FAO/ASTF Project: GCP/RAF/510/MUL:

Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture: Intensive Training Course on TiLV

4-13 December 2018. Kisumu, Kenya

in cooperation with Kenya Marine Fisheries Research Institute (KMFRI) and Kenya Fisheries Service (KeFS)

Epidemiology Session
Diagnosis and Screening

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Overview

- Clinicians and pathologists devote substantial time to arriving at the correct diagnosis when investigating disease.

- The diagnosis is usually reached through a process of clinical examination and assessment and the application of various diagnostic tests.
  - Good judgement
  - A thorough knowledge of the literature
  - Past experience
  - Diagnostic tests
  - Intuition

- This section discusses the important epidemiological characteristics of tests and their practical application and interpretation in epidemiological studies and surveillance activities. The epidemiological evaluation of tests is also briefly discussed.

Box 3.1. Some methods used to diagnose disease.

- History
- Behaviour
- Clinical signs
- Physical examination
- Autopsy
- Molecular biology
- Microbiology
- Serology
- Epidemiology
- Response to therapy
- Production
- Economics
- Biochemistry
- Physiology
- Imaging
- Transmission tests

Measurement scale

Dichotomous: pregnancy (yes/no), bacteria isolated (yes/no), serologically positive (yes/no)

Ordinal: serologic titer (1:4, 1:8, 1:16, etc.)

Continuous: red blood cell counts, serum enzyme concentrations, ELISA optical density (OD) values

Screening versus Diagnosis

- Screening begins with apparently healthy individuals whereas diagnostic testing begins with animals showing signs consistent with the disease in question.
- Screening are used for the presumptive identification of unrecognized disease in apparently healthy populations.
- A screening test should have both high sensitivity and precision, be easy to perform and of low cost if a large number of individuals are to be tested.
- A screening test is not intended to be diagnostic: individuals who return a positive result in a screening test should be subject to a more thorough investigation to establish a diagnosis.

Screening versus Diagnosis

- Diagnostic tests are used to confirm a diagnosis in animals presenting with signs of the disease of concern.

- Diagnostic tests usually require a high specificity to minimize the likelihood of animals being incorrectly diagnosed with disease.

- Screening tests are used to screen healthy animals for disease, whereas diagnostic tests are used to confirm a diagnosis in diseased animals.

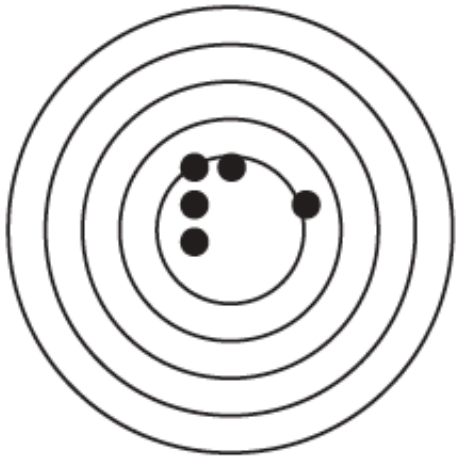
Key characteristics of tests used for screening and diagnosis

Screening	Diagnosis
Applied to healthy population	Applied to sick individual
Seeks unrecognized disease	Differentiates among likely diseases
High sensitivity	High specificity
Large numbers tested	Small numbers tested
Low cost important	Cost not so important

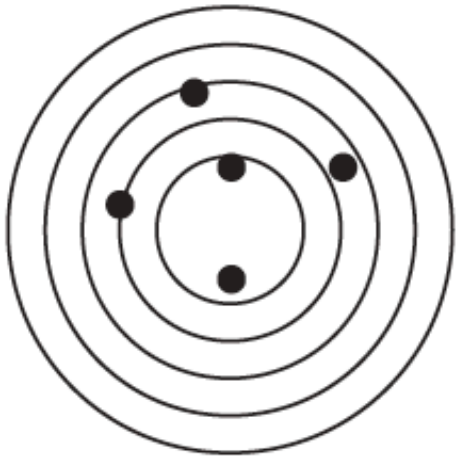
Accuracy of Test Procedures

- The accuracy of a test can be measured in two ways
  - Validity
  - Precision
- An accurate test is both precise and valid.
- In other words the result is repeatable (a measure of precision) and also gives a true measure of the value being measured (sensitive and specific – measures of validity).
- Precision is defined as a lack of random error (high repeatability) while validity is a lack of systematic error or bias (high sensitivity and specificity).

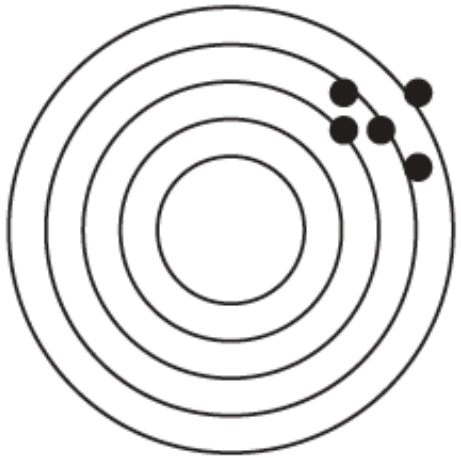
Validity and precision in test procedures



Valid
and
precise



Valid
but
imprecise



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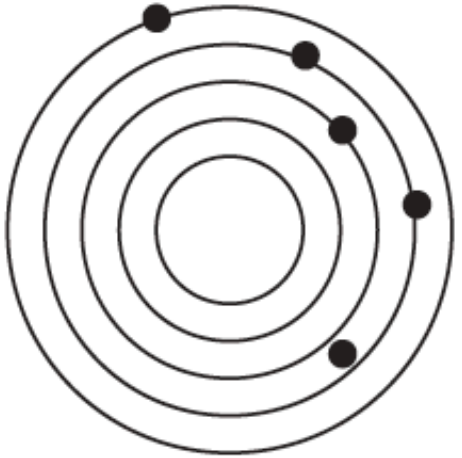


Invalid
and
imprecise

Precision

- Tests performed on presumably identical material under apparently similar conditions are expected to produce very similar but not identical results.
- This variation is attributed to unavoidable random error.
- Factors that contribute to the variability of a test procedure:
  - Uniformity of test material
  - Transport and storage of test material
  - Reagents
  - Equipment and its calibration
  - Operator
  - Environmental conditions such as temperature, humidity, light, air pollution.

