AVIAGEN ANADOLU AŞ

POULTRY DIAGNOSTIC AND ANALYSIS LABORATORY

SAMPLING MANUAL – Key Points

SAMPLING MANUAL

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RECOMMENDED HATCHING SAMPLES FROM THE HATCHERY

As a principle, samples should be collected on the hatch day.

To make sure that each hatch is represented, care should be taken to collect the samples randomly and from multiple trays of the same hatch.

1- Culled Chick Samples

- From each source
- From each line
- From each hatcher
- On each hatch day

Collecting at least 6 chicks as sample is recommended; the viscera (intestines, heart, liver, and stomach) of the chicks should be removed with gloved hands (without using scissors) and placed in a sterile container. The recommended weight for the container is maximum 25 g including the samples. To ensure the required representative quantity, a representative number of containers should be used, with maximum 25 g of sample in each container instead of lots of grams of specimen in the same container.

Before they are sent out to the Aviagen laboratory, each container should have a label containing necessary information, or relevant information should be written on the top lid with a suitable pen. It is strongly recommended that you change your sterile gloves after each sample collection. If conditions are favourable, it is recommended that 6 other chicks, collected in the same manner, are placed in a suitable bag and put in the freezer, where they should be kept for 1 month as replicate (witness) samples by the client sending the sample.

2-Fluff Samples

Fluff samples, enough to fill maximum 1/3 of a sterile container, should be collected from various parts of each hatcher on every hatch day; after writing the necessary information on the container, it should be sent to the Aviagen laboratory. The recommended weight for the container is maximum 25 g including the samples. To ensure the required representative quantity, a representative number of containers should be used, with maximum 25 g of sample in each container instead of lots of grams of specimen in the same container.

3- Hatch Tray Paper

- From each source
- From each line
- From each hatcher
- On each hatch day

Enough sample to fill the sterile sample bag (Whirl-Pak bags are recommended, but not mandatory) should be collected especially from the areas of the paper that are soiled with faecal matter, and then sent to the Aviagen laboratory. Preferably, the paper should be sent in its entirety, without breaking into pieces.

All samples should be sent to the Aviagen laboratory within maximum 6 hours. Typically, 250 ml polystyrene sterile sampling containers or sampling bags should be used.

Each container or bag must be labelled with necessary information. Informing our laboratory about your coding system will have a significant effect on the speed and accuracy of your results. For example:

KLCK 16 May

XX 308

This label informs us that the samples inside were collected on 16 May at the Kalecik hatchery on the hatch day of the 308 eggs bought and hatched from Farm XX.

In order for all samples to be processed, they must be accompanied by the sample submission forms provided by our Laboratory.

If samples are sent via courier, care should be taken so that they do not arrive at the laboratory on a weekend. It is recommended that the samples are sent so that they arrive at the laboratory on weekdays, preferably on the first day of the week.

TRANSFERRING THE SAMPLES TO THE LABORATORY WITHIN MAXIMUM 24 HOURS AFTER COLLECTION IS VITAL FOR CONDUCTING THE ANALYSES PROPERLY AND PRODUCING ACCURATE RESULTS.

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RECOMMENDED FARM SAMPLES AT ARRIVAL OF CHICKS

It is recommended that samples are collected without delay upon arrival of the chicks at the farm.

For each 500 chicks, it is recommended that samples are collected from 10 box liners through random sampling. Samples taken from box liners or dead on arrivals should be labelled with farm, house and pen codes and any other information that may be relevant for the chick, and then placed in a sterile bag and sent to the laboratory. All DOAs (maximum 60) should be labelled with farm, house and pen codes and any other information that may be relevant for the chick, and then placed in a sterile bag and sent to the laboratory.

First week mortalities (maximum 60) should also be labelled with farm, house and pen codes and any other information that may be relevant for the chick, and then placed in a sterile bag and sent to the laboratory; or, if viscera is preferred as sample, the viscera, especially livers, intestines, crops and spleens, should be collected and sent to the laboratory in containers holding the viscera of 6 chicks at the most (so that each is around 25 g).

