Why do I have to sample so many pigs for accurate serology results?

Dr. Locke Karriker, DVM, MS
Veterinary Diagnostic and Production Animal Medicine
Iowa State University
College of Veterinary Medicine

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10 Key Points

1. Don’t test without a plan to use the test results.
2. Levels of antigens vary over time.
3. Levels of antibodies vary over time.
4. No test is perfect.
5. Not all of the animals are infected at the same time.
6. Tests are more likely to find positive animals when more animals are infected. Early detection is more difficult.
7. Use the right test.
8. Sample size depends on: confidence needed in answer, number of animals infected, size of the population.
9. Larger sample sizes increase confidence in the answer.
10. Failure to detect positives does not mean the population is all negative.

What is the goal of testing?

• Detect disease
  – Are the gilts infected with PRRS?
• Determine how many of the group is infected or affected
  – Should I vaccinate for ileitis or treat?
• Refine timing of infection
  – When is the best time to vaccinate growing pigs for influenza?

Definitions

• Antigen
• Antibody
• Diagnostic sensitivity
• Diagnostic specificity
Antigen

- **AKA:**
  - Immunogen
  - Hapten
  - Infectious disease
  - Viral particle
  - Virus
  - Bacteria
  - Disease agent

- Basically, the actual disease organism
  - Reacts with antibody

<table>
<thead>
<tr>
<th>Fate of most antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clearance after 1st exposure</strong></td>
</tr>
<tr>
<td>- Equilibrium phase</td>
</tr>
<tr>
<td>- Catabolic decay phase</td>
</tr>
<tr>
<td>- Immune elimination phase</td>
</tr>
<tr>
<td><strong>Clearance after 2nd exposure</strong></td>
</tr>
<tr>
<td>- More rapid onset of immune elimination phase</td>
</tr>
</tbody>
</table>

Key Point #2:
Levels of antigens vary over time

This timeframe varies by disease!

Basic Functions of Antibodies

- **Humoral Immune Responses**
  - Antigen binding
  - Protection
  - Non-protection
  - Immune regulation

- **Effector functions**
  - Complement fixation
  - Phagocytosis
  - Cell binding
Antibody Responses against Antigen

- Lag phase
- Log phase
- Plateau phase
- Decline phase
* Specificity

This timeframe varies by disease!

Key Point #3:
Levels of antibody vary over time!

Diagnostic sensitivity

- Likelihood that the test correctly identifies infected individuals as positive
  - 5/5 test positive = 100%
  - 4/5 test positive = 80%
  - 3/5 test positive = 60%
  - ‘Infected population’

Diagnostic specificity

Likelihood that the test correctly identifies uninfected individuals as negative

- 5/5 test negative = 100%
- 4/5 test negative = 80%
- 3/5 test negative = 60%
- ‘Uninfected population’

Field samples = unknown disease status
**Diagnostic sensitivity/specificity**

**DISEASE STATUS**

| Key Point #4: No test is perfect. |

Field samples = unknown disease status

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**Stage of infection affects diagnostic sensitivity**

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**Disease Outbreak in a 1000 hd Barn**

**Key Point #5:** Not all of the animals are infected at the same time.

**Diagnostic sensitivity is DYNAMIC**

<table>
<thead>
<tr>
<th>SAMPLE (n = 28)</th>
<th>ASSAY</th>
<th>DAY POST INOCULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERUM</td>
<td>VI</td>
<td>71.1%</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0%</td>
</tr>
<tr>
<td>POR</td>
<td>32.1%</td>
<td>14.3%</td>
</tr>
<tr>
<td>TONSIL BIOPSY</td>
<td>VI</td>
<td>25.0%</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>3.6%</td>
</tr>
<tr>
<td></td>
<td>POR</td>
<td>96.4%</td>
</tr>
<tr>
<td></td>
<td>75.0%</td>
<td></td>
</tr>
</tbody>
</table>

Data adapted from Wills RW, Osorio FA, Doster AR, Sur J-H. 1998. Persistent PRRSV infection

**Key Point #6:** The test is more likely to find positive animals when more animals are infected. Early detection is more difficult.
Understand the test

• What does the test detect?
  – Antigen
    • PCR (polymerase chain reaction)
    • Culture
    • IFA (immunofluorescent antibody test)
    • IHC (immunohistochemical test)
  – Antibody
    • ELISA (enzyme linked immunosorbent assay)
    • SN (serum neutralization)

• Are the gilts infected with PRRS?
  – Antibody
    • Could use ELISA
  – Virus
    • Could use PCR

Understand the result

Key Point #7:
Use a test that will detect antibodies if exposure might be longer ago.
Use a test that will detect antigen if you want to find current infection.
Know what the particular test detects.

Sampling To Detect Disease In A Group

• We ask:
  – How many animal should I test to be 95% or 99% that a herd is disease free?
  – What maximum level of disease might still exist in the herd if none of the samples animals test positive?

\[
n = \left[1 - (1 - a)^{\frac{1}{2}}\right] \times \left[N - \frac{D - 1}{2}\right]
\]

- \(n\) = required sample size
- \(a\) = confidence level of observing at least one diseased animal in a sample where disease affects at least \(D/N\) animals in the population
- \(D\) = number of diseased animals in population
- \(N\) = total number of animals in population
Key Point #8 - Sample size depends on:

\[ n = \text{required sample size} \]

\[ a = \text{confidence level of observing at least one diseased animal in a sample where disease affects at least} \frac{D}{N} \text{animals in the population} \]

\[ D = \text{number of diseased animals in population} \]

\[ N = \text{total number of animals in population} \]

Sampling To Detect Disease In A Group

\[ n = \left[ 1 - (1-a)^\frac{1}{2} \right] \times \left[ N - \frac{(D-1)}{2} \right] \]

\[ a = 0.95 \]

\[ D = 10 \]

\[ N = 1000 \]

Sampling To Detect Disease In A Group

\[ n = \left[ 1 - (1-0.95)^\frac{1}{2} \right] \times \left[ 1000 - \frac{(10-1)}{2} \right] \]

\[ n = 258 \]

\[ a = 0.95 \]

\[ D = 10 \]

\[ N = 1000 \]

Assumes a perfect test!

Key Point #9: Larger sample sizes increase confidence in the answer!
These impacts and more have the most effect on interpretation of negative test results

Consider the practice of testing 10 nursery pigs in a room of 1000 to detect PRRS in a flow with a mixed history. Assuming the test is 100% specific and 100% sensitive (a big assumption), and we would like to be at least 95% confident about the answer, this sample size could allow up to 258 animals to be positive in the group while still producing 0/10 positive samples at testing. In other words, the ten sample approach is capable of missing a prevalence of 25.8% at the time of testing.

Key Point #10: Failure to DETECT positives does NOT mean the population is all NEGATIVE!

The following people graciously contributed content to this presentation:

Dr. Jeff Zimmerman ISU-VDL
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Dr. Annette O’Connor ISU-FSVS

College of Veterinary Medicine
Iowa State University