

Microbiological Monitoring for a Hatchery QA Program

Microbiological monitoring is an essential element of any hatching egg quality assurance program. The contamination of the eggs or the chicks can come from the breeder house, egg holding facilities, or the hatchery. Microbiological monitoring evaluates the hatchery sanitation program, the quality of the eggs entering and chicks leaving the hatchery. The monitoring program needs to be conducted on a routine basis, the results carefully interpreted, and the data filed in a manner which allows for review of data over time. Monitoring programs used in the hatchery can have two levels of intensity. The first level would be for the routine program. The second, more intense level, would be used to solve a specific problem. The first level would include air and surface sampling. The second level would involve egg washes, chick sampling, and bacterial identification.

Monitoring Methods:

A variety of methods can be used in monitoring, depending upon the sample type and personal preference. When sampling the air in an area, air plates or a mechanical sampler may be used. The swab and streak procedure or the RODAC plate procedure can be used to monitor flat surfaces. To monitor eggshell surfaces, the sterile tape procedure, the total egg wash procedure, or the egg roll on media procedure are appropriate. Chicks can be sampled by the yolk sac swab procedure. Liquids may be monitored using media plates.

While a variety of microorganisms can cause problems in the hatchery, not all types of microorganisms can be detected by the monitoring program described here. Molds (including *Aspergillus*) and bacteria (e.g. *Pseudomonas*, coliforms and staphylococci) can be detected, but this program is not designed to detect viruses. Virus isolation is a more complicated procedure. However, monitoring for bacterial and mold presence will assess the efficacy of the sanitation procedures being used.

Media to be Used in Monitoring:

Media used to grow molds or bacteria may be either "liquid" (which is called broth) or "solid" (which is called agar). Although agar media are called "solid" media, their consistency is like that of very hard jello. Agar media are generally used to determine the types and numbers of microorganisms present, while broth media are used to isolate or identify the specific organisms which grow on agar media.

Agar media provides the nutrients required by microbes to grow. The various types of microbes have different nutrient requirements for growth, and/or are inhibited by antibiotics or chemicals. These different nutrient requirements and susceptibility to inhibitory compounds allow one to use a selective type of agar to determine number and type of microbe present in a given location. The type of agar chosen will depend upon which type of organism one wishes to monitor and how specific one wishes to be in the type of organism monitored. However, it is important to consistently use the same type of agar so that comparisons of colony counts can be made over time. Some types of agar are suitable for routine monitoring of the total microbial load in the hatchery, while other types should be used in more intensive problem solving situations to help identify specific types of bacteria.

Tryptic soy agar (TSA) or nutrient agar are general purpose media for bacteria. Neither of these media contain inhibitory chemical compounds and should allow many types of bacteria to grow. However, TSA may be the better of the two since it contains two peptones and is designed to support the growth of a wide variety of delicate or fastidious organisms. General purpose bacterial media are usually incubated at 37°C (98.6°F) for 48 hours before counting.

Sabouraud dextrose agar or potato dextrose agar are general purpose media for molds and yeasts. Potato dextrose agar (PDA) may be slightly superior to sabouraud dextrose agar (SDA) for growing molds since SDA was originally formulated to detect fungi associated with skin infections (such as ring worm). Media which are used to enumerate molds will often include antibiotic(s) [such as chloramphenicol (0.05 g/liter)] to inhibit bacterial growth and allow the molds present to grow. To prevent loss of activity, antibiotics are generally added following autoclaving and cooling of the medium. Molds tend to grow at a slower rate than bacteria. Thus, general purpose mold media are generally incubated at room temperature for 3 to 5 days.

MacConkey agar (MA), violet red bile agar (VRB) or eosin methylene blue agar (EMB) are media commonly used to detect coliform bacteria. Coliform bacteria are those which resemble or are close relatives to Escherichia coli. While all three media are good, MA probably detects a slightly wider array of bacteria than the other two. MacConkey agar is usually incubated at 37°C for 48 hours. Coliforms (including E. coli) produce colonies which are red in color on MA, but clear or colorless colonies should also be counted since these colonies may indicate the presence of other harmful bacteria (e.g. Salmonella).

Consistently monitoring hatcheries with these three media (TSA for total bacteria, PDA for molds and yeasts, and MA for coliforms) will provide a good general picture of the microbial load in a given area. These media can be purchased as pre-poured plates from a number of scientific supply houses. Media for the detection of specific groups of organisms such as Pseudomonas, Staphylococcus, Streptococcus and Aspergillus are also available. While pre-poured media plates are more expensive than dehydrated media prepared by your personnel at your facility, the quality and sterility of these media are generally assured by the companies which prepare them. Since media quality and sterility can affect the count obtained, quality and cost should be taken into consideration when making the decision as to whether to purchase or prepare your own media.

