Are we certain there is disease?
and
What should we call a case?

Introduction to Outbreak Investigation

Module 2 – Confirming the Diagnosis

Module 3 – Case Definitions

Learning Objectives

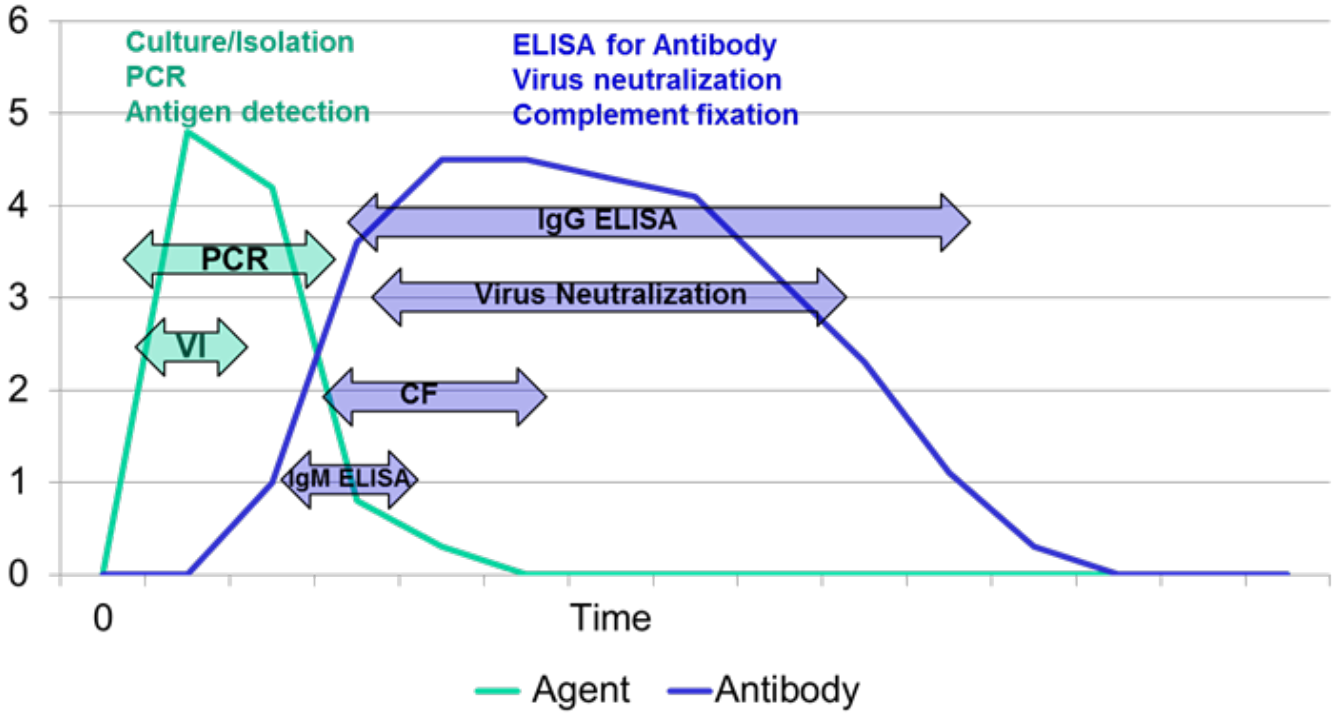
- Steps to confirm the diagnosis to include:
  - Diagnostic testing as it relates to confirming the diagnosis
  - Sensitivity and specificity as they relate to diagnostic testing
- Define case definitions

Confirm the Diagnosis

Clinical findings

Laboratory results and methods

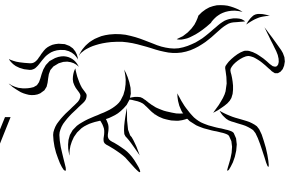
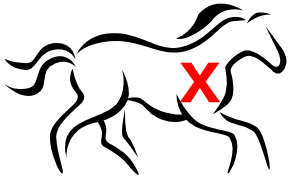
Time course of infection: Test selection



Diagnostic Testing – Fitness for Purpose

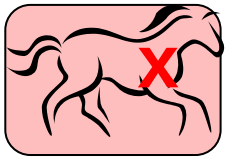
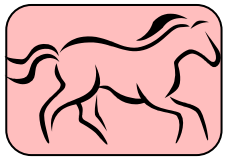
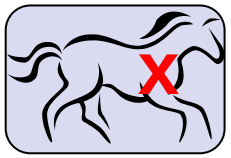
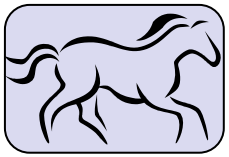
- Species, Sample, and Purpose for which test was designed and validated
- What is measured? Does this relate to risk?
  - Antibody may reflect prior exposure or vaccination and <u>not</u> present risk
- Screening test or Confirmatory test?
  - Sensitivity/Specificity
- Individual test or Herd test?
  - Significance of single result in a group of animals

