.1 L				
Water*	-	5,000 ml				
Agar (Gracelaria)	-	220 g				
Sodium Bicarbonate	-	5 g				
		Group				
		1	2	3	4	5
-	Casein	200 g	-	-	-	-
-	Sucrose	300 g	-	-	-	-
-	Wesson Salt (no FePO)	45 g	-	-	-	-
-	Sorbic Acid	5 g	-	-	-	-
-	Calcium Proprionate	5 g	-	-	-	-
-	Methyl Paraben	5 g	-	-	-	-
-	Potato Starch	200 g	-	-	-	-
-	Soy Flour	-	500 g	-	-	-
-	Cholesterol	-	-	19 g	-	-
-	Wheat Germ Oil	-	-	24 ml	-	-
-	Choline Chloride	-	-	-	4 g	-
-	Vitamin A Beadlets	-	-	-	5 g	-
-	Vitamin Mix (Bio-Serv)	-	-	-	71 g	-
-	Alphacel	-	-	-	-	1,500 g

(Diet recipe from: Keena MA 2015. Great Lakes Entomologist *48(1-2):9-33*

*autoclaved RO water

Appendix 10

(Note: the contents of this electronic form change when selecting the "Diet Size" from the drop-down menu)

Regular Diet Checklist

Diet Size: **Full** ***Amount:*** **28.9 L**

Date Made: _____ **Prepared By:** _____
Date into Container: _____ **Checked By:** _____

Kettle

Water	17856 mL	☐	☐	
Agar	500 g	☐	☐	Lot #: _____

Mixer

					unit # of trays
Water	6336 mL	☐	☐		
Casein	1008 g	☐	☐	Lot #: _____	_____
KOH	144 mL	☐	☐	Lot #: _____	_____
Alphacel	144 g	☐	☐	Lot #: _____	_____
Wesson Salt	288 g	☐	☐	Lot #: _____	_____
Sugar	1008 g	☐	☐	Lot #: _____	_____
Wheat Germ (toasted)	864 g	☐	☐	Lot #: _____	_____
Choline Chloride	28.8 g	☐	☐	Lot #: _____	_____
Ascorbic Acid	115.2 g	☐	☐	Lot #: _____	_____
Formaldehyde	14.4 mL	☐	☐	Lot #: _____	_____
Methyl Paraben	43.2 g	☐	☐	Lot #: _____	_____
Aureomycin	62.5 g	☐	☐	Lot #: _____	_____
Linseed Oil	144 mL	☐	☐	Lot #: _____	_____
Vitamin Solution	288 mL	☐	☐	Date: _____	_____
Anti-Fungal Spray			☐	Date: _____	_____

Equipment

- Lab Coat ☐
- Latex Gloves ☐
- Aprons ☐
- Rubber Boots for Kettle ☐
- Safety Gloves for Kettle ☐
- Face Shield ☐

Distribution

Name	# of Trays	Fill	Comments
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

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Appendix 11

(Note: the contents of this electronic form change when selecting the "Diet Size" from the drop-down menu)

Bell Diet Checklist

Diet Size: **Half** ***Amount:*** **16.4** L

Date Made: _____ **Prepared By:** _____
Date into Container: _____ **Checked By:** _____

Kettle

Water	9450 mL	☐	☐	
Agar	225 g	☐	☐	Lot #: _____

Mixer

					cmt # of trays
Water	4050 mL	☐	☐		
Casein	375 g	☐	☐	Lot #: _____	_____
Wesson Salt	120 g	☐	☐	Lot #: _____	_____
Sorbic Acid	30 g	☐	☐	Lot #: _____	_____
Methyl Paraben	15 g	☐	☐	Lot #: _____	_____
Ascorbic Acid	57.6 g	☐	☐	Lot #: _____	_____
Choline Chloride	14.4 g	☐	☐	Lot #: _____	_____
Wheat Germ (toasted)	1800 g	☐	☐	Lot #: _____	_____
Linseed Oil	144 mL	☐	☐	Lot #: _____	_____
Vitamin Solution	144 mL	☐	☐	Date: _____	_____
Anti-Fungal Spray			☐	Date: _____	_____