Each sample must be labelled with necessary information. For example:

CR 9 CBL GP

This label informs us that the samples inside have been collected on the 9th day of the month at the Grandparent (GP) Stock CR farm from the CBL house. Informing our laboratory about your coding system will have a significant effect on the speed and accuracy of your results.

In order for all samples to be processed, they must be accompanied by the sample submission forms provided by our laboratory.

If samples are sent via courier, care should be taken so that they do not arrive at the laboratory on a weekend. It is recommended that the samples are sent so that they arrive at the laboratory on weekdays, preferably on the first day of the week.

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RECOMMENDED FARM SAMPLES FROM WEEK 6 THROUGH TO DEPLETION

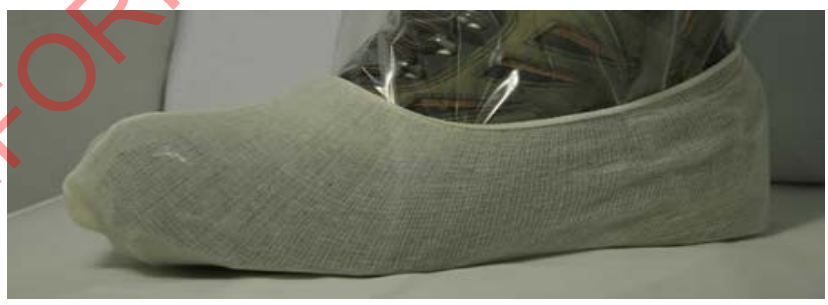
Samples should be collected via random sampling from various different points in the house where the flock is located, ensuring that they represent the entire flock. House dust and drag swab samples should be collected for all sections in all houses. Cloacal swabs, a procedure that is gradually being abandoned as a common practice, is not really recommended since it can only detect salmonella in dissemination period, and might lead inaccurate test results.

At least 2 **house dust samples** (collected in 2x 250 ml sterile containers) per house are recommended. Dust samples should be collected to fill maximum 1/3 of the sterile container, labelled with the relevant information, and then sent to the Aviagen laboratory. The recommended weight for the container is maximum 25 g including the samples. Collecting dust samples from at least 10 separate locations inside a house is strongly recommended to increase the representativeness of the samples. Sterile gloves should be worn when collecting samples, and a new pair of gloves should be used for each container. To ensure the required representative quantity, a representative number of containers should be used, with maximum 25 g of sample in each container instead of lots of grams of specimen in the same container.

Collecting at least **6 drag swabs (taken with socks or via boot swab)** per house is recommended. Drag swabs should be collected from the litter surface. It is recommended that maximum 3 drag swabs are placed in one sterile container of 250 ml. This means 2x 250 ml containers for each house.

Sock or boot swab material should never be touched with bare hands; sterile gloves should be used at all times, and care should be taken to ensure that the sock and/or boot swab does not contact any disinfectant. A walk through the entire length and width of the house should be ensured. When moving onto the next sample, the sterile gloves should be changed with a new pair. Before collecting the sample, the boot swabs or socks should be moistened with Buffered Peptone Water. The Buffered Peptone Water (BPW) used for this purpose should be fresh and prepared under laboratory conditions; any BPW not used should be destroyed to prevent it from being used again. As an alternative to sock or boot swabs, the tubular elastic bandages, traded under the name Tubigrip, can also be used. Tubigrip bandages are sold in 10 metre-rolls; when needed, they can be cut into smaller lengths and can be easily adapted for boots or shoes due to their elasticity.

Although no longer a very popular practice, if **cloacal swabs** are also desired, care should be taken to ensure that cloacal swabs are collected from each section of the house with at least 10 cotton swabs, which should all be placed in a single 250 ml sterile container and sent to the laboratory. At least 60 cloacal swabs should be collected from each house, which means at least 6 sterile containers. Although no longer a preferred method due to its very small chance of isolation, faecal samples can also be sent to the laboratory; to this end, samples should be collected from fresh faeces using sterile gloves, and sent to the laboratory in 250ml containers. It is recommended that a separate container is used for each section of the house, making sure that each container holds maximum 25 g of faeces.