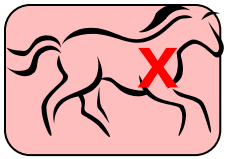
Assay Parameters

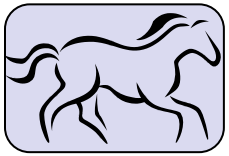
- Situation: 100 horses
  - 90 horses are healthy 
  - 10 horses have the disease 

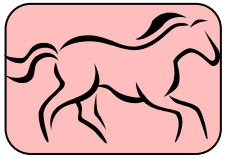
  - Positive test result
  - Negative test result

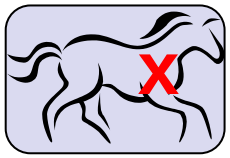
Four Possible Test Outcomes

	Infected	Not Infected
Test Positive	True Positive 	False Positive 
Test Negative	False Negative 	True Negative 



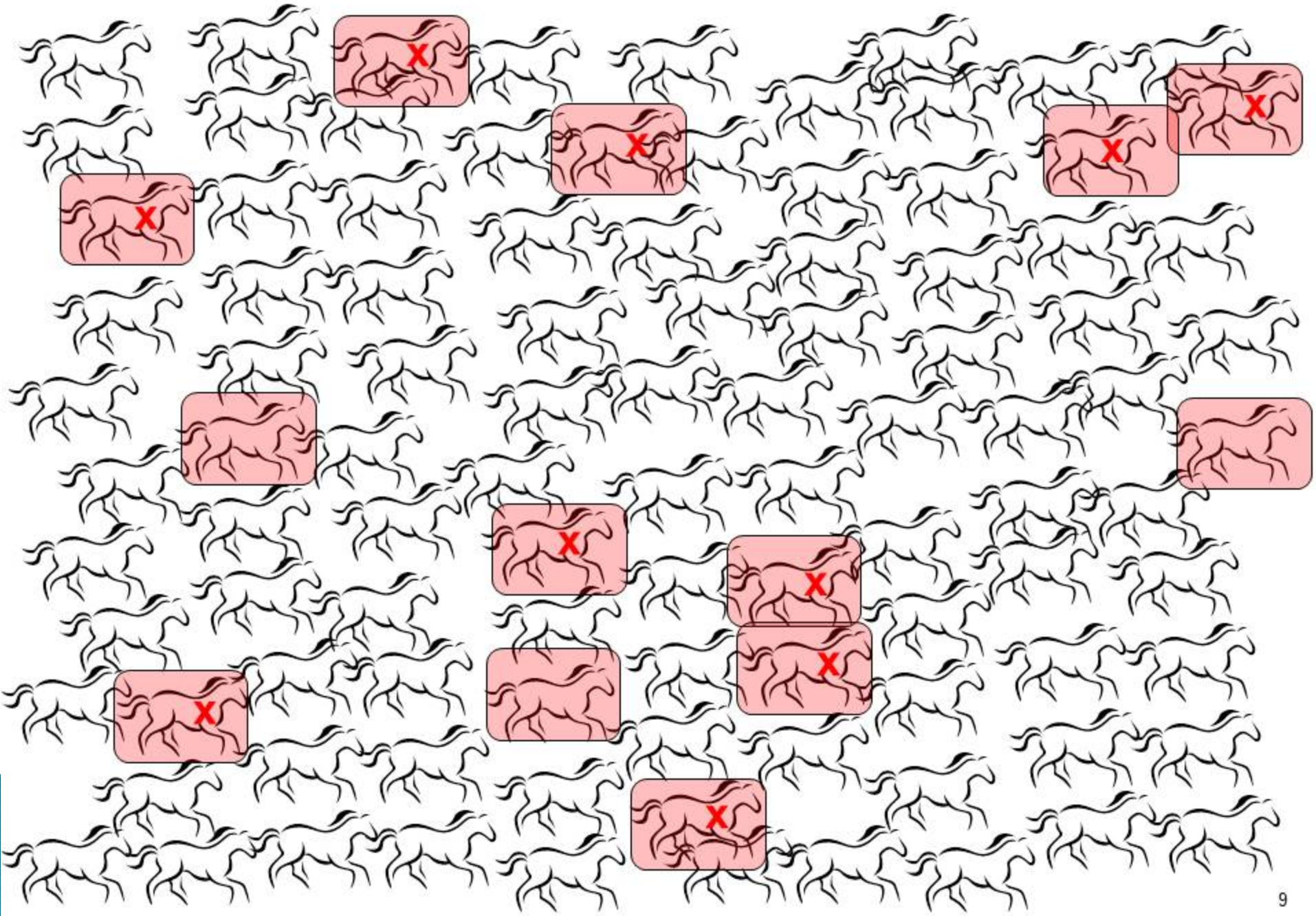






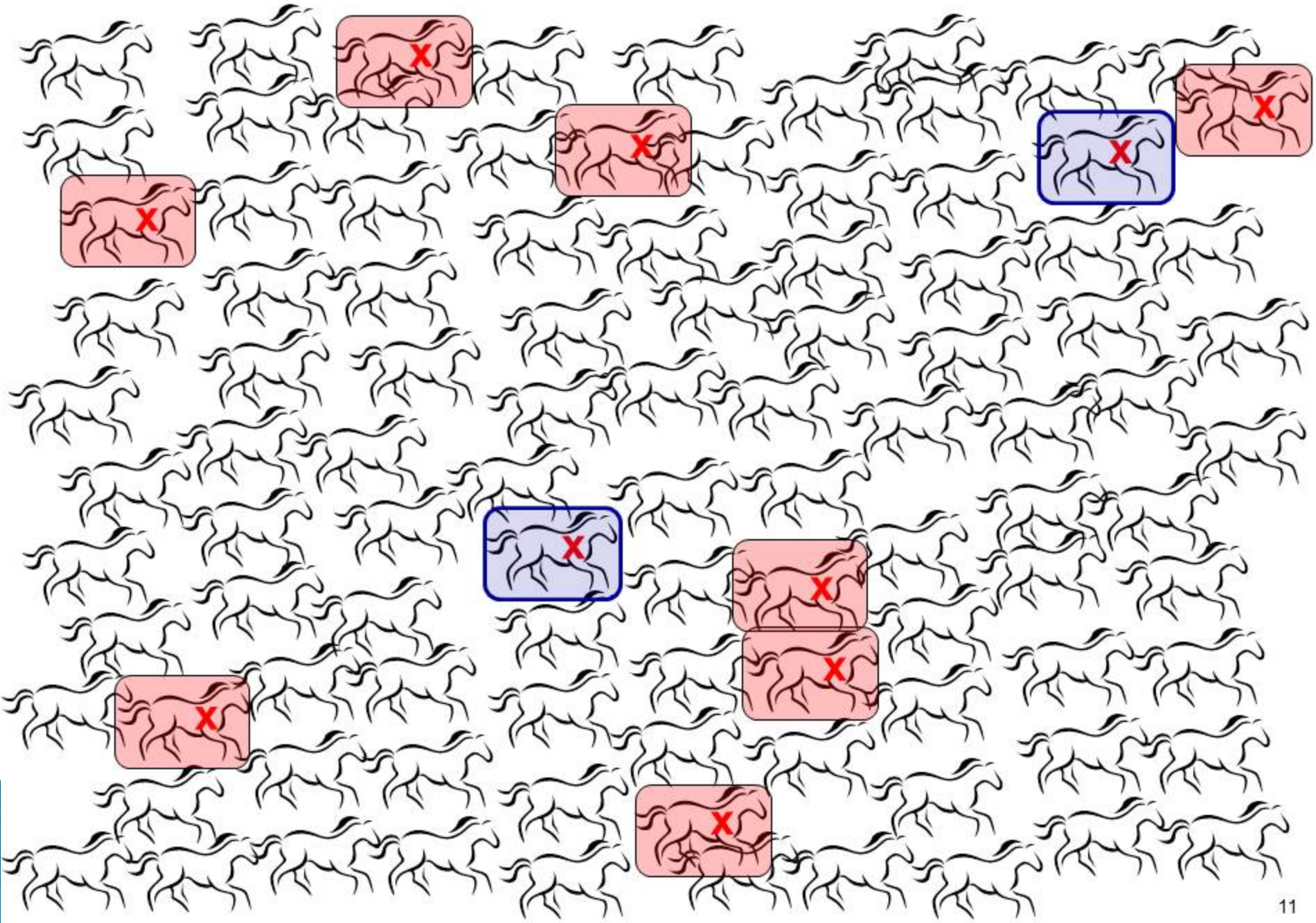
Assay Sensitivity

- Sensitivity is the ability of a test to identify individuals who have a given disease or condition.
- If a test is 100% sensitive, all infected horses will be found by a positive test result.
  - Horses testing negative do not have the infection. There will be no "false negative" tests



