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Application of PCR tests for monitoring large herds

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Introduction

New diagnostic tests provide practitioners with opportunities for higher levels of diagnostic confidence or confusion, depending on the characteristics of the new test and the interpretation of the results. Dramatic advances in molecular biology and immunology have paved the way for endless possibilities in diagnostic sensitivity, specificity, economy, and rapidity. Researchers are, in good faith, applying molecular techniques to develop newer and better diagnostic tests to detect the presence of disease agents, disease conditions, and even genetic predisposition or resistance to disease. The scope of these applications and the rate of new test introductions are certain to expand substantially in the next few years.

On one hand, there is nothing new about the application of new diagnostic tests in production animal medicine. We have seen a steady stream of new test introductions over the past decade for detecting presence of pseudorabies virus (PRV) or antibodies, for example, and have applied them successfully in a national eradication program. Because PRV is a reportable disease, the tests undergo intensive scrutiny (i.e., validation) before being accepted as an official test by state and federal government officials.

The proverbial other hand is that diagnostic tests for "non-program" diseases do not undergo the degree of validation under field conditions that is required for tests of reportable diseases. Further, the various labs running a given test are not required to have the test validated in their specific lab. Finally, the interpretation of the results of a new test is not always fully understood at the time the test is offered for use. As a result, diagnostic results may be more likely to be mistaken or misinterpreted if internal controls or external interpretation have not been worked out adequately before a test is implemented. If we are constantly working out the capabilities and limitations of new tests by trial and error, we run the risk of not only wasting time and money, but also of alienating producers as they lose faith in diagnostic tests specifically and veterinarians in general.

Dr. Francis Collins, Director of the Human Genome Project, described a form of diagnostic pathology in which new tests for genetic predisposition to various diseases can actually cause "toxicity" in people because the technical capability of the test has outpaced our capacity to interpret what it means.[2] A simpler form of diagnostic pathology can result when a test has not been sufficiently validated to ensure that the results are reliable and accurate. Specifically, one author notes that in the case of PCR tests "none of these tests have been taken to a level of national or international standardization or validation."[1] Arguably, swine practitioners can suffer from the pathology caused by using tests of undetermined accuracy and reliability, or attempting interpretation where none has been clearly established.

So, what does this have to do with the application of PCR tests in large herds? This introduction is not intended to throw cold water on the phenomenal advances that are being made in molecular diagnostics, because the potential benefits are truly astounding. Rather, it is intended to

- remind practitioners that we must evaluate any new test with the same healthy skepticism that has welcomed each new diagnostic assay already in use;
- encourage practitioners to stay close to new diagnostic developments that will improve the safety and efficiency of pork production; and,
- assure practitioners that veterinarians will play an ever larger role in successful implementation of technical innovations in the pork industry.

What is PCR?

PCR stands for Polymerase Chain Reaction, a process used for amplifying nucleic acid. The objective of the process is to allow detection of minute quantities of nucleic acid in biologic samples, resulting in extremely sensitive and specific diagnostic tests. The concept has been compared to traditional culture techniques by DH Persing: "The promise of in vitro amplification techniques is the replacement of the process of biological amplification—growth in culture—with enzymatic duplication and amplification of specific nucleic acid sequences."[3] The key advantages of PCR techniques over culture methods are

- speed (the process of amplification can be reduced from days and weeks to several hours),

- sensitivity (theoretically a single copy of a nucleic acid sequence can be detected, and obstacles to culture such as some growth inhibitory factors in semen can be overcome with adequate technique), and
- specificity (probes can be constructed to replicate only sequences that have been carefully selected for specificity).

The need for speed

The marked advantage of a test that detects disease agents directly and rapidly is that the status of an individual animal can be determined with some degree of certainty and speed. **Figure 1** illustrates the limitations of the use of serology for determining health status of an individual animal; the period from exposure to development of detectable antibodies requires several days to weeks for most disease agents. Maternal antibodies can further interfere with the process of accurately determining the exposure history of an individual or group. Detecting the antigen directly circumvents the problem posed by the latent period to seroconversion, as well as the potential confusion from colostral antibodies.

The advent of rapid diagnostic tests coincides with changes in the industry that take advantage of such capabilities. **Figure 2** illustrates the variety of movements that are now common in the swine industry. For example, moving pigs at weaning, either as breeding stock or commercial pigs, is standard practice in many production systems. In some instances, determining the health status of pigs prior to weaning is of value as a tool for deciding the wisdom of co-mingling sources, or for determining the health status of a group of pigs where disease elimination is being attempted. Given the short "shelf-life" of pigs prior to weaning, PCR tests have an advantage over culture methods because of the speed of the test.

Figure 1. Generic timeline from exposure to detection of a disease agent

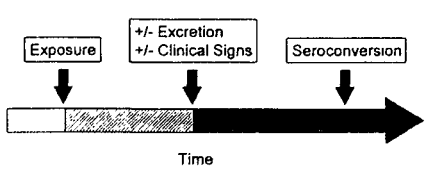
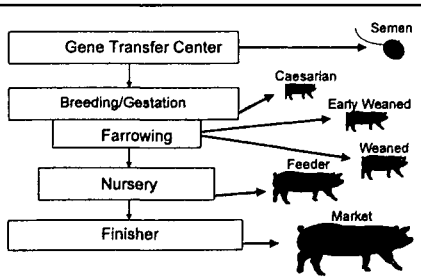


Figure 2. Types of current swine movements



This is even more significant in the case of fresh semen. With more producers moving toward purchase or use of semen from separate boar studs, the ability to detect disease agents present in semen before insemination is of increasing importance. Methods are available for testing several agents potentially transmissible by semen, but have not developed to the point where real time testing (<12 hours) is achievable at a commercial level. This technology will continue to advance to the point where such testing is available.

