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Cleaning and Disinfection & Biosecurity
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III. 

Environmental Sampling in Pullet and Layer Houses
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Introduction
The procedures outlined below are meant to be used as guidelines as it may not be economical to institute ideal C and D procedures for all farms. These procedures can be adapted to individual farms on a step-wise basis- any reduction in the overall pathogen load will be beneficial. Rodent control will be addressed in the Vector Management Training Team.

Cleaning and Disinfection
There are three major steps in C and D:
Removal of debris
Thorough cleaning of surfaces
Disinfection

Removal of debris
-Ideally complete removal of debris will be performed after every cycle (between bird placements)
-During cycles and while birds are present, at minimum, carcasses, trash, spilled feed, rodent nests and other loose debris should be removed on a regular basis
-Remove caked, wet or fouled litter regularly- rows can be alternately cleaned
-Reusing litter is not recommended for layer/breeding flocks-in cases where old litter is used for seeding in insect control programs, the old litter must be dry and free of carcasses (both rodent and chicken). Drying and/or exposure to heat/sunlight will kill many pathogens
-Spray insecticide immediately after removing fowl and wait before removing litter (if insect problem is out of control or chemical vector control is used rather than the “old litter seeding” method)
-Dispose of litter far from the facilities- if necessary, wet with a disinfectant or burn- with most disease agents composting is okay
-Dispose of carcasses- pit or tank away from ground water with cover so no one falls in-cover sealed with a foot of soil overlay; electrically heated septic tank; burning (EPA approved incinerator); rendering (cans used pt haul carcasses must be sterilized

Cleaning of surfaces
-The purpose of cleaning surfaces is to remove all organic matter-most disinfectants will not work in organic matter and many pathogens are able to live for long periods when protected by the organic matter
-Dry cleaning can be used alone of in combination with wet cleaning if the dry cleaning is able to remove all debris (egg matter generally cannot be removed with dry cleaning)
-With wet cleaning, allowing a soaking time will loosen up debris so that it is more easily removes with a pressure sprayer or brush
Pressure sprayers should not be used with birds still in the building as it can aerosalize pathogens.

- Vents, fans outlets, feed lines and augers and manure pits require special attention - those items (such as electrical) which cannot be wet may need fumigation after dry cleaning.
- Hot water (200 degrees F) pressure spray has been proven to be better at killing bacteria than cold water.
- Detergents will facilitate cleaning but must be compatible with disinfectant.
- Better to clean house with out removing large equipment, if possible.
- If detergent is used, a clean water rinse is recommended.
- Standing water should be mopped up and residual water removed from pits before disinfectant is applied.

**Disinfection/disinfectants**

- Must have clean surfaces.
- Disinfect as soon as possible after cleaning.
- It is best to have 2-4 weeks down time after drying of disinfectant.
- Need to consider pH, water hardness, environmental temperature, compatibility with detergent, EPA implications, ease of application, effectiveness in the presence of organic matter, environmental surface porosity, traffic flow, type of microbial flora, contact time, OSHA implications and corrosiveness.
- Rotating low pH with high pH COMPATIBLE disinfectants has proven to be more effective than continually using the same disinfectant.
- Calculate the total surface area of the building then add 30% for the cages.
- Flush water lines with disinfectant.

**Types:**

Phenol (carbolic acid): made from coal tars. Usually in water solutions, expensive, other disinfectants generally compared to it (phenol coefficients), little residual activity, good for high risk areas, effectiveness reduced by presence of cations, may present disposal problems, non sporicidal, good in organic matter, slightly corrosive, not affected by heat, most common disinfectant used for SE control, can be applied by fogging, can be tuberculocidal.

- Cresols: related to phenols, similar bactericidal properties, not very soluble, residual activity, corrosive and staining, good in organic matter, not affected by heat.
- Bisphenols: two phenols hooked together, good when combined with chlorine, some combinations have good antifungal activity, usually combined with other phenols.
- Pine oil: not as caustic as cresol, better odor, insoluble in water and must be emulsified with soap or other emulsifier.