Assessing precision

- Two complementary measures are used to assess the precision of test methods: repeatability and reproducibility.
- Repeatability refers to a test being performed on the same sample(s) under conditions that are as constant as possible in the one laboratory by one operator using the same equipment over a short period of time.
- Reproducibility is the ability of a test on the same sample(s) to give consistent results in repeated tests under widely varying conditions in different laboratories at different times and by different operators.
- Thus, repeatability and reproducibility are two extremes, the first measuring the minimum and the second the maximum variability in results due to random error.

Assessing precision

- Reproducibility provides a measure of the robustness of a test: that is, how well it performs under varying conditions of environment and equipment.
- Measures of precision on continuous scale:
  - Error standard deviation (duplicate measurements for each specimen)
  - Coefficient of variation (error standard deviation as a percentage of the mean)
  - Line of identity (where the fitted regression line for the duplicate measurements is compared to the line of identity that has a slope of 1 and passes through the origin).
- Measures of precision on qualitative scale:
  - Kappa statistic to assess the level of agreement of repeated tests conducted on the same samples.

Test Validity

- The validity of a test procedure is a measurement of the amount of bias in a test result and is quantified by the test's diagnostic **sensitivity** (DSe) and diagnostic **specificity** (DSp).
- DSe of a test is the proportion of animals with the disease (or infection) of interest that test positive (i.e. proportion of true positives).
- This contrasts with the laboratory definition of analytical sensitivity, which is the ability of an analytical method to detect very small amounts of the material (such as an antibody or antigen).
- Sensitivity is also defined as the conditional probability that a test will correctly identify those animals that are infected **(Pr T+|D+)**.

Factors affecting sensitivity in antibody assay estimates

- Number of animals in study
- Method used to determine disease or infection status
- Stage of disease
- Cut-off point selected
- Anti-species conjugate type
- Non-specific inhibitors
- Incomplete antibody
- Suppression of immunoglobulin production

Specificity

- **DSp** of a test is the proportion of animals without the disease of interest that test negative (i.e. proportion of true negatives).

- **Specificity** is also defined as the conditional probability that a test will correctly identify those animals that are not infected **(Pr T–|D–)**.

- This compares with the laboratory definition of analytical specificity, which is the ability of the test to react only when the particular material is present and not react to the presence of other compounds.

Factors affecting specificity in antibody assay estimates

- Number of animals in study
- Method used to determine disease or infection status
- Cut-off point selected
- Anti-species conjugate type
- Non-specific inhibitors
- Group cross-reactions
- Non-specific agglutinins

Estimating sensitivity and specificity from animals of known disease status

- To estimate sensitivity and specificity, one must conduct the test on specimens from a number of animals for which the status of infection or disease is known.
  - Experimental infections
  - Field samples are much better

- Results from such a study can then be tabulated in a 2 x 2 table

Calculation of sensitivity and specificity estimates from results of testing on animals of known disease status

	State of nature		
Test result	Diseased (D+)	Not diseased (D–)	Total
Positive (T+)	a	b	a + b = T+
Negative (T–)	c	d	c + d = T–
Total	a + c = D+	b + d = D–	n = a + b + c + d

a = true positives; b = false positives; c = false negatives; d = true negatives

Test characteristics	
Sensitivity (Se)	= a/(a+c)
Specificity (Sp)	= d/(b+d)
Infection probabilities	
Probability of having disease (Prevalence)	= (a+c)/n
Probability of not having disease (1–Prevalence)	= (b+d)/n

Accuracy of a diagnostic test

- Measured in terms of
  - Sensitivity and specificity
  - Area under the receiver-operating characteristic (ROC) curve
- Laboratory context
  - Analytic sensitivity (*sin: minimum detection limit*)
  - Analytic specificity (*sin: cross-reaction profile)*

Accuracy of a diagnostic test

Measured relative to a “gold standard”

- Diagnostic method or combination of methods which determines absolutely and without error whether a disease/infection is present
- Doesn’t exist for all diseases
- May not be practical due to cost, labor or invasiveness
- Often use terms such as “reference test”, “criterion standard”, “definitive test” to recognize imperfections in the gold standard

Classification of individuals according to test results (T+, T-) and infection status (I+, I-)

		State of Nature: Infected	State of Nature: Non-infected		
Test results	+	a	b	a+b	= T+
	-	c	d	c+d	= T-
		a+c = I+	b+d = I-	a+b+c+d = n	

a = true positives; b = false positives;
c = false negatives; d = true negatives

Classification of individuals according to test results (T+, T-) and infection status (I+, I-)

		State of Nature: Infected	State of Nature: Non-infected	
Test results	+	TP	FP	= T+
	-	FN	TN	= T-
		= I+	= I-	N