Multi-site production systems also have applications for PCR diagnostic testing that will capture the benefits of rapid testing. A key element of production on multiple sites with all-in—all-out production flow will be definition of populations as lots for food safety and health management. Defined populations can be tested for presence or absence of specific disease agents near to the time when pigs are to be shipped as commercial pigs or breeding stock, providing current information on the specific health status of that particular lot.

Test sensitivity and specificity

An accepted truism in veterinary medicine states that "you shouldn't run a test unless you know what you're going to do with the results." This holds true for PCR testing as it does for any other diagnostic assays. In light of many new tests that are being added to our diagnostic toolbox, I would propose a second criteria when deciding to run a test—don't use a test that you don't understand. This sounds terribly simplistic (or worse), but enthusiasm over the many new tests on the part of the developers and consumers has overwhelmed healthy skepticism on more than one occasion. This criteria poses two challenges: 1) test developers must have an objective basis for evaluating tests under field conditions; and 2) test consumers need to critically review that basis before applying the test as part of a decision-making process.

A minimum basis for understanding the capabilities of a new test is a measure of the sensitivity and specificity of the test. A table used for calculating sensitivity and specificity values is shown in **Figure 3**.[4] The sensitivity of a test is defined as its ability to detect diseased animals in a population, stated numerically as a/(a+c). Specificity is defined as the ability of a test to detect non-diseased animals, stated as d/(b+d).

In order for test developers and consumers to develop informed judgements about a particular test at a particular diagnostic lab, specific information about the population used to generate the sensitivity and specificity data is required. From that basis, practitioners can then develop their own data and understanding of the test based on experience in field settings.

Figure 3. Sensitivity and Specificity of tests

	ACTUAL HEALTH STATUS (Disease *X*)	
TEST Result	**Present (D+)**	**Absent (D-)**
Positive (T+)	*a*	*b*
Negative (T-)	*c*	*d*
	a + c	*b + d*

(Martin *et al*, 1987)

Case examples of applications

PCR diagnostic tests are routinely performed in several veterinary diagnostic labs. Use of PCR tests for monitoring production herds within PIC has primarily been for confirmation of the presence or absence of specific disease agents from samples submitted for diagnosis of a clinical case. However, several projects have been undertaken by PIC that include PCR testing for PRRS virus as an integral component of the monitoring protocol, both in Europe and in the United States.

A PRRS virus PCR test was used extensively for the establishment of a new genetic nucleus farm in Europe. The validation of the test preceded its use in the population program. The GN project and several others that were run concurrently included PCR testing of caesarian or Isowean-derived pigs from eight different herds in three countries.

From one of the farms, 12 groups of hysterectomies were performed involving 123 sows to procure 805 pigs. All 805 pigs were tested using umbilical blood by PCR for PRRS virus and all came back negative. From another farm, seven groups of pigs were obtained through a medicated early weaning program with PCR testing of all 668 pigs at weaning. Again, all pigs in all groups tested negative. Pigs were moved into isolation grow-out facilities for additional testing by serology, which also produced entirely negative results for PRRS antibodies.

A second European nucleus farm was populated using a similar protocol with two source farms using medicated early weaning. In this case, 12 groups were included, totaling 1452 pigs. Of the 12 groups, two had pigs test positive for PRRS by PCR at weaning. In one group, one pig out of 125 tested positive, and in another, two of 115 pigs tested positive. The groups and sentinels in contact subsequently tested positive for antibodies to PRRS virus.

In the US, a project to establish a PRRS-negative boar stud population from two PRRS-positive sow farms by Isowean was undertaken. Two groups of boars were weaned at 12–18 days of age into separate isolated nurseries. Approximately half (50) of the pigs were tested by PCR two weeks after weaning. All of the pigs were tested by PCR and ELISA for PRRS four weeks later. All of pigs in both groups tested negative on all tests. Placement with sentinels for further isolation and testing also produced negative results and the boars were placed in the stud at approximately 6 months of age for a final 30-day isolation period.

The lessons learned from these experiences were that PCR tests for PRRS virus at two labs are both sensitive and specific. Over 6000 PCR tests were run in the European lab over the course of the projects described and others. Based on subsequent testing by other means, it appears that the test correctly identified, on a group basis, the true status of each lot of pigs—a sensitivity and specificity of 100%. While no test is truly 100% sensitive and specific, these results certainly formed the basis of a high level of trust in the accuracy of the test.

Fewer tests have been run in the US lab, and questions of test specificity have arisen under certain circumstances that may have been affected by sample quality. The need to evaluate the test results in the light of clinical observations and other pertinent diagnostic information will always be necessary, but PCR testing has become an integral tool for monitoring health status in defined populations of pigs.

Acknowledgments

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