

Your pigs are sick. They are coughing, have fever and some have even died. By the time the vet arrives, it has spread throughout the barn. You are watching a disaster unfold. The vet quickly recognizes the classic signs of App (*Actinobacillus pleuropneumoniae*) and remembers that the herd is not vaccinated for this organism. Didn't seem necessary – never had a case before. Several animals that had recently died were chosen for post mortem sampling. Lungs and hearts were removed for shipment to the diagnostic laboratory for confirmation of the diagnosis.



What is the best specimen?

As soon as the animal dies the microorganisms begin taking over, particularly as the carcass remains warm. If several hours elapse before the samples are taken, these post mortem contaminants can outgrow the organisms that caused the problem in the first place. Therefore, choosing an animal that has recently died or selectively euthanized, chilling the organs quickly to slow down the growth of contaminants and getting it to the lab as quickly as possible, will greatly increase your chance of getting an accurate result from the lab. If the best samples are available on a Friday in most cases it would be better to freeze the organs and send them on Monday, so they do not remain in the courier warehouse. This is particularly important during the warm summer months. The growth of the contaminating organisms is slowed down or halted by chilling and freezing as they grow best at body temperature. This is critical to the diagnostic process since the quality of the result is entirely dependent on the quality of the sample received.

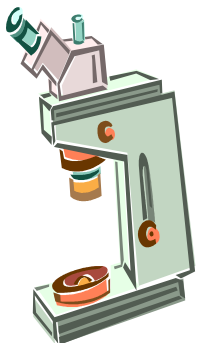
Why does the lab take so long getting bacteriology results back?

Unless the samples are delivered immediately to the lab it will take a courier 24 – 48 hours to get the samples to the lab, depending on how far you are away. The lab will usually process the samples the same day they arrive but once the test is set up, they must incubate to allow the bacteria to grow for further study and identification. *Actinobacillus pleuropneumonia* will take 24 hours to grow in the lab, but other bacteria such as *Haemophilus parasuis* can take up to 48 hours. It can be as many as 4 days before the lab can proceed with evaluating the organisms from the sample. If a week-end occurs during this time it can add 2 more days to the process. Identification of bacterial organisms can take a minimum of two days as the technician performs various tests that are typical for the species of interest. If the samples have deteriorated in transit, contaminating organisms may have to be removed in a sequential process that purifies the culture. Valid results cannot be obtained on a mixed culture. Generally an interim report can be issued once the organism(s) has been identified, so we know what we are dealing with. Sensitivity results that provide information on which antibiotics could be used to control the organism will take another 24 hours following identification.

A skilled laboratory technician will use the diagnosis given by the vet, the condition and appearance of the organ specimens and the appearance of the organisms grown in culture in the lab to make a quick prediction of the causative organism. They will use their experience to quickly identify the organism(s) and get a result as soon as possible to the vet. Unfortunately, each step takes time using routine bacterial isolation and sensitivity testing. If the laboratory offers the service, once the organism is identified it can be further characterized by serotyping. This can be useful in deciding whether to use a licensed bacterin or an autogenous bacterin. If the serotype is included in a licensed bacterin then that may be the best choice for a vaccination programme, if the serotype is not covered by the licensed product you can choose to use an autogenous bacterin. The autogenous bacterin will be made from the organism(s) isolated by the diagnostic laboratory and therefore is specific to your farm.

Lab reports

The information on the lab report can be confusing when all you want to know is "What do I have and how do I get rid of it?" . The report from Gallant Custom Laboratories may look like this:



Sample ID	Culture Results	Serotype / Genotype Results
1-heart	Heavy mixed bacterial growth- <i>Streptococcus suis</i> , <i>Haemophilus parasuis</i>	<i>Streptococcus suis</i> serotype 1 <i>Haemophilus parasuis</i> serovar 4
2-lung	Heavy mixed bacterial growth- <i>Streptococcus suis</i> and <i>Haemophilus parasuis</i>	<i>Streptococcus suis</i> serotype 1 <i>Haemophilus parasuis</i> serovar 4
3-liver	Scant mixed bacterial growth- <i>Streptococcus suis</i>	<i>Streptococcus suis</i> serotype 1
4-intestine	Heavy growth of <i>Escherichia coli</i>	Enterotoxigenic <i>Escherichia coli</i> strain O149:K91:F4

What bug came from my animal?