TP = true +ve; FP = false +ve; FN = false –ve; TN = true -ve

		Disease +	Disease -	
Test	+	a	b	a+b
	-	c	d	c+d
		a+c	b+d	a+b+c+d

Test performance characteristics

Sensitivity (Se) = a/(a+c); Specificity (Sp) = d/(b+d)
Likelihood ratio (positive) = Se / 1-Sp
Likelihood ratio (negative) = (1-Se) / Sp

Prior (pre-test) probabilities

Probability of having disease (Prev) = (a+c)/n
Probability of not having disease (1-Prev) = (b+d)/n

		Disease +	Disease -	
Test	+	a	b	a+b
	-	c	d	c+d
		a+c	b+d	a+b+c+d

Posterior (post-test) probabilities (*syn: predictive values*)

Predictive value positive:

= a/(a+b) = PSe /[PSe + (1-P)(1-Sp)]

Predictive value negative:

= d/(c+d) = (1-P)Sp/[(1-P)Sp + P(1-Se)]

Apparent (test) prevalence (AP)

Proportion of test positive results = (a+b)/n

Exercise

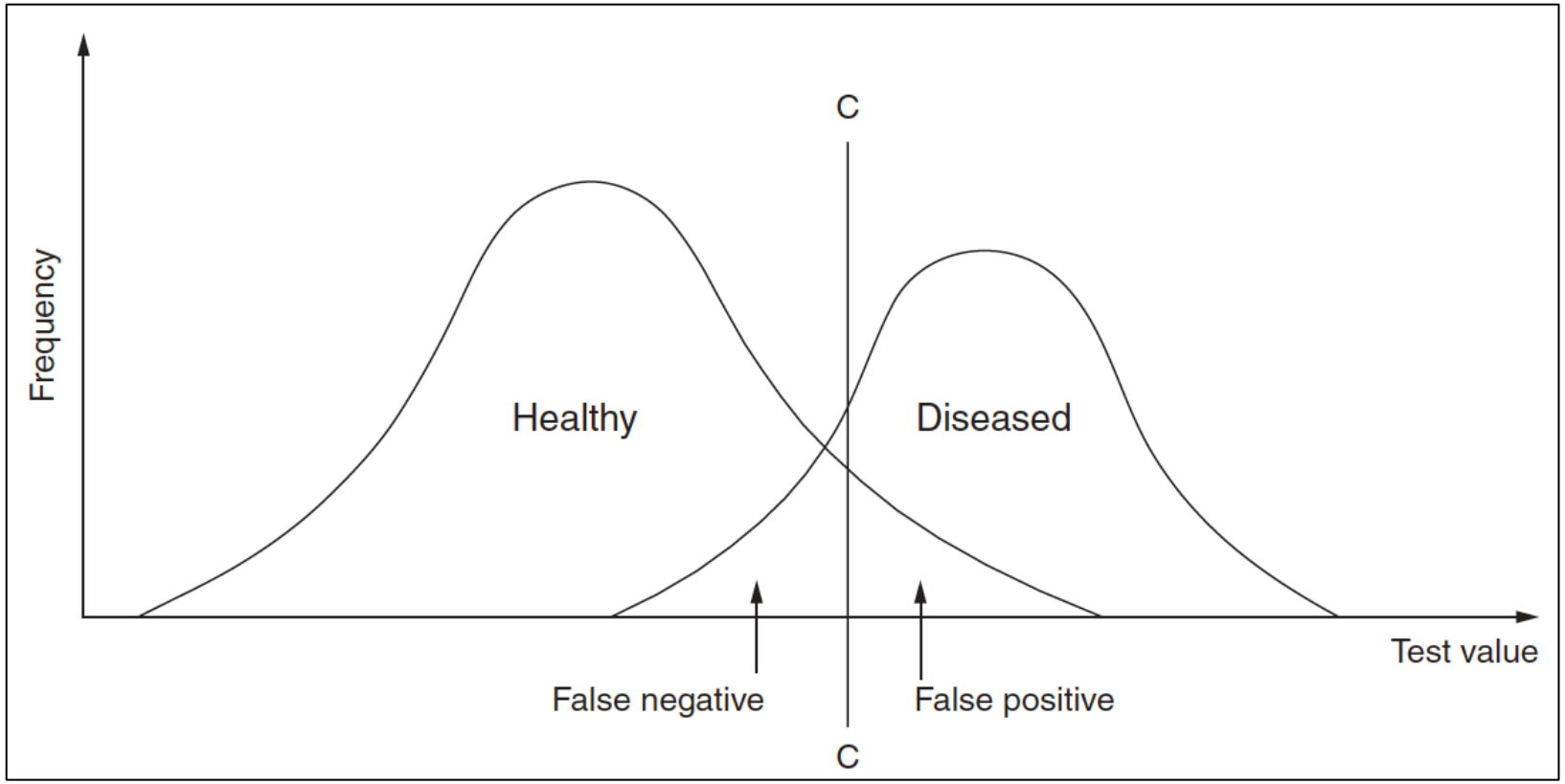
	True disease status		
Test result	D+	D–	Total
Positive (T+)	33	2	35
Negative (T–)	4	141	145
Total	37	143	180

Estimated sensitivity and specificity are:

Se = 33/37 = **89.2%**
Sp = 141/143 = **98.6%**

Relationship between Se and Sp

- For tests where the raw result is presented as a value on a continuous scale, such as an ELISA, there is an inverse relationship between Se and Sp