Although there are boot swabs and socks manufactured for this purpose, if none are available it is acceptable to use sterile gauze in conformity with pre-defined rules.

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HUMAN FAECAL SWAB SAMPLES

Swabs collected individually from maximum 10 people should be cut with a sterile scissor, using sterile gloves, pooled in a 250 ml specimen container. Instead of the names of the swab donors, the numbers assigned to each donor should be indicated on the label attached to the container, and then the container should be sent to the Aviagen laboratory. Swabs must be definitely smeared with faeces. It is recommended that staff members give individual swabs in addition to collective swabs other than those taken for regular check-ups. Ensuring that staff members give swab samples 1 week before starting work following a leave of absence, vacation or sick leave is important in terms of establishing your system. Disposable empty plastic sterile swabs are recommended as swab sticks. In case of long distances, swab sticks with transport media can be used.

Each sample must be labelled with necessary information. For example:

MA 2, 7, 4, 9

This label informs us that the samples have been sent in one shared container, and have been collected from individuals assigned with code numbers 2, 7, 4 and 9 who are employees at the MA plant. Informing our laboratory about your coding system will have a significant effect on the speed and accuracy of your results.

In order for all samples to be processed, they must be accompanied by the sample submission forms provided by our laboratory.

If samples are sent via courier, care should be taken so that they do not arrive at the laboratory on a weekend. It is recommended that the samples are sent so that they arrive at the laboratory on weekdays, preferably on the first day of the week.

TRANSFERRING THE SAMPLES TO THE LABORATORY WITHIN MAXIMUM 24 HOURS AFTER COLLECTION IS VITAL FOR CONDUCTING THE ANALYSES PROPERLY AND PRODUCING ACCURATE RESULTS.

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COLLECTING WATER SAMPLES FOR MICROBIOLOGICAL INSPECTION FROM HATCHERIES AND FARMS

From Municipal Water Supply

Disposable plastic water sampling bottles of at least 250 ml with or without thiosulfate, depending on whether the municipal water is chlorinated or not, should be used to collect water samples. Before taking the sample, the tap should be left to run for at least 2-3 minutes to dispose of any water collected in the pipes. Then, the bottle should be filled to the neck level. The bottle should never be filled to the brim.
AFTER CAREFULLY WRITING DOWN THE LOCATION, DATE AND HOUR OF COLLECTION ON THE LABEL, THE WATER SAMPLES SHOULD BE TRANSFERRED TO THE LABORATORY WITHOUT BREAKING THE COLD CHAIN.

Care should be taken to prevent the mouth of the bottle from contacting the water which causes uncontrolled contamination .

From the Hatchery

The main concern here is to test the water used in each setter and hatcher unit.
Also, as with the samples taken from the municipal water supply, another important detail is the use of bottles with or without thiosulfate depending on whether the water is chlorinated or not.

From Inside the House

Ideally, water samples should be collected from a connection point between the water tank and the first drinker. **Taking the sample by directly dipping into the tank should be avoided at all times.** Today, it is a well-known fact that, due to advanced drinking systems and tanks, taking samples directly is impractical and causes problems with regard to occupational safety.

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Technical reasons for avoiding direct sampling from tanks in the house:

It is difficult to open and doubly difficult to turn the caps of water tanks located at the apexes of houses. Usually, even when you manage to turn the caps, there is the problem that a lot of dust and unwanted particles tend to fall inside the tank. Although it is recommended to collect the water sample from a connection point (joint) between the water tank and the first drinker, when this is not practically possible, samples are taken either from the first drinker or the very last point of the drinkers.

When samples are taken from drinkers, it is recommended to open the drinker tap to run for some time, and take the sample after a powerful flushing to remove any debris. It is strongly recommended to employ utmost care to ensure that the water sample to be sent to the laboratory is not contaminated with any dust or other waste materials whatsoever.

AFTER CAREFULLY WRITING DOWN THE LOCATION, DATE AND HOUR OF COLLECTION ON THE LABEL, THE WATER SAMPLES SHOULD BE TRANSFERRED TO THE LABORATORY WITHOUT BREAKING THE COLD CHAIN.