Sample ID - Any information to identify the samples is transcribed by the technician during the initial set up. This is useful to group organs from the same animal, location of the animal and date – such information can be important to better understand the results.

Culture Results - The technician makes a judgment about the amount of bacterial growth and whether or not it is a mixed or “pure” culture. It is expected that the organism(s) that caused the disease would be predominant and present in large numbers. A mixed culture may indicate post mortem contamination or more than one organism causing the problem. Organs such as the heart, liver, brain, or spleen should typically not have any bacterial growth, therefore, the presence of bacteria – either mixed or pure is significant. Organs such as the intestines or lungs are exposed to many different organisms so the presence of bacteria is not that remarkable, therefore, the technician must use their skills to pick the problem organisms from the normal bacteria. If the report states “No significant isolate” the technician has determined that the organisms in the sample are not typically a problem and may be from post mortem contamination or normal in that organ.

Serotyping - This can be very useful information. Some serotypes are typically more of a problem than others. If you receive a report that *Streptococcus suis* was found in all the samples and they were all serotype 1 then it was clearly a problem, particularly if it is found in clean organs such as the heart, brain, liver or spleen. *Escherichia coli* is part of the normal bacteria found in the intestine and is necessary for good digestion but some strains are capable of causing disease and serotyping for Enterotoxigenic *Escherichia coli* (ETEC) can be useful in identifying these isolates. Knowing the serotype of *Haemophilus parasuis* can be helpful in tracking new strains in a herd or deciding the best vaccine solution. Serotype information can also tell you if there is more than one strain of the organism in your herd.

Susceptibility testing - This result predicts the sensitivity or resistance of the problem bacterial organism to specific antibiotics available for use. The test used by many labs is the Kirby-Bauer method. The technician evaluates the bacteria’s ability to grow next to a paper disc that has been impregnated with the antibiotic. If it has no problem growing next to the disc it is considered resistant but if it is unable to grow near the disc, the distance is measured and it is determined if it is sensitive (based on just how far away from the disc it is). It is a useful tool but must be

Antibiotic	Streptococcus suis Type 1
Chlorotetracycline	R
Amoxicillin	S
Amikacin	R
Ampicillin	S
Apramycin	R
Ceftiofur (XNL)	R
Lincomycin	R
Tylosin	R

evaluated with caution as a lab test cannot always predict what would happen in the animal. Based on these results only Amoxicillin and Ampicillin would be useful to try against this strain of *Streptococcus suis*. Laboratories can test more types of antibiotics and choose them based on the type of organism and species of animal.

Alternate methods - is there anything faster and better?

Diagnostic technology is advancing rapidly and it is now possible to identify the presence of many organisms without the time consuming process of culturing and identification. However, without the culture it is impossible to perform sensitivity testing. Therefore, if antibiotic therapy is important or you are considering an autogenous bacterin, you still have to go through the isolation process. However, it could give valuable information quickly, that the organism is present in the sample. PCR testing (Polymerase Chain Reaction) is now available for many veterinary organisms and is a specialty of some laboratories. Depending on the service level of the lab, the results can be available in as little as 8 hours- but call ahead to understand their policy. Caution must be used when evaluating the results, since some organisms such as *Escherichia coli*, *Streptococcus suis* and *Haemophilus parasuis* can be present and not causing any disease problem. PCR testing is highly specific and can be a valuable tool in early detection.



Real-time PCR testing of PRRSV is widely used to monitor boar studs and identify the disease status of production facilities. Since the presence of PRRSV in a swine herd is always a problem this method of detection is particularly valuable as results can be issued the same day. Gallant offers this service under contract for clients performing weekly routine monitoring. It is now possible to sequence the virus to compare it to previous breaks or common strains in a geographical area. This will be valuable in control and epidemiological studies. PCR technology is advancing to provide rapid identification, genetic information for comparison of isolates, predict the pathogenicity capabilities, and even quantification of organisms, in some cases. Generally, in the hands of a skilled technician the PCR method is very accurate but cannot take the place of an experienced veterinary evaluation, observations of the animal care personnel, disease history and appearance and quality of the samples. All the pieces of the puzzle must be considered to make an educated decision on the current disease problem. This will take a little time.

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