Perfect test

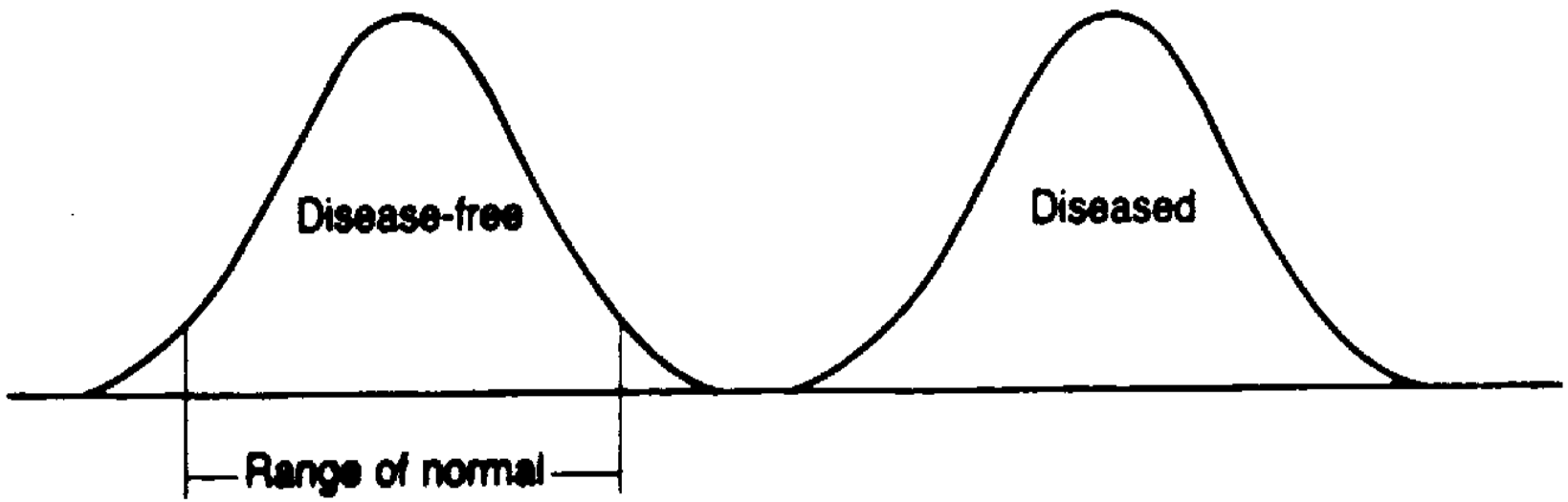


FIGURE 15-3 *A test with complete separation of populations results in perfect diagnostic discrimination.*

Typical highly accurate test

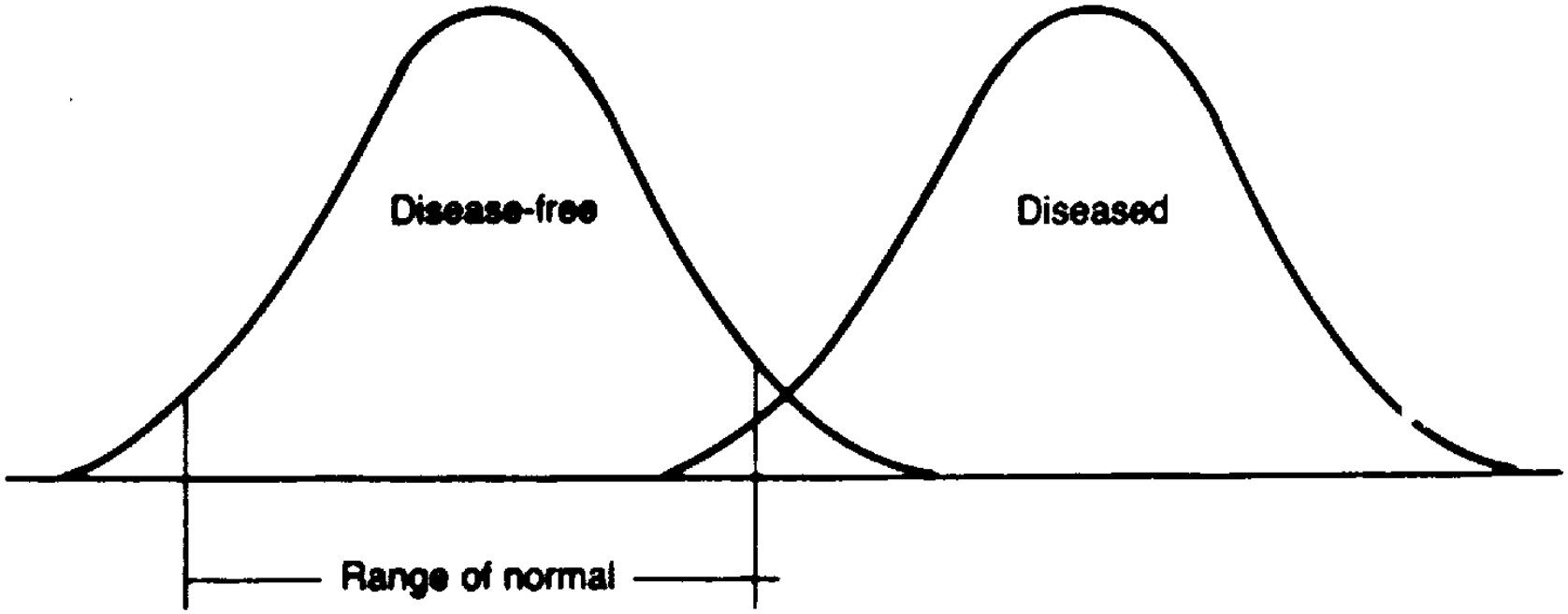


FIGURE 15-4 *A test with partial separation of the populations results in partial diagnostic discrimination.*

