§ 147.13 Procedure for bacteriological culturing of eggshells for colon bacilli organisms.

Proper precautions to avoid environmental contamination of the samples during the collection and laboratory process, and proper handling of the samples following collection are essential. Each State Inspector involved in eggshell culture activities must receive instruction in the necessary sanitation procedures, sampling procedures, and sample handling by the authorized laboratory involved. The Official State Agency will maintain a record showing that the required instruction was given to each State Inspector.

(a) Sample selection. Forty (40) eggs in the top flats of each of three randomly selected cases of sanitized eggs from each flock will be utilized for each sampling.

(b) Swab procedure. A 2.5 centimeter diameter circular area of the large end of each of the eggs will be rubbed with a sterile swab previously moistened with sterile lactose broth, or other suitable liquid media provided by the authorized laboratory. One swab will be used for five eggs, and four swabs will be pooled to each sterile, capped tube provided by the authorized laboratory.

1. From the tube containing four swabs and lactose broth or other suitable media, 1 ml will be transferred to 10 ml lactose in a fermentation tube.

2. Incubate at 37 °C for 48 hours. The presence of acid, and gas in the amount of 10 percent or more after 24 and 48 hours indicates the presence of colon bacilli organisms.

Obtain procedure for preparing double strength skim milk from USDA-APHIS “Recommended Sample Collection Methods for Environmental Samples” available from the National Poultry Improvement Plan, Veterinary Services, APHIS, USDA, 1498 Klondike Road, Suite 200, Conyers, GA 30012.
§ 147.15 Laboratory procedure recommended for the bacteriological examination of mycoplasma reactors.

(a) Turbinates, trachea, air sacs, sinuses, nasal passages, respiratory exudates, synovial fluid, eggs (including yolk, yolk sacs, membranes and allantoic fluid), should be directly sampled with sterile swabs. Aseptic techniques are very important as some organisms may not be suppressed by the antimicrobial agents used in this procedure. Tissue suspensions from large volumes are sometimes desirable from the sites listed above and occasionally from the oviduct and cloaca. Tissues should be ground or blended completely in 10 times their volume of Mycoplasma Broth Medium (MBM). (See paragraph (f) of this section.) Specimens submitted to referral laboratories in order of preference for recovery of the mycoplasma organisms are: (1) live birds, (2) refrigerated fresh tissues, (3) tissue specimens packed with dry ice.

(b) Inoculate 5–10 ml of MBM with a swab, wire loop or 0.1 ml of the tissue suspension. When evidence of growth is observed (lowered pH or turbidity of broth) transfer each broth culture as needed to maintain the original isolates. Incubate tubes at 37 °C for at least 21 days before discarding as negative. When growth is first observed or if no growth occurs by the 4th or 5th day of incubation, inoculate broth culture onto a plate of Mycoplasma Agar Medium (MAM). (See paragraph (g) of this section.) Several cultures may be inoculated on one plate by using a wire loop or a cotton swab. Incubate plates 3–5 days at 37 °C in a high humidity chamber. If preferred, 5 percent CO₂ may be added or a candle jar may be used. Tiny circular and translucent colonies with elevated centers are very suggestive of mycoplasma. Indirect lighting and a low power or dissecting