Making Media for Hatchery Monitoring:

Should you decide to prepare your own agar media, equipment (hot plates, autoclaves and gram scale) and supplies (such as glassware and plates) may be purchased from scientific supply houses. Prepare media in a room within the hatchery which

has minimal traffic flow and minimal airborne contamination. The vaccine room might be a good media preparation room. Media should be made in tempered glass containers (such as pyrex) using the directions on the media package as a guide. Media should be mixed with an appropriate amount of water and heated using a stirring hot plate. Media should be completely melted and dissolved prior to autoclaving. Media are usually completely melted and dissolved when they are clear not cloudy, but caution should be taken not to overheat after clearing. Media should then be autoclaved at 15 psi of steam (121°C or 250°F) for 15 minutes or as indicated in the package directions. **Caution:** media can be autoclaved too long, when this happens sugars in the media may caramelize, peptones may degrade and/or other heat sensitive media components may be destroyed. Thus, overheated media should be discarded. Media is extremely hot following autoclaving and should be allowed to cool to the point where it can be handled with bare hands (approximately 110°F to 115°F).

Filling the Petri and RODAC Dishes: Petri dishes (15 x 100 mm) to be filled with media should be placed on a level counter top or table. The plastic sleeves which contained new dishes should be saved for plate storage after media is dispensed. About 20 ml of media should be poured into each plate. This amount of media will fill each plate 1/2 to 3/4 full. Care should be taken to observe aseptic technique when pouring plates. Plates spill easily prior to hardening and should be handled carefully prior to solidification. However, to save space, plates may be stacked as you pour them.

RODAC plates are filled with media in similar fashion to standard plates except 16.5 to 17.5 ml of agar are poured into each plate. In addition, special care must be taken to avoid bubble formation and overflow of media. The objective when pouring RODAC plates is to make a perfectly convex surface with the agar. Thus, a level surface is important.

After plates have cooled to room temperature they should be incubated overnight at 37°C to check for sterility. Plates which have colonies on them following incubation should be discarded, while those without colonies should be stored in the plastic sleeves you saved from new plates. Sleeves should be dated, inverted to prevent condensation from falling on the media surface and placed in a refrigerator to minimize drying.

Sampling the air:

The air plate is the most commonly used

procedure. Areas commonly sampled using this method are hatchery hallways, egg rooms, chick rooms, setters and hatchers. The bottom of the plate should be marked with the location to be monitored. The petri plate with the selected media is exposed by carefully placing the plate (media half on the bottom) on a flat surface within the environment to be monitored and gently removing the cover, letting it rest on a clean surface. The length of time that the media is exposed to the air will depend upon the expected contamination level of the area. It is extremely important that the length of exposure is consistent when sampling the air in a particular environment from one time to the next to allow for meaningful comparisons. If the area is a relatively clean environment such as a setter, hallway, or cleaned hatcher, a ten minute sampling time is suggested.

Mechanical air sampling devices (such as the Anderson Air Sampler and the RCS unit) are generally battery powered and allow a set volume of air to pass over a set of agar wells which allows the operator to assess the contamination level. The standard time is one minute. The type of agar media in the wells can be varied depending on the sampling goal.

Sampling flat surfaces:

Flat surfaces such as incubator walls, farm racks, cases, or fan blades may be monitored following cleaning and drying. All plates should be marked on the bottom (the half plate containing the media) indicating the type and location of surface being monitored. A sterile swab which has been moistened in a sterile solution or a manufactured sterile culturette may be rubbed gently over a predefined area of the sample surface (one or two square inches). The swab is then gently streaked over the surface of the plate several times in a zig zag fashion.

Another method of sampling flat surfaces employs the RODAC contact plate. RODAC plates should be made so that the agar is slightly higher than the edge of the plate. The cover of the plate should be removed, and the agar pressed gently upon the surface to be monitored. The plate must not be moved in any direction once contact is made. The cover should be replaced after the impression is made, taking care not to touch the agar.

Sampling liquids:

To sample vaccine and other liquids, a 0.5 ml to 1.0 ml aliquot should be pipetted onto the surface

of a sterile agar plate and spread gently with a sterile swab or a sterile "L" shaped glass rod and the plates should be incubated for two hours with the media on the bottom to allow the excess moisture to soak into the media. Label the bottom of the plate to identify the substance being monitored.

Sampling eggshell surfaces:

Eggshell surfaces generally contain microbial contamination unless they have been treated appropriately with a chemical sanitizer. The level of microbial contamination may be checked to assess the eggshell sanitizing program or to help solve a contamination problem identified by the egg breakout program.