From water treated with UV or chemicals

Collection of samples from water treated with UV or water treatment chemicals is slightly different, and requires taking two samples before and after the treatment in order to understand whether the treatment works or not.
In this kind of sampling processes, collecting the samples from the tap rather than the drinker is a much healthier option, though samples can also be collected from drinkers if there are no alternatives.

SERUM SAMPLES

A serum sample requires 3-4 ml of blood sample, which should be collected from the large vein running superficially under the wing.

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It has been observed through experience that keeping the sample left in slanted position for 6-10 hours at room temperature after clotting produces the densest and most high quality serum samples.

The photograph above shows a transparent, good quality serum.

At least 60 serums should be sent for monitoring mycoplasma. Samples should be collected via random sampling, and should be plenty enough to truly represent the flock.

When monitoring diseases and the immune response to vaccines:

In serological monitoring observing an immune response or a disease challange, a realistic and effective result can only be possible through a correctly implemented, valid and random sampling process. For effective results, a successful sampling process should be ensured and extra care should be taken about the following:

1) Using a statistically valid sampling method.

2) Taking random samples in numbers sufficient to realistically represent the flock.

3) Samples must be labelled to avoid any mix ups during transport or handling.

Sampling method

Using statistically valid sampling methods is vital in terms of the reproducibility and reliability of the serological analysis results and/or the true mean flock titre. The statistical validity of a sampling method is dependent on two main conditions:

1) Selecting random animals for sampling,

2) Taking enough number of samples to truthfully represent the flock.

Random selection of animals for sampling means each animal in the flock has equal chances of being selected for sampling.

Although the number of samples and the sample collection time are generally determined by the responsible veterinarian of the establishment, cost/benefit analyses and ability to meet requirements play an important role in determining these aspects. In general, micro-centrifuge tubes are used for transporting samples to the laboratory. Tightly sealed tubes screw cap type of tubes should be preferred over standard capped tubes. Using tightly sealed tubes will prevent accidental opening of the caps and spilling or scattering of samples during transport.

If possible, it is best to centrifuge to ensure that the serum is collected on the top side,

or even better to extract the serum and send only the serum part to the laboratory. To prevent serums from yielding incorrect results, especially in Mycoplasma tests, it is recommended that the serum samples are inactivated through using water bath at 56°C for 30 minutes.

Sampling at Farms

Every establishment may collect samples according to its own monitoring programme, but if you are going to send samples to our laboratory, we recommend that you also send us your vaccination and monitoring programmes together with the lot numbers and expiry dates of applied vaccines as well as the names or codes of the staff members performing the vaccines. Sending this information is important in terms of enabling a healthy evaluation of the results by the sender and informing us better as the party performing the analyses.

It is recommended that each client determines, together with their technical teams, the number of serums for monitoring according to their farm structures and number of animals. When determining the number of serum samples, nests and perches also affect the structure. So, instead of an automatically determined number, the sample number should be determined in view of the specific structure of the house.

As a guidance, various different house plans and corresponding sampling plans are shown below in Figures 1 through 4.

Grandparent (GP) and Parent Stock (PS)

If the establishment where samples are taken is a GP or PS establishment, it is recommended that 60 serum samples from each house are sent to the laboratory. In case of vaccine monitoring, sending 30 samples will help in obtaining an optimum result.

Where house population exceeds 6000 birds, 1% of the total population should be sampled, rounding up to the closest decimal number - for example, if there are 7567 birds in the house, 80 samples should be sent.

An example is given below as to ensure representation of the entire house population in cases of structural components such as dividers, nests etc. and for various house shapes.

BR-Broiler

If samples are being collected from a broiler house, it is recommended that 30 serum samples are sent for optimum results.

If there are pens in the houses, the total number proposed should be divided into the number of pens; the result should be rounded to the closest whole number, and equivalent number of samples should be collected from each section.

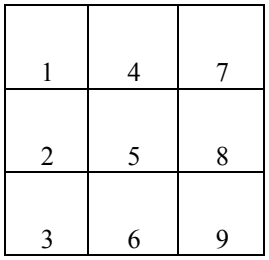
For example, if 90 serum samples were foreseen in the monitoring programme of a flock housed in a house with 7 pens:

90/7 =12.8; thus, 13 birds should be selected through random sampling for blood collection.