Equipment

- ☐ Lab Coat
- ☐ Latex Gloves
- ☐ Aprons
- ☐ Rubber Boots for Kettle
- ☐ Safety Gloves for Kettle
- ☐ Face Shield

Distribution

Name	# of Trays	Fill	Comments

IPS Form Number 0017/004

Appendix 12

(Note: the contents of this electronic form change when selecting the "Diet Size" from the drop-down menu)

Addy Diet Checklist

Diet Size: **Half** ***Amount:*** ***16.8*** **L**

Date Made: _____ **Prepared By:** _____
Date into Container: _____ **Checked By:** _____

Kettle

Water	9450 mL	☐	☐	
Agar	250 g	☐	☐	Lot #: _____

Mixer

					omit # of trays
Water	4050 mL	☐	☐		
Casein	588 g	☐	☐	Lot #: _____	_____
Dextrose	588 g	☐	☐	Lot #: _____	_____
Wesson Salt	168 g	☐	☐	Lot #. _____	_____
Cholesterol	30 g	☐	☐	Lot #: _____	_____
Sorbic Acid	20.4 g	☐	☐	Lot #: _____	_____
Methyl Paraben	10.2 g	☐	☐	Lot #: _____	_____
Choline Chloride	16.8 g	☐	☐	Lot #: _____	_____
Alphacel	300 g	☐	☐	Lot #:	
Linseed Oil	72 mL	☐	☐	Lot #: _____	_____
Sodium Alginate	73 g	☐	☐	Lot #: _____	_____
Ascorbic Acid	73 g	☐	☐	Lot #: _____	_____
Formaldehyde	7.6 mL	☐	☐	Lot #: _____	_____
Aureomycin	23.5 g	☐	☐	Lot #: _____	_____
Ferric Phosphate	15 g	☐	☐	Lot #: _____	_____
Wheat Germ (toasted)	840 g	☐	☐	Lot #: _____	_____
Vitamin Solution	240 mL	☐	☐	Date: _____	_____
Anti-Fungal Spray			☐	Date: _____	_____

Equipment

- Lab Coat ☐
- Latex Gloves ☐
- Aprons ☐
- Rubber Boots for Kettle ☐
- Safety Gloves for Kettle ☐
- Face Shield ☐

Distribution

Name	# of Trays	Fill	Comments

IPS Form Number 0144/003

Appendix 13

(Note: the contents of this electronic form change when selecting the "Diet Size" from the drop-down menu)

ALB Diet Checklist

Diet Size: **Full** ***Amount:*** **22.9 L**

Date Made: _____ **Prepared By:** _____

Date into Container: _____ **Checked By:** _____

Kettle

Water	16500 mL	☐	☐	
Agar(Gracilaria)	525 g	☐	☐	Lot #: _____

Combine in kettle and bring to a full boil, add to mixer.

Mixer

Group 1					omit # of trays
Raw Wheat Germ	1275 g	☐	☐	Lot #: _____	_____
Torula Yeast	675 g	☐	☐	Lot #:	
Group 2					
Wesson Salt	67.5 g	☐	☐	Lot #: _____	_____
Sorbic Acid	46.5 g	☐	☐	Lot #: _____	_____
Methyl Paraben	46.5 g	☐	☐	Lot #: _____	_____
Sugar	375 g	☐	☐	Lot #: _____	_____
Casein	225 g	☐	☐	Lot #: _____	_____
Sodium Proplonate	28.5 g	☐	☐	Lot #:	
Group 3					
Wheat Germ Oil	105 mL	☐	☐	Lot #: _____	_____
Cholesterol	22.5 g	☐	☐	Lot #: _____	_____

Mix for 4 minutes to allow diet to cool.