Chlorine, hypochlorites, chlorinated lime: available as powders, liquid, germicidal activity dependent upon concentration of available chlorine and the acidity of the solution (increase pH/decrease biocidal activity) hypochlorites have about 70% available chlorine, corrosive, no residual activity, irritating but broad spectrum, good for egg washing, not good in the presence of organic matter, fresh solutions must be made daily, solutions need to be stored in dark, cool areas, usually not affected by hard water, poor cleaning ability, bad odor.
Iodines; when combined in organic complexes they are called tamed iodines, an iodophore is a combination of iodine with a solubilizing agent that slowly liberates free iodine, amber color fades when the solution is used up, can be mixed in cold and hard water, staining and corrosive/irritating properties mellowed by the organic combination, heat inactivated, OK with hard water, not corrosive, not effective in organic matter. -Quaternary ammonium: broad spectrum, clear odorless, nonirritating, good detergent activity, stable, relatively non toxic, cannot be used in soapy solutions, not good in hard water, widely used, residual activity good, affected by heat, not good in organic matter, surfaces must be thoroughly rinsed of detergent before use, may be bacteriostatic, nonirritating to skin.

-Formaldehyde: great but not allowed without special permit, eggs still fumigated in order to decrease Salmonella – however – aeration afterwards may result in excess removal of carbon dioxide in the egg and reduce hatchability – humidity is essential, formaldehyde is generally combined with potassium permanganate to generate gas – both substances and the gas are toxic, volatile, caustic, good in organic matter.

Glutaradehyde: non-staining, non-corrosive, not stable in solution, sporocidal, tuberculocidal, virucidal, can be corrosive in low pH solutions, irritating to skin and mucous membranes, not good in organic matter, bad odor

Others:

Alcohol: too flammable, good broad spectrum activity, may have residual activity when formulated with quats or phenolics.

Ethylene oxide: too flammable

Copper Sulfate: ineffective as a disinfectant, may limit spread of fungal infections when used in the drinking water.

Quicklime: must be used in damp areas as depends upon water to liberate heat, caustic to birds until dry, can be used in drains.

Disinfectants approved for use in California (not an all inclusive list): Environ, One Stroke Environ, LPH Ag, Advantage 256, PHD 22.5, Tek Trol, Discan 256, Pantek, DC+R, Process NPD, Oxysept 333, Iodu 2, Quorum Clear, Environ D.

**Microbial Monitoring**

-To evaluate the efficacy of any C & D program, a system needs to be established for monitoring (see below)

**Biosecurity / Disease Surveillance**

Facility location/accessibility/security systems – factors to be considered:

- Neighbors
- Flyways
- Large highways
- Natural barriers
- Prevailing wind currents
- Location of poultry units on the farm
- Movement of vehicles and equipment both onto and within farm
- Work traffic of caretakers, vaccination crews, etc.
- Fencing
- Alarm systems both for equipment failure and invaders
Managing employee accessibility
One way doors
Foot pans
Shower in/ shower out
Daily change of clothing
Hand washing
Wear hats
Non contact with other animals / birds of same species
Good relationship between management and employees
Limit/ eliminate visits to other farms

Control of non-human visitors
Bird proof (screening, etc) including feed areas and crate storage sheds
Decrease insects, flys
Rodents – important in Salmonella control
Household pets
Inanimate sources – trucks, feed, crates (avoid re-use of cardboard trays)
Pelleting does not sterilize feed
Remove trash and spilled feed
Repair feed troughs
Use an apron of treated soil / concrete around buildings – prevent vegetation around buildings

Control of human visitors
Work crews and their equipment
Veterinarians
Feet and hands are the worst offenders (Newcastle disease virus can last several days in the human respiratory tract)
Inseminators

Stocking of birds
Use disease free stock (National Poultry Improvement Plan) to reduce likelihood of recovered carriers
Do not obtain from multiple sources
All in all out in same building
No multiple ages in same building
Quarantine (4-6 weeks, no medicated feed, can put naïve sentinels in with quarantined animals in order to detect disease)
No mixed species (chicken are less susceptible to enterohepatitis than turkeys)
No hospital pens that keep birds from multiple sources
Cull pens in buildings only for birds in that building or keep cull pens separate
Since some diseases are egg borne, treat eggs as if they are already hatched

Disease surveillance
Monitor stock serologically
Monitor the environment (swabs, open culture plates) – in processing rooms, open plates and expose to environment for 10 minutes on an off day (hot weather and older flocks will generally be correlated with a higher microbial load)
Monitor eggs for disease and other aspects such fertility
Monitor egg residues in hatchers
Culture a representative population of chicks immediately post hatch
Monitor vaccines and diluents
Monitor after sanitation

Environment
Housing type
Impervious surfaces
Covered light
If possible, cover all items with impervious surfaces that cannot be exposed to a wet disinfectant

Temperature
In California, can generally be controlled by ventilation

Ventilation
Ammonia buildup = tracheal cilia destruction
Long term dust and dry litter exposure – colibacillosis
Wet litter = coccidiosis