SAMPLING MANUAL

Sample Plans per House Structures

Figure 1.



House

This house consists of 9 sections accommodating different strains.

Broiler

Mycoplasma – 30 samples

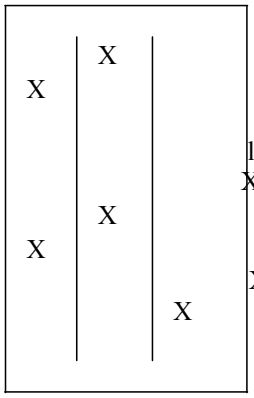
9 sections – 4 samples from each section

GP/PS

Mycoplasma – 60 samples or 1%

9 sections – 6 or 7 samples from each section

Figure 2.



House

Feed line/Nests

This is a house with 2 feed lines running in the middle and with nests.

Broiler

Mycoplasma – 30 samples

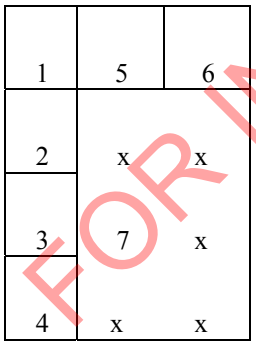
3 pens – 10 samples from each pen pens divided into smaller segments, randomly selected from shown sampling points)

GP/PS

Mycoplasma – 60 samples or 1%

3 sections – 20 samples from each pen. (With pens divided into smaller segments, 5 10 randomly selected from shown sampling points)

Figure 3.



House

This house has one large pen and numerous smaller pens.

Broiler

Mycoplasma – 30 samples

Pen 1-6 – 3 samples from each pen

Pen 7 - 12 samples

GP/PS

Mycoplasma – 60 samples or 1%

Pen 1-6 – 6 samples from each section

Pen 7 - 24 samples

Figure 4.

1	6
2	7
3	8
4	9
5	10

House

10 pens

This house has 10 pens.

Broiler	**GP/PS**
Mycoplasma – 30 samples	Mycoplasma – 60 samples or 1%
3 random samples from each pen	6 random samples from each pen

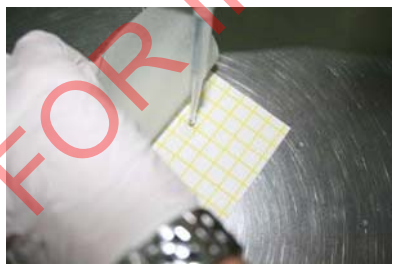
Please keep in mind that the plans shown above are provided only as guidance, and that every establishment must formulate their own monitoring plans.

Summer months require extra vigilance with regard to samples. After they are collected, samples should not be left outside. It is recommended that thermostatic boxes kept in a cold chain are used, and that plastic ice boxes or, preferably, dry ice are placed inside the thermostatic boxes to ensure the samples arrive at the laboratory while maintaining a low temperature.

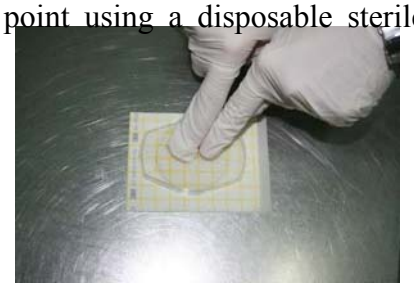
MONITORING THE FARM/HATCHERY- CLEAN-OUT SWABS FROM FARMS/HATCHERIES

Clean-out swabs are processed using the Petrifilm technique at our laboratory.

a) Start preparing by putting all needed materials on a table disinfected with alcohol. **To avoid errors and to ensure accurate results, it is crucial that the laboratory follows the instructions provided in bullets b through h and delivers the prepared petrifilms with secured cooling to the establishment where sampling will be done.**



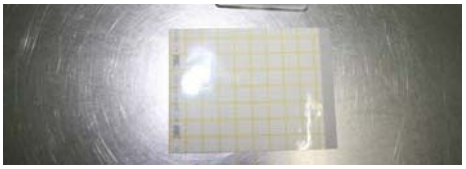
b) Remove the outer layer on the Petrifilms and drop 1ml of sterile distilled water on its central point using a disposable sterile Pasteur pipette.