Worthless test

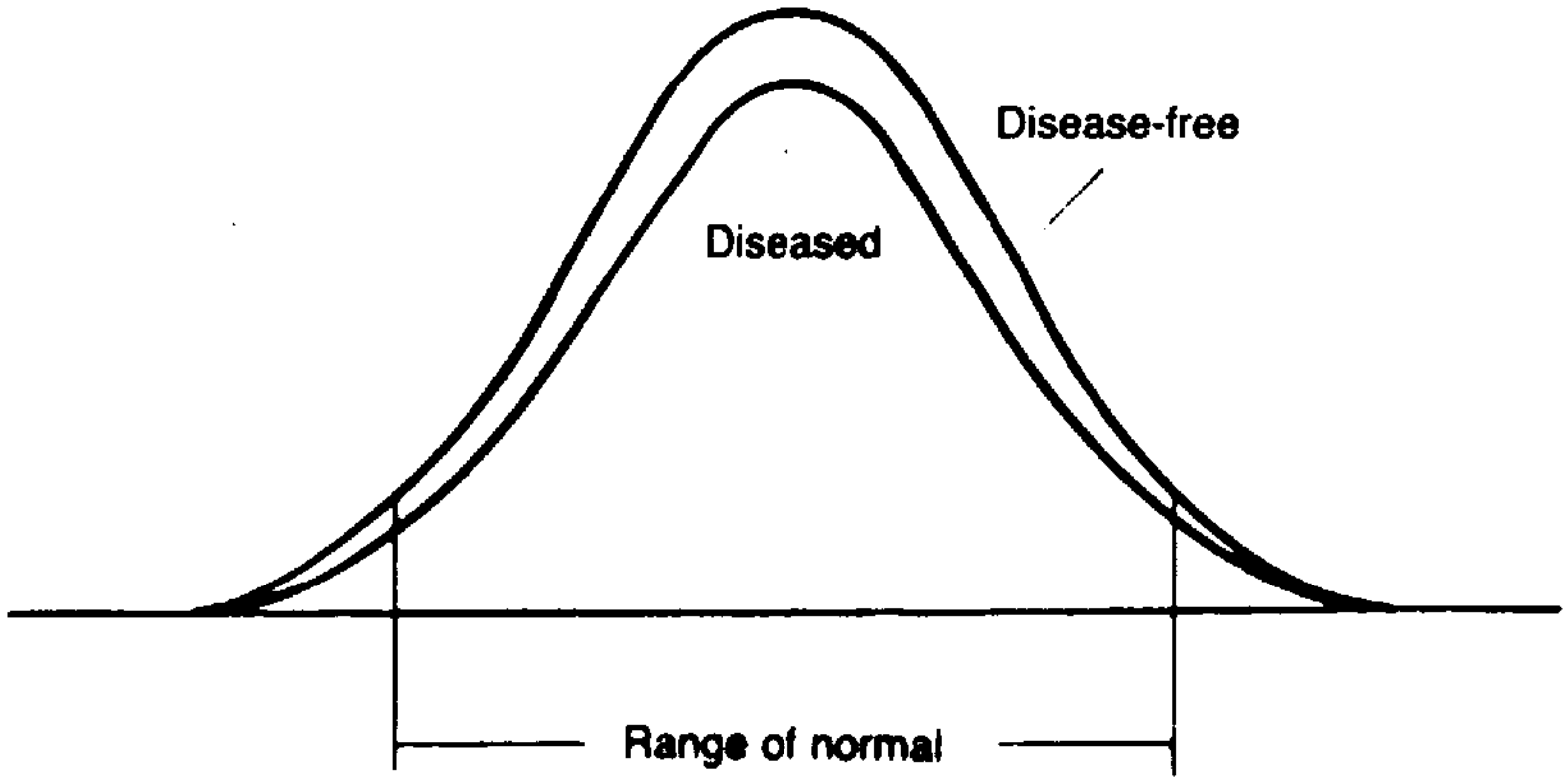
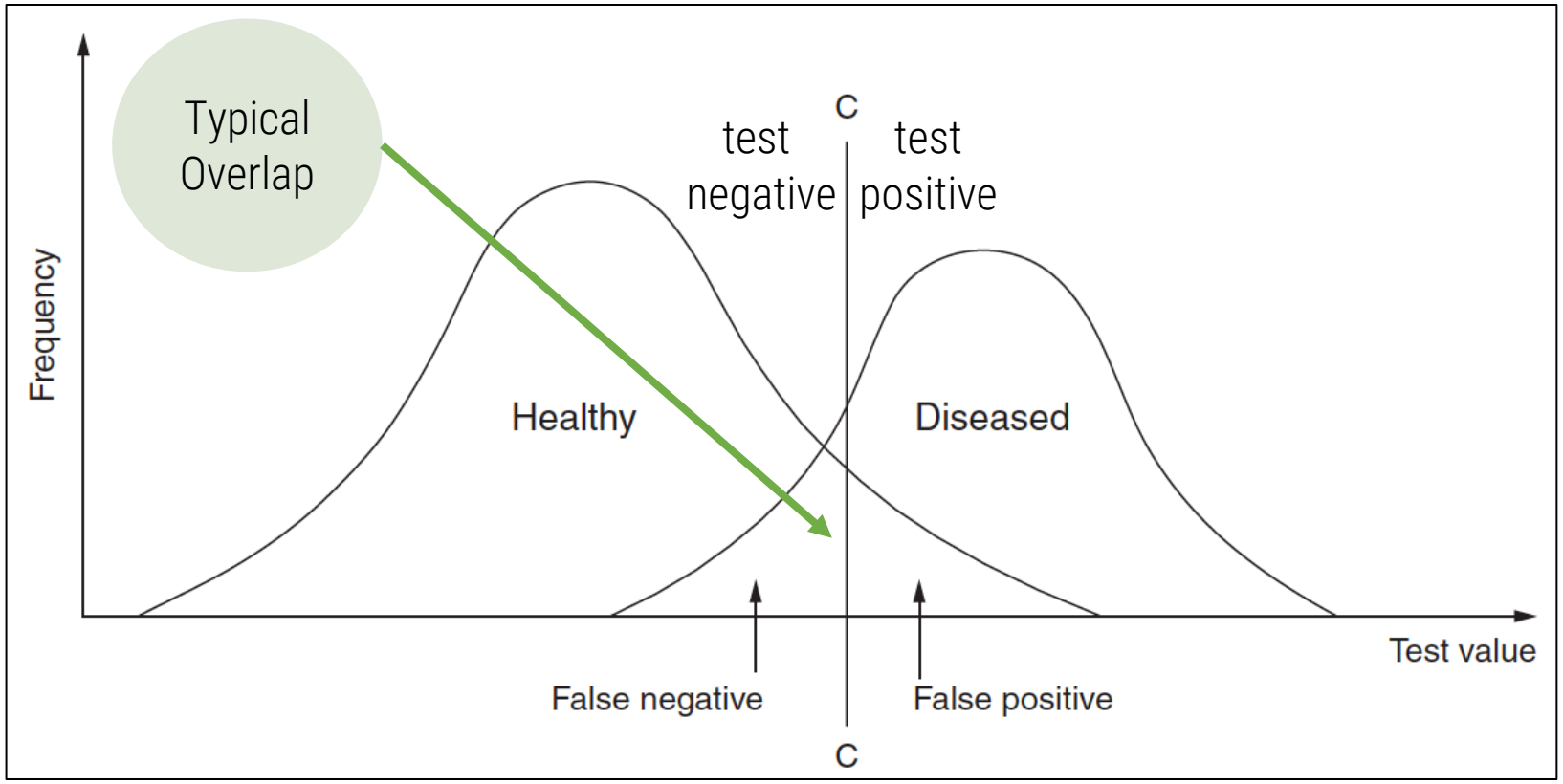