Bird density
Do Not Overstock

Common Disinfectants, Spectrums and Physical Characteristics

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Broad</th>
<th>Irritating</th>
<th>Residual</th>
<th>Corrosive</th>
<th>Staining</th>
<th>Active in Presence of Organic Matter</th>
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<tbody>
<tr>
<td>Chlorine</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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<td>Iodine</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Phenol</td>
<td>Yes</td>
<td>Yes</td>
<td>Slight</td>
<td>Slight</td>
<td>No</td>
<td>Yes</td>
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</tr>
<tr>
<td>Quaternary Ammonium Compounds</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes (reduced)</td>
<td>Heat affected</td>
</tr>
<tr>
<td>Cresol</td>
<td>Moderate</td>
<td>Yes</td>
<td>Yes (in oil)</td>
<td>Yes</td>
<td>Yes</td>
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<td>No</td>
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<tr>
<td>Formaldehyde</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Very</td>
<td>No</td>
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</table>
Environmental Sampling in Pullet and Layer Houses
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Variations in poultry house design and/or unsuitable manure pit conditions will require appropriate adaptations for collecting representative manure environmental samples. Unsuitable manure pit conditions could include situations where manure is piled very high, liquid, or semi-liquid manure.

The following describes various situations:
A. Deep pit manure collection houses

This protocol is modeled after the Pennsylvania Egg Quality Assurance Program (PEQAP)

1. Protocol for collection of manure samples in pullet and layer houses with deep pit manure collection.
2. Purpose: The purpose is to collect representative manure samples in pullet and layer houses.
3. General
   a) The PEQAP calls for two manure samples per bank. These samples must be representative of all birds in the house. To make the samples representative of all the birds, dragging swabs along each row of manure for the entire length of the house is recommended.
   b) It is important to maintain as much consistency as possible when collecting samples. This helps ensure valid results.
4. Equipment required
   a) Standard biosecurity equipment and guidelines (see attached)
   b) One small cooler with 2 frozen ice packs
   c) One small garbage bag containing the following:
      1) Whirl-Pak 2 per bank, pre-numbered these with the house collection number. Number the banks of the house from left to right as you face the banks.
      2) Laboratory gloves – one pair per bank.
      3) Large garbage bag to serve as a table cloth or a table cloth of some sort.
   d) Sterile 4”x 4” 12 ply gauze sponges pre-prepared as drag swabs 2 sponges per bank. These should be pre-prepared in autoclave packs with the required number of sponges plus two extra.
   e) One can of lite evaporated skimmed milk. One alcohol swab (to disinfect the can before opening).
   d) One pair of scissors.
   e) One can opener.
   f) One felt tipped marker.
   g) One zip-lok or equivalent type one gallon plastic bag.
h) One set of manure drag poles. These can be constructed from 3/8” x 42” solid aluminum rod with ¼” hole drilled ½” from one end. Alternatively, they can be constructed from ½” x 3” conduit with a ¼” hole drilled ½” from one end. The solid aluminum rods are easier to clean and disinfect.

5. Procedure
   a) Suit up and disinfect before entering the house in accordance with standard biosecurity practices (see attached).
   b) Bring all materials to the bottom floor. Use the bottom utility area if the house has one. Bring bucket filled with a disinfecting solution such as “Environ 1 Stroke” and brush for disinfecting equipment. Spread out the large garbage bag and arrange the material on it. Number the Whirl-Pak bags with the bank number if they have not been pre-numbered.
   c) Open the alcohol swab and wipe the top of the can of lite evaporated skimmed milk.
   d) Disinfect the can opener and scissors with the sanitizing solution in disinfection bucket. Use the can opener to open the can. Use the scissors to cut open the autoclave pack of swabs near the top of the pack.
   e) Moisten the swabs in the pack by pouring the milk into the pack and massaging the outside of the pack. Lay the pack on the garbage bag.
   f) Tear off the top of the two Whirl-Paks for bank 1.
   g) Put on a pair of laboratory gloves.
   h) Banks will be sampled from left to right. The bank at the far left will be labeled bank 1.
   i) Tie the swabs to the pole. Tie one swab slightly ahead of the other one (if two swabs are used simultaneously on one pole) to give maximum surface area.
   j) Walt the length of the house dragging the swabs along one side of the top ridge of the manure. Sample one or two banks at a time. Drag the other side of the ridge on the way back.
   k) Place each swab in a Whirl-Pak without touching the swab. Cut attaching string. Add approximately 5 ml of milk and close Whirl-Pak. Place Whirl-Pak in a one gallon plastic bag.
   l) Use the bucket and brush to disinfect poles and scissors.
   m) Remove gloves, tear off top of the next Whirl-Pak, put on a clean pair of gloves and remove two additional swabs from the autoclave pack.
   n) Drag the remaining banks as noted above.
   o) Seal the one gallon plastic bag and place it in the cooler.
   p) Put all the discarded material in the garbage bag.
   q) Place the cooler outside the house, clean and disinfect the coolers, then load them into vehicle.
   r) Follow standard biosecurity procedures when leaving.
   s) Transport samples to the processing facilities within 24 hours.