Assay Sensitivity – less than 100%

- If a test is not 100% sensitive, some of the infected horses will not have a positive test result.
- There will be some "false negatives" – horses with the infection that have a negative test result

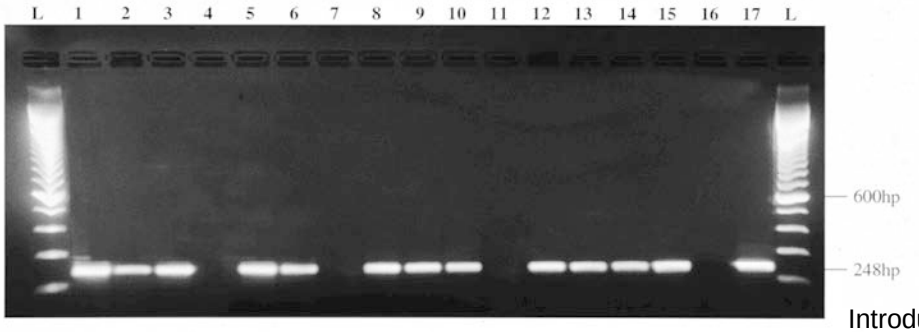

X
X
X
X
X
X
X
X
X
X

Impacts of Assay Sensitivity on Investigating Outbreaks

- If sensitivity is low, infected horses may not be identified in testing
  - Risk spread of disease either within a population or between populations if infected horses contact naïve horses
- Examples
  - Tests that rely on culture of bacteria or viruses: e.g. CEM, equine arteritis virus
  - Tests that are easily affected by sample quality: e.g. PCR
  - Methods that lack amplification of reaction: e.g. agar gel immunodiffusion

Impact of Assay Sensitivity on Investigating Outbreaks

- If <u>sensitivity is high</u>, infected horses will likely be identified in testing provided the test is fit for purpose
  - Tests with high sensitivity are desirable to exclude introduction of infected animals
- Highly sensitive tests may not accurately reflect risk for transmission
  - Could unnecessarily exclude horses that are not infectious
    - Example: PCR that detects nucleic acid fragments beyond the time when the horse is shedding the pathogen