FIGURE 15-5 *A test with no separation of the populations results in no diagnostic discrimination.*

Relationship between Se and Sp



Selection of the appropriate cut-off value

- Relative cost of false positives and false negatives
- Stage of an eradication program
- Availability of other tests.

Test Interpretation at the Individual Animal Level Predictive Values

- When interpreting test results, we are more interested in how well we can rely on the test result. *Is a positive result indicative of an infected animal and, conversely, is a negative result truly indicative of an uninfected animal?*

- Predictive values are the conditional probabilities that answer these two related questions:
  - What is the probability that a test-positive animal is truly infected (the positive predictive value or PPV)?
  - What is the probability that a test-negative animal is truly not infected (the negative predictive value or NPV)?

Predictive values
(*syn*: post-test or posterior probabilities)

- Using probability notation, the predictive value of positive test results (PPV) is the P(D+|T+); for negative test results (NPV) it is the P(D−|T−).

- Formulae for calculating predictive values are based on Bayes' theorem of conditional probability.

$$\text{Positive predictive value} = \frac{P \times Se}{P \times Se + (1-P) \times (1-Sp)}$$

$$\text{Negative predictive value} = \frac{(1-P) \times Sp}{(1-P) \times Sp + P \times (1-Se)}$$

where Se = sensitivity, Sp = specificity and
P = pre-test probability of disease (sometimes estimated true prevalence in the population).

Predictive values

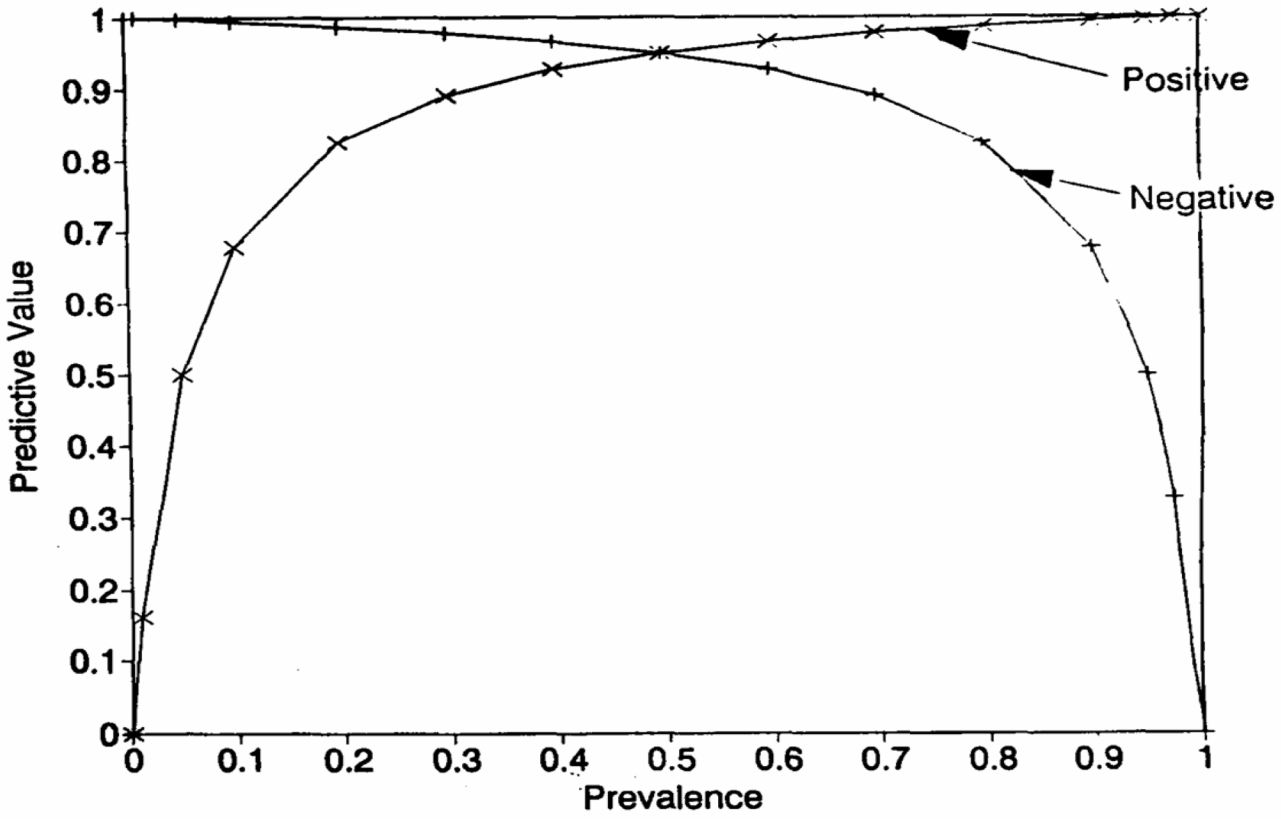
- PPV is the probability that a test-positive individual is truly infected.
- NPV is the probability that a test-negative individual is truly uninfected.
- Pre-test probability of disease (P) is a critical parameter

