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## Environmental Monitoring for Salmonella

Determining the status of a flock is a very important control point within a salmonella reduction program. In the case of breeders, if salmonella has been isolated from the flock then increased biosecurity and sanitary measures can be put in place to help stop the spread within the farm, to the progeny or to the next flock placed in that barn. Without a good representative sample, flocks with a low level of contamination will be missed. There are many different ways of monitoring a flock.

**Swab sampling** can be done on freshly produced cecal droppings as salmonella prefer to colonize this area of the gut. This way of swabbing guarantees an individual bird sampling. The individual swabs are pooled usually 5 or 10 per pool and put into pre-enriched fluid. Although swab samples will show which birds are shedding salmonella at the immediate time of testing, sampling the environment is a more reliable indication of the true salmonella status of the flock. This is especially important within the laying barn, as the eggs will be exposed to the barn environment. Although an individual hen may not be shedding salmonella, the environment into which the egg is exposed may be positive. In practice it is more important to know whether a flock is salmonella positive than the incidence of positive birds within the flock.

In the USA, **drag swab sampling** is commonly used to determine the salmonella status of a flock. A moistened, sterilized cloth (10 x 14 in./25 x 35cm)

attached to a length of string, is dragged over the litter throughout the house. This piece of cloth, with the attached litter and feces is considered to be the sample and incubated completely.

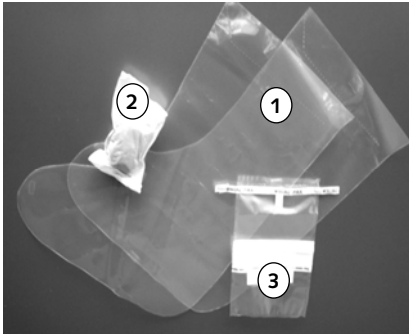
The **overshoe** method first described by Aho has been shown to be a practical, sensitive technique to collect samples from the barn environment. He used plastic overshoes to walk across the poultry house and incubated the overshoes with the attached organic material intact. The amount of organic material depends on factors such as the humidity of the litter and roughness of the shoe surface. The number of samples that need to be taken was estimated according to Aho (1992). Five pairs of overshoes will detect salmonella in a flock with only 1% of contaminated individuals. One pair of overshoes however, will detect salmonella in a flock if 5% of the flock is contaminated. This level is sensitive enough for practical monitoring of commercial and breeder flocks.

In a study done in 1996, Caldwell and associates compared the frequency of salmonella isolation from overshoes versus conventional drag-swab methods. Their results showed that salmonella was detected with equal frequency by both methods on nine individual broiler farms over three separate sampling periods. Both methods were equally as likely to detect Salmonella when houses were either vacant (awaiting the placement of the next flock) or occupied.

At Hybrid, we use overshoes that are normally used in surgery rooms in hospitals. We make our own environmental monitoring kits, which contain the following:

a. 2 Disposable plastic boots

b. 2 Sterile gauze surgical overshoes or booties (we pack the booties in a 4" x 8" sterilization pouch, pictured below)



c. 1 Sterile Whirl-pak bag with a label to record important information such as flock identification, flock age, date, barn, farm and name of the person collecting the sample.

Kits should be stored in a clean dry place. When sampling a flock, never use a kit which has been opened or if the bag containing the sterile booties has been torn. You must ensure that the kit is not contaminated before it enters the environment to be sampled. It is recommended to store the kits in a sealed container of some kind if you are going to be carrying some with you in your vehicle.

### Procedure for Overshoe or "Bootie" Sample Collection:

1. Wash and disinfect footwear before entering the barn.
2. It is very important to either sanitize your hands using alcohol hand gel or if preferred wear sterile gloves.
3. Open the kit and put the disposable plastic boots over your clean sanitized footwear.

4. Open the bags containing the booties and put them on.

5. Walk throughout the barn for at least 15 minutes.



6. Remove the booties and put them into the sterile Whirl-pak bag.

7. Seal the Whirl-pak bag. Write necessary identification on the bag such as: date, farm name, barn or flock number.

8. Refrigerate the sample until sent to the lab or place in a small cooler with an ice pack.

### Laboratory Processing:

When samples arrive at the lab 45 ml of buffered peptone water is added to each Whirl-pak bag and they are put in the incubator for 24 hours at 37°C (98.6°F). After this a 1 ml sub-sample is put into 9 ml of tetrathionate broth with brilliant green and incubated for 24 hours at 43°C (109.4°F). From here samples are plated onto selective media and/or a sub-sample put into M broth for ELISA testing.

### Our Experience

We have been using this technique at Hybrid Turkeys to collect environmental samples for the past 15 years. The advantages of the overshoe method are the following:

Any individual can be easily trained to take this type of sample. It does not require laboratory personnel to do it. As such, additional time is not

expended to take the samples. The farm manager or service person can wear the booties during the course of making a routine barn check or flock inspection.

It is physically easier than the drag swab method. Squatting hens, aggressive toms and curious commercials can often limit the amount of a “floor” drag swab actually contacts. With the overshoe method, wherever you go, the swab goes and samples that area.

They can be easily sent by mail or courier to a laboratory for culturing without worry of leakage, as they do not contain any liquid media (versus drag swabs). The Hybrid laboratory has easily recovered salmonella from bootie samples taken 48 hours earlier.

Preplanning is not required. Because the kits can be carried in a container in a service vehicle (keep sterile!) or stored on farm, samples can be taken at any time. Drag swabs, which are received frozen, must be used once thawed.

In conclusion, the overshoe or bootie method is a convenient and reliable method of monitoring the barn environment for the presence or absence of salmonella. Knowing the status of all flocks on a farm is a crucial critical control point in a salmonella reduction or eradication program.

## References

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