



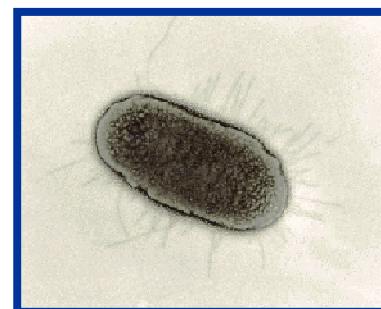
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MICROBIOLOGICAL TOOLS FOR QUALITY ASSURANCE IN HATCHERY: Laboratory Methods

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Most of the time, the monitoring analysis are focusing on bacteria (e.g. coliforms, *Pseudomonas* and *Staphylococcus*) and molds (including *Aspergillus*). Virus isolation and/or PCR are indeed much more complicated to implement routinely, and costly as well. And somehow, the bacterial and fungi presence by itself will assess the efficacy of the sanitation procedures applied.

There are different kinds of procedure, depending upon the target to be sampled: air, flat surfaces, eggshell, liquids, fluff or chicks. And, according to the procedure, different material and media will be used for growing the germs monitored.



*Figure 1: Enterobacteriaceae
(Transmission Electron Microscopy)*

MATERIAL REQUIRED FOR THE MICROBIOLOGICAL MONITORING

The samples will be taken thanks to material chosen, according to the procedure decided by the laboratory. For growing the molds or bacteria, different kind of media can be used:

- ✎ either agar, a solid medium whose hard jello consistency allows a direct sampling and then, incubation on the same material
- ✎ or broth, which is a liquid medium that will be preferred for isolating and identifying the specific organisms which grow on agar media.

The agar media are generally used to determine the type and the number of microorganisms present. In order to make comparisons of colony counts over time, it is important to use always the same kind of agar. Agar media provides the nutrients required by microbes to grow. The various types of microbes have different nutrient requirements, and their susceptibility to inhibitory compounds allow one to use a selective type of agar to determine number and type of microbes present in a given location.

WHICH MEDIUM SHOULD BE USED?

Choosing the type of agar will be done according to the purpose of the monitoring:

- 🔗 for troubleshooting problems and helping in identification of specific types of bacteria, specific media should be used. Media for the detection of specific groups of organisms such as *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Aspergillus* are commercially available as pre-poured plates from a number of scientific supply houses.
- 🔗 but for a routine monitoring of the total microbial load in the hatchery, as a follow up of the sanitation and biosecurity procedure efficiency, three types of media are particularly interesting: TSA for total bacteria, PDA for molds / yeasts and Mac Conkey for coliforms.

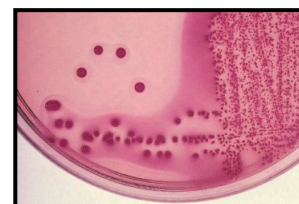


Figure 2: *E. coli* (Mac Conkey)

First, general purpose media for bacteria like **Tryptic soy agar** (TSA) or nutrient agar don't contain any inhibitory chemical compounds and 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. Non-selective media are usually incubated at 37°C (98.6°F) for 48 hours before counting.

As regards **Mac Conkey agar** (MA), it is commonly used to detect coliform bacteria. Violet red bile agar (VRB) and eosin methylene blue agar (EMB) can also be used, but Mac Conkey probably detects a slightly wider array of bacteria than the other two. Mac Conkey 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 should also be counted since these colonies indicate the presence of other harmful bacteria (e.g. *Salmonella*).

Finally, **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 infection (such as ring worm). Media which are used to enumerate molds will often include antibiotic(s) (such as chloramphenicol 0.05g/l) 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.

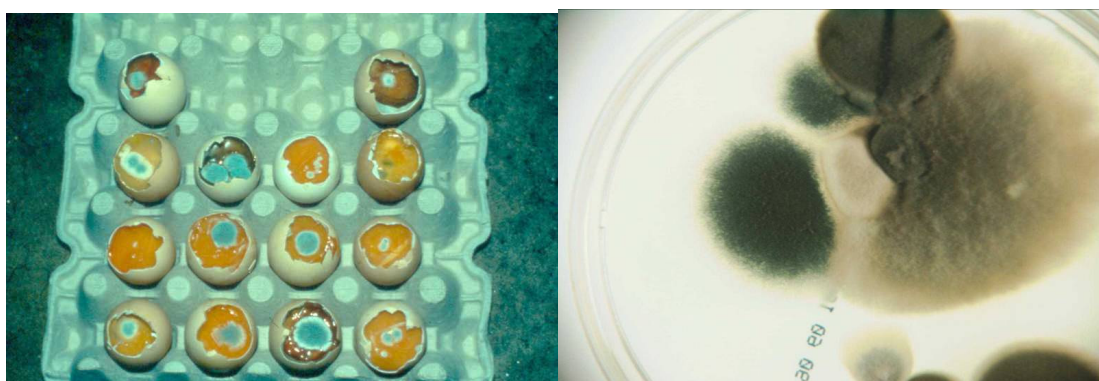


Figure 3: *Aspergillus* on eggs (left) and on Potatoe Dextrose Agar (right)

While pre-poured media 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 to purchase or prepare your own media.