c) Stick the top layer carefully, avoiding sudden movements, and ensure that the growth area in the middle of the Petrifilm absorbs the water thoroughly by applying equal pressure with a "spreader".

d) To control sterilization, one of the Petrifilms should be left in the incubator at 37ºC ± 1ºC for one night.

e) There should be no growth on the Petrifilm after this stage. If growth is observed, it means there is a problem with how the procedure has been carried out.



f) Petrifilms that have water absorbed in their media should be kept for maximum one day before they are discarded.

g) Until they are used, petrifilms should be kept chilled at 5±3ºC either in a fridge or in a cool box.

h) When preparing Petrifilms, care should be taken to not compromise sterility and to ensure that sterile water is added to the Petrifilm that will go through quality control. Sterile gloves should be worn at every phase of the process.

Total Viable Counts (TVC) or Total Mesophilic Aerobic Bacteria Count from Houses

It is recommended that samples in the numbers and from the locations below are collected, as a minimum, from each house:

- 4 x Samples from Walls
- 4 x Samples from Floors
- 4 x Samples from stanchions
- From fan shafts
- From Nest boxes

(These are the minimum points of inspection for house cleanliness testing and may be increased as desired).

Total Viable Counts (TVC) or Total Mesophilic Aerobic Bacteria Count from Hatcheries

Inspection of the cleanliness of the hatcher and setter is recommended as a minimum for all hatcheries.
In addition, it is recommended to take samples from all chambers that are considered as dirty areas and especially from all areas that are involved in the hatching process, and also from chick handling and loading sections. It is recommended that each establishment/client develops its own sampling plan. Please do not forget to add your sampling plan when sending samples to our laboratory.
Sampling points can be increased based on the company's cost/benefit analysis. It is not recommended to take samples from work areas where all disinfectants are present in concentrated amounts after cleaning and disinfection, or from drains that already contain concentrated amounts of disinfectants, as there will be no bacterial growth due to their high disinfectant concentrations.

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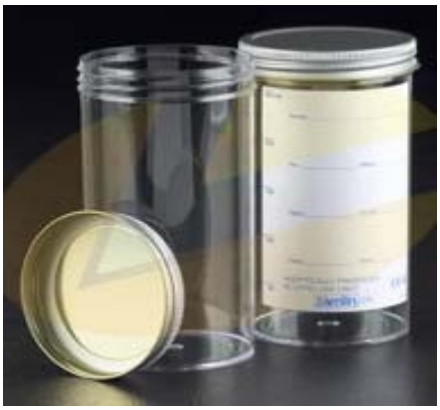
Sampling Method for TVC/TMAB

1. On the Petrifilm, write down the full and accurate names of the areas that will be tested.
2. Samples should be taken from the test site at least 6 hours after cleaning and disinfection.
3. Carefully lift the outer film on the top, press the media section onto the sampling point and wait for 30 seconds.
4. After taking the sample, reseal the thin transparent layer on the Petrifilm. Be careful not to touch the film or gel under the film so as not to contaminate it with bacteria from your own skin.
5. Make sure that the samples arrive at the laboratory within maximum 24 hours. It is recommended that thermostatic boxes kept in a cold chain are used, and that plastic ice boxes or, preferably, dry ice are placed inside the thermostatic boxes to ensure the samples arrive at the laboratory while maintaining a low temperature.
6. Maximum 20 films can be stacked on top of each other with their transparent sides facing up.

MONITORING FARM/HATCHERY - ENVIRONMENTAL SALMONELLA SWABS FROM FARMS/HATCHERIES

It is recommended that hatcheries and farms are tested using environmental swab samples to learn about their statuses in terms of Salmonella, at least once a month. When there is a widespread Salmonella infection in the vicinity, it is recommended that samples are sent in on a weekly basis according to a cost-benefit analysis. In hatcheries, collection of samples on hatch days in particular will yield important clues in terms of reaching the root of the problem. Typically, samples should be taken from walls, floors, and the equipment used inside in both the houses and the hatcheries. As mentioned before, it is of tremendous importance in terms of the results obtained that every establishment's monitoring programme has its own sampling plan developed according to their own cost/benefit analysis, and that this plan is sent to the laboratory together with the samples.