Group 4					
Choline Chloride	6 g	☐	☐	Lot #: _____	_____
Vitamin Mix (Bioserv)	99 g	☐	☐	Date: _____	_____
Vitamin A Beadlets	7.5 g	☐	☐	Date: _____	_____
Group 5					
Alphacel	2925 g	☐	☐	Lot #: _____	_____

Equipment

Lab Coat	☐
Latex Gloves	☐
Aprons	☐
Rubber Boots for Kettle	☐
Safety Gloves for Kettle	☐
Face Shield	☐

Distribution

Name	# of Trays	Fill	Comments

IPS Form Number 0145/003

Appendix 14

EAB Diet Checklist

Diet Size: **Full** ***Amount:*** **8.1 L**

Date Made: _____ **Prepared By:** _____
Date into Container: _____ **Checked By:** _____

Kettle

Water	5000 mL	☐	☐		Unit # Lites
Agar(Gracilaria)	220 g	☐	☐		
Sodium Bicarbonate	5 g	☐	☐	Lot #: _____	_____
	0				

Mixer

Group 1					Unit # Lites
Casein	200 g	☐	☐	Lot #: _____	_____
Sucrose	300 g	☐	☐	Lot #:	
Wesson Salt (no FePO)	45 g	☐	☐	Lot #. _____	_____
Sorbic Acid	5 g	☐	☐	Lot #: _____	_____
Calcium Propionate	5 g	☐	☐	Lot #: _____	_____
Methyl Paraben	5 g	☐	☐	Lot #: _____	_____
Potato Starch	200 g	☐	☐	Lot #:	
Group 2					
Soy Flour	500 g	☐	☐	Lot #: _____	_____
Group 3					
Cholesterol	19 g	☐	☐	Lot #: _____	_____
Wheat Germ Oil	24 mL	☐	☐	Lot #: _____	_____
Group 4					
Choline Chloride	4 g	☐	☐	Lot #: _____	_____
Vitamin A Beadlets	5 g	☐	☐	Lot #: _____	_____
Vitamin Mix (Bioserv)	71 g	☐	☐	Lot #: _____	_____
Group 5					
Alphacel	1500 g	☐	☐	Lot #: _____	_____

Equipment

- Lab Coat ☐
- Latex Gloves ☐
- Aprons ☐
- Rubber Boots for Kettle ☐
- Safety Gloves for Kettle ☐
- Face Shield ☐

Distribution

Name	# of Trays	Fill	Comments

IPS Form Number 0163/002

Appendix 15

4M KOH Solution
(1 Litre)

Ingredients:

- RO Water (autoclaved) 1 L
- Potassium Hydroxide pellets 224 g

Procedure:

1. Pour 1 L sterile water into a 1 L beaker.
2. Add 224 g KOH pellets.
3. Stir until dissolved (caution: solution gets hot).
4. Prepare aliquots in sufficient volumes for full, half and blender batches (refer to Appendices 5-7 for diet recipes).
5. Label bottles with the ingredient name and batch size, and maintain in an opaque bin (labeled as 4M KOH) in the walk-in cold room.
6. Label the bin with the date that it was prepared.

Appendix 16

Anti-fungal Spray Solution
(4 Litres)

Ingredients:

- Sorbic Acid 60 g
- Methly Paraben 24 g
- Ethyl Alcohol (95%) 4000 ml

Procedure:

1. Add 60g Sorbic Acid and 24g Methyl Paraben to a large plastic jug.
2. Add 4000 ml 95% ethyl alcohol and stir until powdered ingredients have dissolved.
3. Pouring some of the solution into a 1L spray bottle, as required.
4. Label both containers as Antifungal Spray Solution and with the date that it was prepared.
5. Store both containers in the chemical fume hood. (No expiry date is required).

Appendix 17

Month:

Diet Orders

Week of:

Name	Diet Type	# of Trays	Special Request

Week of:

Name	Diet Type	# of Trays	Special Request

Week of:

Name	Diet Type	# of Trays	Special Request

Week of:

Name	Diet Type	# of Trays	Special Request

Week of:

Name	Diet Type	# of Trays	Special Request

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