Pre-test probability of disease (P)

Possible starting values

- P = 0.9 if full clinical signs are present and they are pathognomonic for the disease
- P = 0.5 if 2 possibilities are equally likely, or if clinician has multiple diagnostic possibilities and wants to rule them “in” or “out”
- P = 0.1 if known risk factors are present
- P = 0.01 if risk factors are absent and disease is unlikely

As prevalence decreases, the PVP decreases but the PVN increases



Predictive values

- Specificity exerts a greater influence on the PVP than does sensitivity
- Animal diseases --- in the final stages of disease eradication use tests or testing strategies with higher specificities to minimize unnecessary culling

Example – *Piscirickettsia salmonis* infection in farmed salmon

- Goal: to validate an indirect fluorescent antibody test (IFAT) to detect systemic disease attributable to the bacterium *Piscirickettsia salmonis,* in farmed salmon.

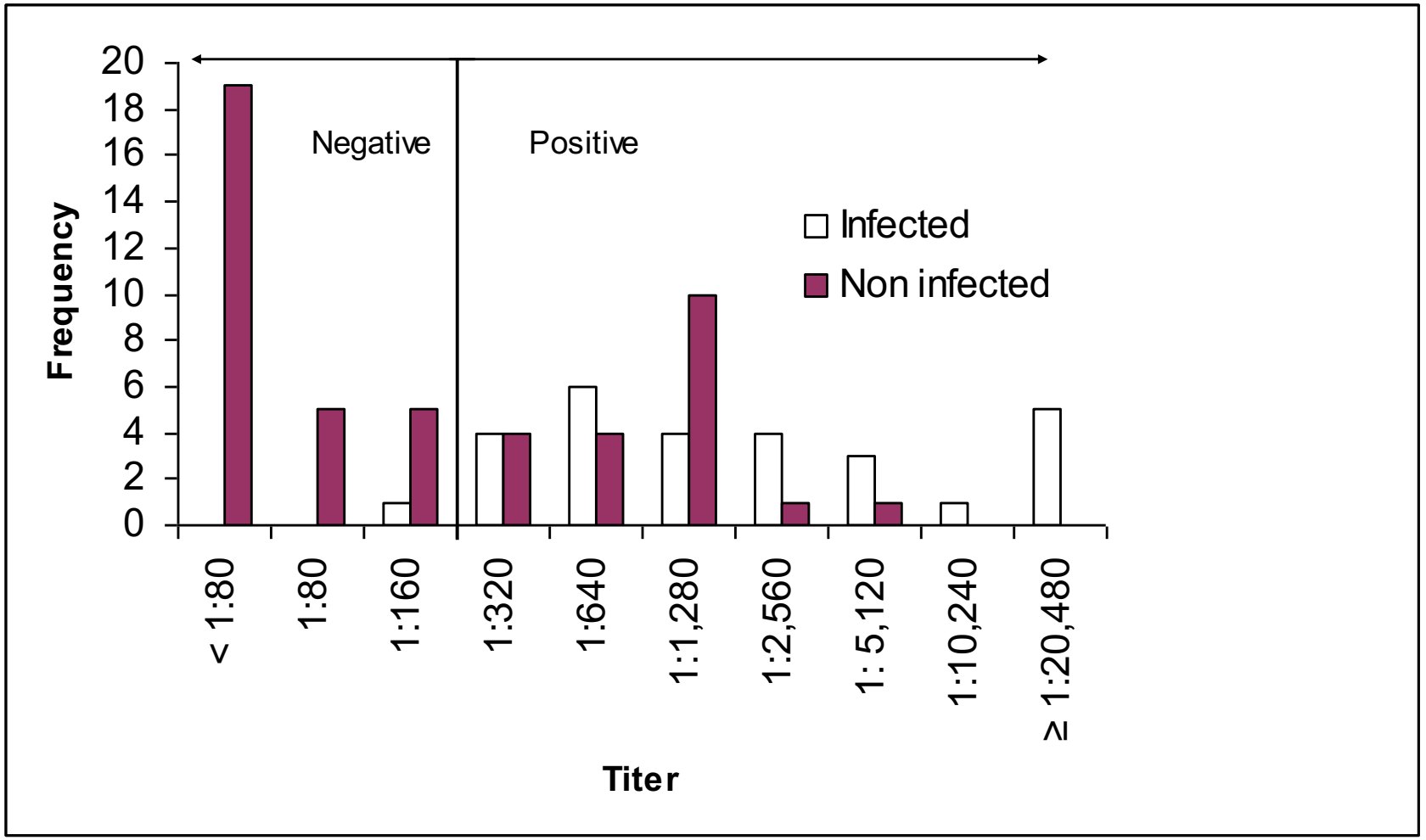
- For the validation, 28 dead salmon with confirmed *P. salmonis* liver lesions and 49 salmon without *P. salmonis* lesions were evaluated.