It is recommended that sterile blue sponges soaked in an enrichment medium are used for collecting samples. Although blue sponges are manufactured specifically for this task, in cases where conditions are not suitable or where this material cannot be obtained, samples can be collected using sterile gauze and/or cleaning cloths with intact packages or, as the best alternative, using the previously mentioned elastic tubular bandages.



Another important point is the quality of the containers in which samples are stored, and the number of sponges/cloths that should be put in a single container. The recommended weight for the container is maximum 25 g including the samples. To ensure the required representative quantity, a representative number of containers should be used, with maximum 25 g of sample in each container instead of lots of grams of specimen in the same container. Disposable 250 ml PS containers found on the market (or any other disposable and lidded container with the same holding capacity) will yield the best result for this type of samples.

It is recommended that sampling points are constantly changed in order to allow for random sampling, which is important in itself, and in order to increase the chance of finding a positive result as different points are likely to increase the chances even in a regular sampling programme.

If samples are collected using cloth or gauze instead of a blue sponge dosed with neutralizing buffer, these materials must be moistened with a pre-enrichment liquid. However, this does not mean that a sample cannot be collected dry. Moistening may not be needed for samples that have a high chance of arriving at the laboratory immediately after collection. It is important to know

that buffering/pre-enrichment liquids carry a high risk of contamination and can be spoiled easily even if they are kept cold or under the best conditions; thus, they must be produced under laboratory conditions and only in small amounts. Alternatively, sterile pre-prepared buffer/enrichment liquid can be used. If any liquid remains, it should not be used again, and should be immediately discarded. During sampling, effort should be made to drag the sampling material on hard-to-reach and hard-to-clean surfaces. It is particularly important to take samples from cracks and corners. Sterile gloves should be worn during the procedure, and gloves should be changed at each different sampling area and for each different sampling type.

Hatchery Air Quality Samples

Existence of fungal species in the air of the hatchery may cause serious problems in terms of both production quality and hatching performance. So, fungal checks are recommended as part of hygiene measures. Sampling is also vital for assessing current situation and taking due measures.To this end, our laboratory provides clients with prepared Plate Count Agar plates for detection of bacterial load in the air and Sabaroud Dextrose Agar plates for detection of fungal loads in the air.

It is recommended that every room in the hatchery is tested for air quality. Sampling by uncapping the agar plate and waiting for a certain amount of time is not recommended, since it is an outdated method that is gradually being abandoned as it can result in contamination and incorrect results.

Moreover, the limitations of the standards employed by our laboratory are based on limitations due to air quality measurements done by machines; hence, results are given in m^3.

It is recommended to perform a more intensive sampling in the initial phase to identify your specific limitations, and afterwards follow up with controls once a month.

In order for all samples to be processed, they must be accompanied by the sample submission forms provided by our laboratory.

If samples are sent via courier, care should be taken so that they do not arrive at the laboratory on a weekend. It is recommended that the samples are sent so that they arrive at the laboratory on weekdays, preferably on the first day of the week.

TRANSFERRING THE SAMPLES TO THE LABORATORY WITHIN MAXIMUM 24 HOURS AFTER COLLECTION IS VITAL FOR CONDUCTING THE ANALYSES PROPERLY AND FOR OBTAINING ACCURATE RESULTS.

MICROBIOLOGICAL INSPECTION OF FEED MILLS

At feed mills, it is recommended to perform weekly controls to check for hygiene and for continuation of the salmonella-free status. However, as mentioned before, clients should develop their own monitoring programmes based on their cost-benefit analysis, and collect their samples in accordance with their own programmes.