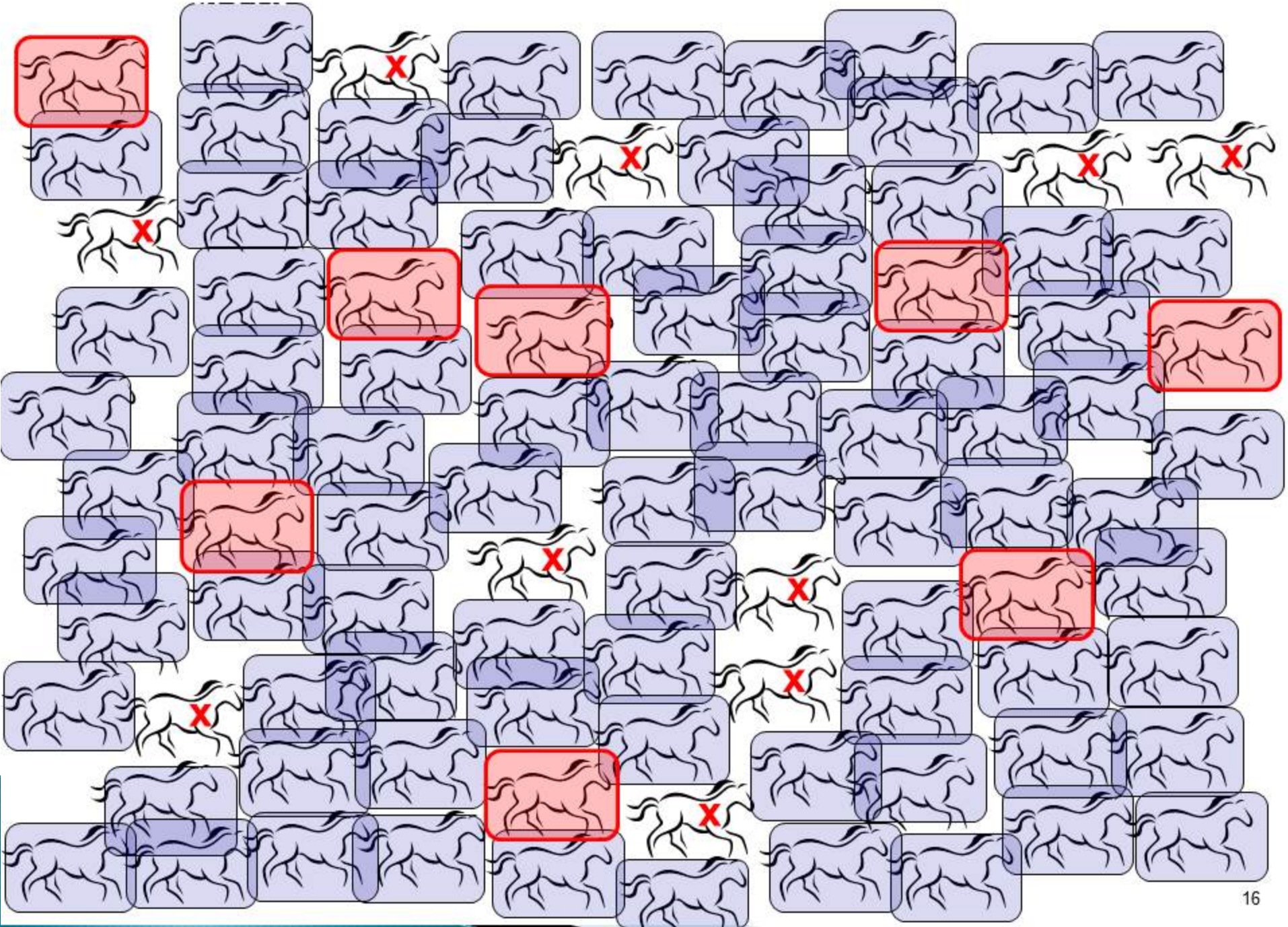


Assay Specificity

- Specificity is the ability of a test to correctly exclude individuals who do not have a given disease or condition.
- If a test is 100% specific, all negative results will represent uninfected horses.
- There will be no false positives.

Assay Specificity

- If a test is not 100% specific, some of the horses that are not infected will be falsely identified as positive.
- There will be false positives.



Impact of Assay Specificity on Investigating Outbreaks

- If specificity is low, animals that are not infected may be labeled as positive
  - May result in perception that outbreak is larger than it really is

- If specificity is high and the test is fit for purpose, there will be false negative test results
  - May result in perception that outbreak is not as large as it really is

Case Definitions

- Individual outbreaks require standardized case definitions
- Case definitions should include criteria for:
  - Animal/Farm
  - Place
  - Time
  - Diagnostic criteria

- APHIS-Veterinary Services' Outbreak Surveillance Toolbox is a good resource for case definitions
- Do not include potential risk factors in the case definition

Classifying Cases

- Can have suspect, presumptive positive, confirmed positive
- Case Definition for Equine Piroplasmosis
  - *Suspect case:* Any equid with:
    - Clinical signs consistent with equine piroplasmosis; **OR**
    - History of exposure; **OR**
    - An inconclusive or positive test (Giemsa-stained blood film, or CF, or IFA, or c-ELISA, or PCR) performed on sample taken during routine screening or surveillance for equine piroplasmosis.
  - *Presumptive positive case:* Any suspect case with:
    - Epidemiological information consistent with equine piroplasmosis; ***AND***
    - A positive test for equine piroplasmosis (Giemsa-stained blood film, or CF, or IFA, or c-ELISA, or PCR).
  - *Confirmed positive case:* Any equid tested positive for equine piroplasmosis by NVSL or a laboratory designated by the Secretary of Agriculture by at least one of the following methods: CF ***OR*** cELISA.

Summary

- Confirm the diagnosis
  - Identify clinical signs
  - Diagnostic testing
    - Select test that is fit for purpose
    - Choose test with appropriate sensitivity and specificity
    - Understand limitations of test
  - Farm visits
  - Consult with experts

- Case Definition
  - Utilizes type of animal, clinical sings, diagnostic test results, place and time to set up criteria for classifying animals with the disease in question.

References

- Dohoo, I., Martin, W., and H. Stryhn. *Veterinary Epidemiologic Research*. Canada: AVC Inc., 2003, print.
- Thrusfield, M. *Veterinary Epidemiology*, *Third edition.* Ames: Blackwell Science Ltd., 2007, print.
- Ostlund, E. "*Role of Diagnostic Laboratories in Providing Services for the Equine Industry*". Panama: Presentation for Regional Workshop on Facilitation of International Competition Horse Movement, 2012.