Choosing and Interpreting Diagnostic Testing in an Environment of Increasing Technology

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Agenda

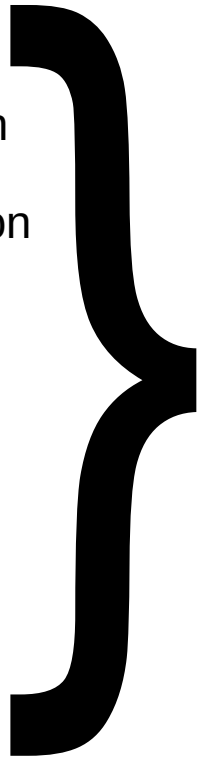
- Review of fundamental testing methods
- Current and emerging technologies
- Strategic use of testing for increased information
- Clinical examples of concurrent test usage
- Interpretation of parallel testing
- Summary

Options for Diagnostic Testing Increasing

- Veterinarians and Diagnosticians continue to develop and sometimes struggle with the best use of available technologies to accurately diagnose animal disease
- As faster and more accurate technologies' become available it is important to understand how these fit into the diagnostic picture
- Technology and information sharing has expanded greatly with the invention and adoption of internet resources and online scientific forums
- New developments in diagnostics can be used to support and enhance fundamental diagnostic testing results when the user understands interpretation in context

Review of Fundamental Testing Methods

- Antibody Detection
  - ELISA
  - Agar Gel Immunodiffusion (AGID)
  - Hemagglutination Inhibition (HI)
- Antigen Detection
  - Conventional PCR
  - Virus Isolation
  - ELISA
- Confirmatory Testing
  - Culture
  - Virus Neutralization
  - Necropsy

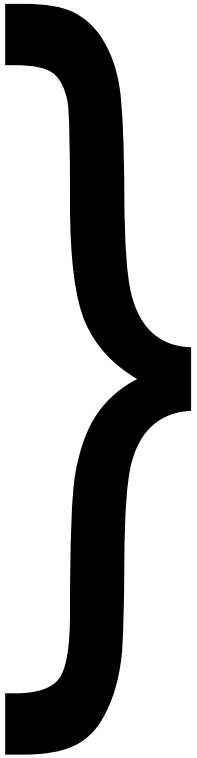


Considerations

- Advantages
  - Often less expensive to run
  - Less equipment investment
- Disadvantages
  - Specificity
  - Sensitivity
  - Time to results

Current and Emerging Technologies

- Real time PCR
- High Throughput Sequencing
- Antibody testing with Infrared Fluorophore Testing
- Next?



Considerations

- Advantages
  - Increased sensitivity
  - Efficiency of testing and time to results
  - Increased availability of Quality Controls
  - More clarity in results, less subjectivity
- Disadvantages
  - Costs of new technologies may be higher
  - New instrumentation is sometimes needed
  - Increased technical difficulty to run/ more technician training needed

Enzyme Linked Immunosorbent Assay (ELISA)

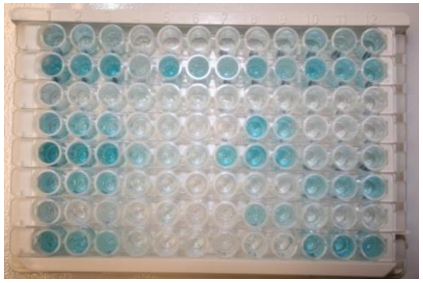
- ELISA detects antibodies after infection.
- Antibodies are found in serum, oral fluids, and GI tract (mucosal).
- The indirect ELISA provides a semi quantitative range of antibody levels when used routinely over time.

Benefits

- Low cost
- Commercial assays—high repeatability and validated test performance

Drawback

- Time to detection of seroconversion in acute infection diagnosis



PCR—Nucleic Acid Detection

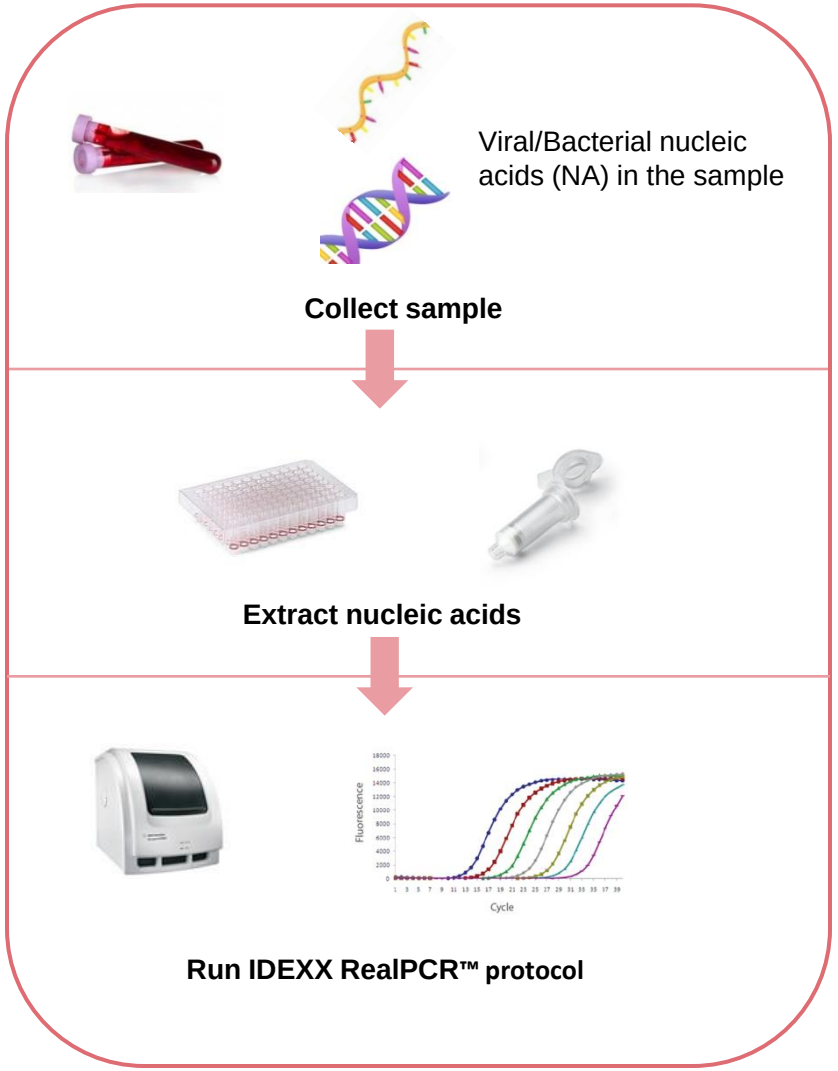
- Detects genetic material of pathogen
  - DNA or RNA
- Target sequence is amplified to enhance detection
- Reported as the number of cycles necessary for detection
  - Cycle threshold value (Ct-value)
  - Lower Ct = more initial target present