HOW TO MAKE MEDIA FOR HATCHERY MONITORING?



First, the place where they should be prepared is very important: at least a room within the hatchery which has minimal traffic flow and minimal airborne contamination. The vaccine room might be a good media preparation room for instance.

Then, tempered glass containers (such as pyrex) should be used, according to the directions on the media package as a guide. The amount of water to be mixed should be appropriate and heated using a stirring hot plate. Prior to autoclaving,

media should be completely melted and dissolved, and they are so when they are clear not cloudy. But caution should be taken not to overheat after clearing. The final 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: in case of too long autoclaving, sugars in the media may caramelize and peptones may degrade like other heat sensitive media components. Thus overheated media should be discarded. Since media are extremely hot following autoclaving, they should be allowed to cool to the point where it can be handled with bare hands (approximately 110°F or 115°F).



Figure 4: Tempered glass container for media preparation

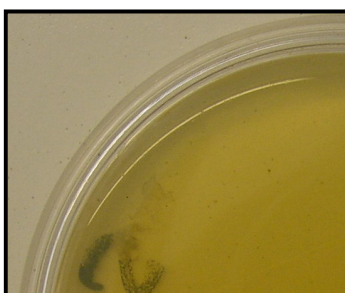


Figure 5: Convex surface on Contact Plate (RODAC procedure)

Regarding RODAC procedure (*Replicate Organism Direct Agar Contact*), Petri dishes (15*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. Each plate will be filled with 16.5 to 17.5 mL ($\frac{1}{2}$ to $\frac{2}{3}$ full). A strictly aseptic technique must be observed when pouring plates. In order to avoid any spilling or bubble formation, the plates should be handled carefully prior to solidification (however, to save space, plates may be stacked as you pour them). 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 that were 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.

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.

MICROBIOLOGICAL PROCEDURE

INCUBATION OF PLATES:

Common rules of microbiology are obviously to be applied. In order to avoid condensation to influence the growth, all plates should be incubated with the media side of the plates on top.

For RODAC procedure, the incubation of large numbers of contact plates can be organized by different ways, like the use of specific tracks.

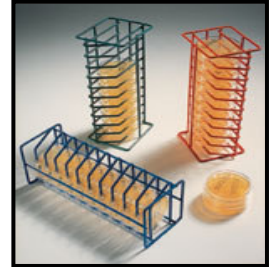


Figure 6: RODAC tracks

- For bacterial monitoring, a microbiological incubator or a setter (if a microbiological incubator is not available) can be used. Indeed, the temperature of incubation should be about 37°C (98.6°F) and the time about 48 H. But if plates have to be incubated in a setter, the workers should be careful not to disturb them. For this purpose, it is possible to protect them by a plastic bag (non fully closed nevertheless).
- As regards the mold monitoring, the incubation should be performed at room temperature for 3 to 5 days.

IDENTIFICATION OF MICROBIOLOGICAL GROWTH:

- In general, bacterial colonies will be smooth, mucoid, and 1-3 mm diameter.
- Mold colonies will be filamentous in appearance and may be much larger due to the extended incubation period.



Figure 7: Escherichia coli on MacConkey Agar

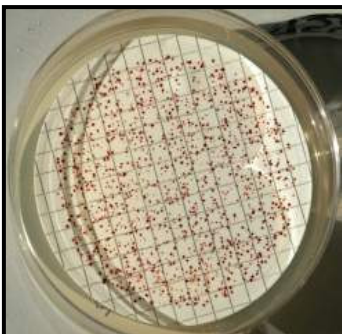


Figure 9: Counting after incubation (liquid sample).

Colonies on air plates, contact plates, liquid spread plates, and swab streaks should be counted and recorded. If a dilution had been used before on the plate, a correction by the dilution factor (10, 100, 1000, etc) has to be brought obviously.

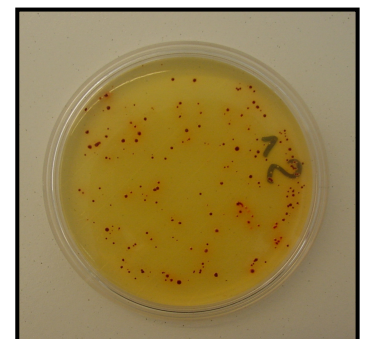


Figure 8: Counting after incubation (surface/contact plate).

CONCLUSION:

Evaluating the microbial monitoring program is the most important step. It is necessary to make sure that all results will be recorded and be able to notify any changes occurring over time. Consequently, the sampling procedure must be well planned prior to its implementation and all the steps respected and recorded with relevant identification of the samples. Only then, the results in each monitored area should be compared with hatchability and chick livability data.

In this issue, the material necessary and the laboratory procedures of this microbiological monitoring have been discussed. Depending on the hygiene and the structure of the monitored hatchery, targets can be posed in a progression way to achieve this reference after a while. Excessive colonies will indicate poor sanitation procedures or a hatching egg production problem.

It is well known that an early detection of contamination can minimize hatchery and chick quality problems, which are of utmost importance for the broiler performance at the end of the day.