A common method of monitoring the shell surface involves rolling the egg surface on a plate. To perform this method, the plate cover should be removed and placed on a clean surface. The egg should then be picked up with your fingertips and the surface which is untouched by your fingers rolled on the surface of the media. Eggs should be rolled in this fashion two times and the cover of the plate should be carefully replaced. This method works as a screening test, but does not provide as accurate a microbial count as the methods described below.

The total egg wash is a more quantitative method for determining the contamination level on eggshell surfaces¹. A clean tissue should be used to pick up the egg and place it in a sterile whirl-pack bag containing 20 milliliters of sterile physiological saline. The egg should be gently massaged in the bag for one minute, let stand for one minute and massage again for one minute. The egg should be removed without further contaminating the wash water and two aliquots of 1.0 ml and 0.1 ml should be pipetted onto the surface of two different media plates for bacteria enumeration and two media plates for mold enumeration. Egg wash water should be spread with a sterile swab or a sterile glass "L" rod and the plates should be incubated for two hours with the media on the bottom to allow the excess moisture to soak into the media. Identification of specific microorganisms is less important than the general trend of groups of microorganisms and changes over time in shell surface contamination. The colonies on the plates should be counted, corrected for dilution factor (1:20 and 1:200), and recorded.

Another method for estimating the bacterial population on the shell surface uses 1.8 cm x 1.6 cm strips of sterile tape². The tape should be applied

to the shell surface, carefully removed, and an impression made on an agar plate. Two taping impressions per egg should be adequate for estimating the total number of bacteria on the eggshell. The total bacterial count using the taping technique tends to be lower than that obtained by using the egg wash technique.

Sampling fluff and other solids:

Solid material may be monitored by aseptically weighing a known quantity (e.g. 1 to 100 g) of the solid and diluted in ten times that amount of sterile saline. Following weighing, the diluted samples should be vigorously mixed by hand or with a vortex for thirty seconds. Dilutions of the sample should then be made by taking a 1 ml volume of the original solution and placing it in 9 ml of sterile saline and vigorously mixing. This procedure is repeated until dilutions of 1:10, 1:100, 1:1,000, and 1:10,000 are made. A 1.0 ml portion of each dilution should then be pipetted and spread on the media surface with a sterile "L" shaped glass rod. The plates should be incubated for two hours with the media on the bottom to allow the excess moisture to soak into the media.

Sampling chicks:

Eight to ten (8-10) first quality chicks from each breeder flock in question should be euthanized by cervical dislocation. The external surfaces of chicks should be soaked with disinfectant to minimize airborne contamination. The abdominal wall should be opened with sterile scissors, and the yolk sac perforated using a sterile, moist swab. The swab should be twirled in the yolk sac, carefully removing the swab, and streaking on the surface of an agar plate. The cover of the plate should be carefully replaced to avoid contamination.

Incubation of plates:

All plates should be incubated with the media side of the plates on top to prevent condensation from influencing growth. Plates which are being evaluated for bacterial contamination should be incubated for 48 hours at 37°C or 98.6°F in a microbiological incubator or a setter if a microbiological incubator is not available. If plates are placed in a setter they should be placed in a plastic

bag and set where they will not be disturbed. Plates being evaluated for mold contamination should be incubated at room temperature for 3 to 5 days.

Identification of Microbial Growth:

In general, bacterial colonies will be smooth, mucoid, and 1-3 mm in diameter. Mold colonies will be filamentous in appearance and may be much larger due to the extended incubation period. Colonies on air plates, contact plates, liquid spread plates, and swab streaks should be counted and recorded. The plating procedures involving dilutions need to be counted, corrected by the dilution factor (10, 100, 1000, etc.), and recorded.

Evaluating the Microbial Monitoring Program:

Make sure that records are maintained of all results so that changes occurring over time can be observed in the different areas monitored. The results should be carefully compared with hatchability and chick livability data. All of the microbiological monitoring which has been performed in supposedly sanitized areas should be 20 colonies or less (you will need to establish your own criteria after some time). Excessive colonies will indicate poor sanitation procedures or a hatching egg production problem. Early detection of contamination can minimize hatchery and chick quality problems.

References:

- ¹Gentry, R. F., C. L. Quarles. 1972. The measurement of bacterial contamination on eggshells. *Poultry Sci.* 51:930-933.
- ²Arhienbuwa, F. E., H. L. Adler, A. D. Wiggins. 1980. A method of surveillance for bacteria on the shell of turkey eggs. *Poultry Sci.* 59: 28-33.

Related information may be found in the following:

- "Essentials of a Hatching Egg Quality Assurance Program" - Poultry Facts No. 7
NCSU Extension Poultry Science
- "Trouble-Shooting Fertility and Hatchability Problems" - Poultry Science and Technology Guide No. 34

Prepared by
Michael J. Wineland
David V. Rives
Frank T. Jones