Benefit

- Highly sensitive and specific

Drawbacks

- Extremely narrow target(s), may miss variants that are slightly different
- Requires continuous field sample monitoring to ensure adequate primers (i.e., RNA viruses)



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Interpretation of Simultaneous Antibody and Antigen Testing Methods (ex. ELISA and PCR)

Antibody/ ELISA	Antigen/ PCR	Interpretation
+	+	**Active viremia** (***circulation and infection***)
+	-	**Previous infection** (***exposed but no evidence of viremia at sampling***)
-	+	**Early infection**
-	-	**Negative** (***no circulation or infection***)

Clinical Examples of Concurrent Test Usage
Overview

- Swine Farm breaks with clinical symptoms consistent with PRRS
- Veterinarian is called to the farm to investigate
- Samples taken are Oral Fluids, Blood, Feces
- Veterinarian would like guidance on how to test these to best understand the clinical picture
- History of the farm is expected to be PRRS negative

Diagnostic Plan:

- PRRS Oral Fluid ELISA on pooled pen samples
- Concurrent PRRS PCR on blood samples
- Consideration given to the farm size and expected disease prevalence to determine optimal number of samples

Clinical Examples of Concurrent Test Usage
Results

Timeline	PCR Results	ELISA Results	Interpretation
Day 1	+	-	Clinically sick pigs, expected PRRS negative premises
Week 1	+	-	Acute infection, no detectable antibody response
Week 2	+	+	Ongoing infection, pigs clearing with veterinary supportive treatment
Week 5	-	+	Acute infection cleared from herd

Ongoing Usage of Diagnostics for Status Monitoring
Interpretation of Parallel Testing

	Classification	Shedding Status (PCR)	Exposure Status (ELISA)
Category 1	Positive Unstable	+	+
Category 2	Positive Stable	?	+
Category 3	Provisional Negative	-	+
Category 4	Negative	-	-

https://www.aasv.org/shap/issues/v19n1/v19n1p44.pdf

Differences Between Antibody and Nucleic Acid Detection

Antibody

- Detects exposure and immune response to target agent
- Detects maternal antibodies, vaccination, pathogen exposure, or reinfection
- High repeatability
- Technically simple
- Cost-effective for continuous health monitoring over time

Antigen/Nucleic acid

- Real time detection of disease agent in the animal/herd
- High sensitivity to identical target agent
  - May have cross-reactions
  - May not detect new variants
- Technically demanding
- Costly

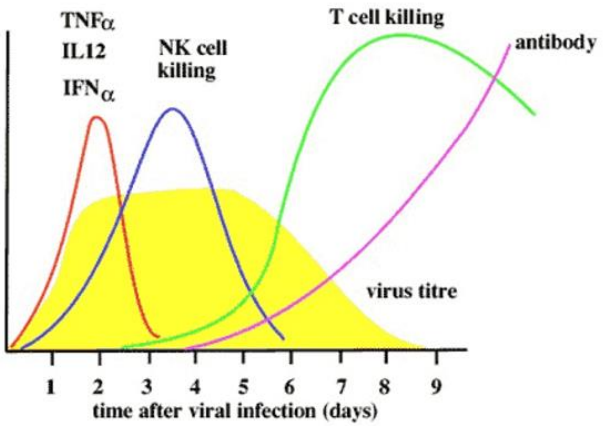
Antibody and PCR assays detect different targets and should not be used as confirmatory tests for each other.

Early in infection, the target may be present with limited antibody production, but as disease progresses, more antibodies will be present and with a low prevalence of the target agent.

Use of Results

Antibodies

- Excellent screening tool
- Gives comprehensive picture of disease status over time
- Early awareness of changes in disease circulation
- Useful for herd health management
  - Disease elimination
  - Vaccination timing
  - Identifying concurrent infections



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Antigen/Nucleic acid

- Investigation for a specific agent
- Point-in-time information
  - Population sensitivity may be low if low prevalence and small sample size
  - Difficult to detect target agent late in infections
- Semi-quantitative assessment of pathogen load
- Sample size and pooling influences ability to detect

Summary

Traditional diagnostics such as ELISA and newer technologies such as PCR are complimentary to one another and are not meant to replace the other

- Surveillance/ monitoring- ELISA
  - Examples: disease eradication programs and FAD surveillance
- Disease detection/circulating pathogen – PCR
  - Example: Early disease outbreak

Know your goals!

Thank you for your time and attention!

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