B. Shallow pit manure collection houses
Attach two drag swabs on the manure scraper assembly and run manure scraper to opposite end of the house where swabs are removed and placed in appropriate Whirl-Pak.

Shallow pit operations all have some type of manure scraper. Some have scraper under each tier, some have floor scraper only, and some have a combination of both. Each scraper blade must be swabbed. Sample only solid manure on the scraper. The ammonia in the pit liquid may inhibit Salmonella growth. Use a separate swab for each scraper, but place all swabs in one Whirl-Pak and label accordingly. Example: H1, B1 M (House 1, Bank 1, Manure). If there is any tier without an operable scraper, the drop board should be sampled. If the drop board is not accessible due to other reasons, then that situation must be dealt with to insure a representative sample collection.

Supplies needed for elevator swabbing remain the same as in deep pit operation. The supplies needed for manure sampling are 2 sterile 4”x 4” gauze sponges per scraper. Sterile drag swab should be available where and when the scrapers are difficult to reach.

C. Manure belt manure collection system
Hook up the swabs into certain spots where they could be in contact with the manure. Turn the manure belt on until the full length of the belt has contacted the swabs. Remove the swabs, identify them, and place them in the Whirl-Pak.

D. Manual egg collection system
In this type of operation there will be no sampling of egg belt, and elevator, because there is none to sample. However two manure samples per bank should be collected.

E. Floor layer operation
Three total samples (2 floor litter, and 1 nest-box) per house or per floor if a house has multiple floors should be collected. Two autoclaved per-prepared drag swags shall be dragged over the litter surface for a minimum of 15 minutes.

One next-box sample shall be collected by using two sterile sponges. The sponges will be used to wipe over the entire surfaces of a minimum of ¼ of the total next-boxes in the house.

F. Manure pit unsuitable for dragging
   a) Hand swab egg belts (approximately 10-12 ft, on each cage level) and the de-escalators on each bank of cages. Sampling time should be from 3 ½ to 5 minutes per each bank of cages. One swab on each side of the bank will make a sample with two swabs in one Whirl-Pak for each bank in house.
   b) Walkway drag swabs. Place two drag swabs on one drag pole and drag walkways. One set of swabs for each two walkways comprise a Whirl-Pak sample.
Basocyte Guidelines

Microbes can be carried on clothing and transferred from poultry house to poultry house. It is essential that personnel develops a sensitivity to the presence of microorganisms and the various means by which they can be spread.

The following guidelines are recommended for individuals when visiting any poultry farm:

1. At each premises put on clean rubber boots and freshly laundered or disposable coveralls. Disinfect boots immediately upon arrival using “Environ 1 Stroke” or another disinfectant.
2. If there is no reason for entering a poultry house DON’T.
3. When entering poultry houses wear freshly laundered or disposable coveralls, head gear, and footwear.
4. Between each poultry house entered on the same premises change disposable footwear (and other outerwear if soiled) wash hands and disinfect rubber boots.
5. Place all disposable items in sealed plastic bags for proper disposal off the premises. Incineration is the ideal method of disposal.
6. Always wash hands, C&D rubber boots and disinfect all equipment when exiting.
7. Do not visit more than one premises per day.
8. After visiting a premises; shower, shampoo, and thoroughly clean hands (particularly under the fingernails).
9. Launder coveralls and clothing before using around another poultry.
10. Vehicle biosecurity:
   a) Keep flies out of vehicle by keeping windows closed while on premises
   b) Each vehicle should be equipped with a bucket, sturdy brushes, a supply of “Environ 1 Stroke” or another approved disinfectant, and water.
   c) Keep interior of vehicle clean; avoid clutter. Disinfect and vacuum the interior between premises as necessary.
   d) Wash the exterior of vehicle before use on another premises.