Salmonella Monitoring

Sample type varies depending on the area where the sample is collected from. If the sampling environment is dusty, it is recommended that dust samples are also collected along with drag swab samples. If the area is typically free of dust, it is recommended to perform drag sampling using sampling materials moistened with a pre-enrichment liquid (blue sponge or special purpose cleaning cloth). Sampling materials should never be touched with bare hands, and sterile gloves should be used at all times. Care should be taken to prevent any contact between the swab and any disinfectant. When moving onto the next sample, the sterile gloves should be changed with a new pair.

SAMPLING MANUAL

a) Dust samples

It is recommended that dust samples are collected by brushing into a sterile container while wearing sterile gloves. Dust sample should be collected to fill maximum 1/3 of the sterile container, labelled with the relevant information, and then sent to the Aviagen laboratory. The recommended weight for the container is maximum 25 g including the samples.

b) Drag Swab Samples

As a general rule, these swabs are expected to cover the raw material acceptance areas, silos and depots, mills, mixers, elevators, coolers, conveyors, conditioners, and in case of a multi-storey plant, the floor dividers and bagging and distribution-storage sections.

If sampling with a cleaning cloth, the cloths should be moistened with Buffered Peptone Water before taking the samples. The Buffered Peptone Water (BPW) used for this purpose should be fresh and prepared under laboratory conditions; any BPW not used should be destroyed to prevent it from being used again.

During sampling, effort should be made to swab the material on hard-to-reach and hard-to-clean surfaces. It is particularly important to take samples from cracks and corners.

The recommended weight for the container is maximum 25 g including the samples. To ensure the required representative quantity, a representative number of containers should be used, with maximum 25 g of sample in each container instead of lots of grams of/too many specimens in the same container. Typically, this 25 g corresponds to the weight reached after collecting samples with 3 blue sponges. Disposable 250 ml PS containers found on the market (or any other disposable and lidded container with the same holding capacity) will yield the best result for this type of samples.

c) Raw material/ finished feed samples

As always, when sending samples from raw materials and/or finished feed, care should be taken to ensure a representative sample quality. Moreover, the difficulty of detecting Salmonella in feeds and/or raw materials that are thermally processed or treated with special chemicals, both of which are methods widely used today, should be taken into consideration.

SAMPLING MANUAL

When collecting samples from thermally processed products, it is recommended to arrive at a final sample by collecting specific amounts of samples at intervals throughout the production phase using automatic sample collectors. In the absence of favourable conditions, samples can also be collected using appropriate probes of various diameters and lengths. However, it is not recommended to collect samples from the surface using a shovel or scoop. Sterile Whirl-Pak bags are the most ideal materials for sending samples; if they cannot be obtained, any bag that can be locked tight and that has the same holding capacity can be used.

In order for all samples to be processed, they must be accompanied by the sample submission forms provided by our Laboratory.

If samples are sent via courier, care should be taken so that they do not arrive at the laboratory on a weekend. It is recommended that the samples are sent so that they arrive at the laboratory on weekdays, preferably on the first day of the week.

TRANSFERRING THE SAMPLES TO THE LABORATORY WITHIN MAXIMUM 24 HOURS AFTER

COLLECTION IS VITAL FOR CONDUCTING THE ANALYSES PROPERLY AND PRODUCING

ACCURATE RESULTS.

HUMAN FAECAL SWAB SAMPLES

Swabs collected individually from maximum 10 people should be cut with a sterile scissor, using sterile gloves, and collectively placed in a 250 ml specimen container. Instead of the names of the swab donors, the numbers assigned to each donor should be indicated on the label attached to the container, and then the container should be sent to the Aviagen laboratory. Swabs must be definitely smeared with faeces. It is recommended that staff members give individual swabs in addition to collective swabs other than those taken for regular check-ups. Ensuring that staff members give swab samples 1 week before starting work following a leave of absence, vacation or sick leave is important in terms of establishing your system. Disposable empty plastic sterile swabs are recommended as swab sticks. In case of long distances, swab sticks with transport media can be used.

SAMPLING MANUAL

Revision 2. Revision status moved to the back page.

Revision 1. On page 6, updated how viscera should be sent to the laboratory in case of first week mortalities. On Page 9, updated how human faecal swab samples should be coded. Changed company name and logo. On page 16, changed the tolerance range for incubation temperature and the cooler temperature required for petrifilms. Added cover.