- Note that a titer represents the highest dilution of the serum that still yields a positive result.

P. salmonis in farmed salmon

P. salmonis IFAT titer	Infected	Noninfected
≥ 1:20,480	5	0
1:10,240	1	0
1: 5,120	3	1
1:2,560	4	1
1:1,280	4	10
1:640	6	4
1:320	4	4
1:160	1	5
1:80	0	5
< 1:80	0	19
Total	28	49

P. salmonis in farmed salmon



Using these data the following table was constructed using a cutoff of 1:320,

- (i.e. titers of ≥1:320 were considered positive and titers of ≤ 1:160 were negative)

		Gold Standard +	Gold Standard -
IFAT	+	27	20
	-	1	29
		28	49

Estimate % (95% CI)

Se = 27/28 = 96.4 (81.6 – 99.9%)

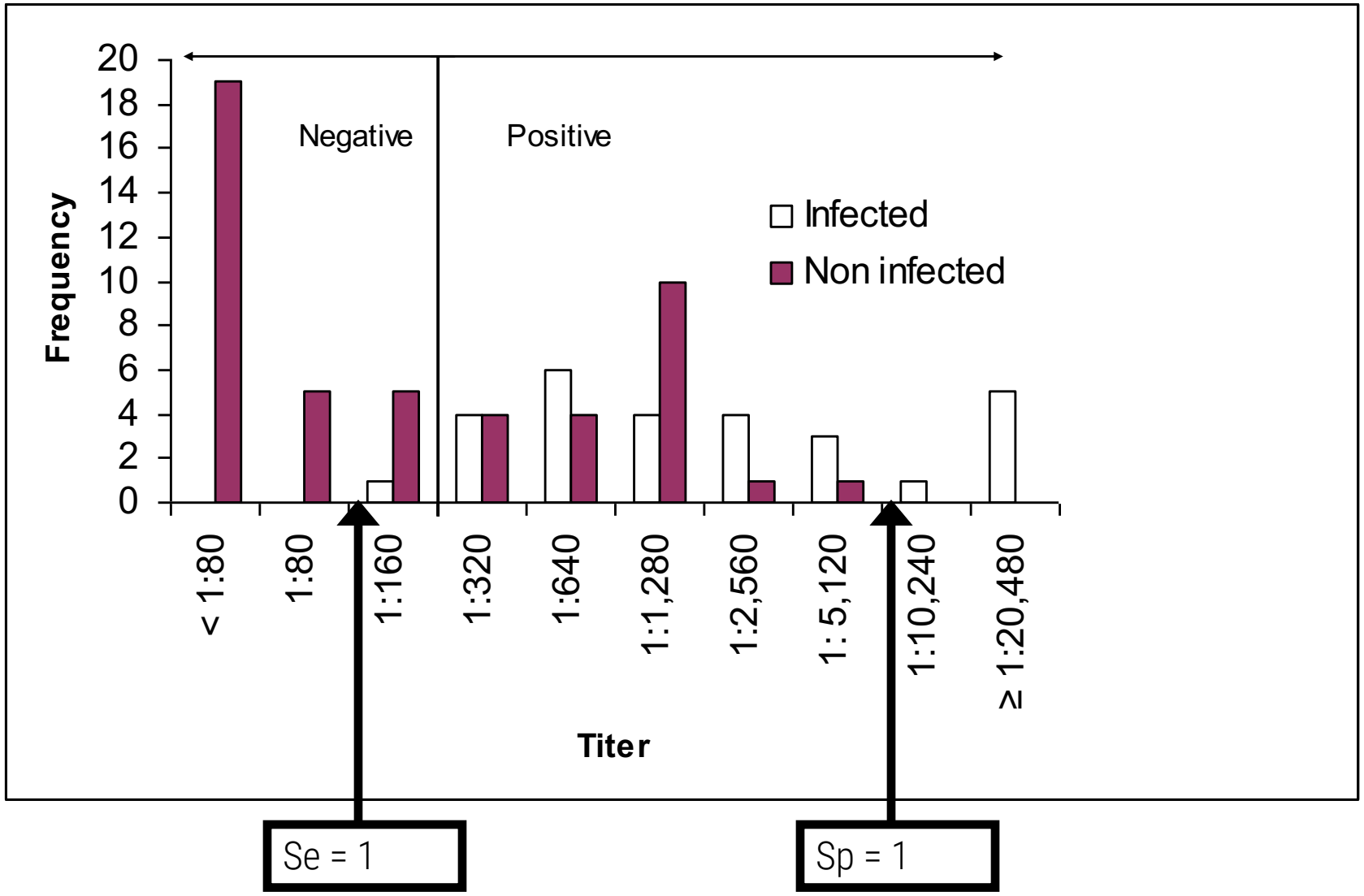
Sp = 29/49 = 59.2 (44.2 – 73.0%)

A cutoff of 1:320 balances false positives

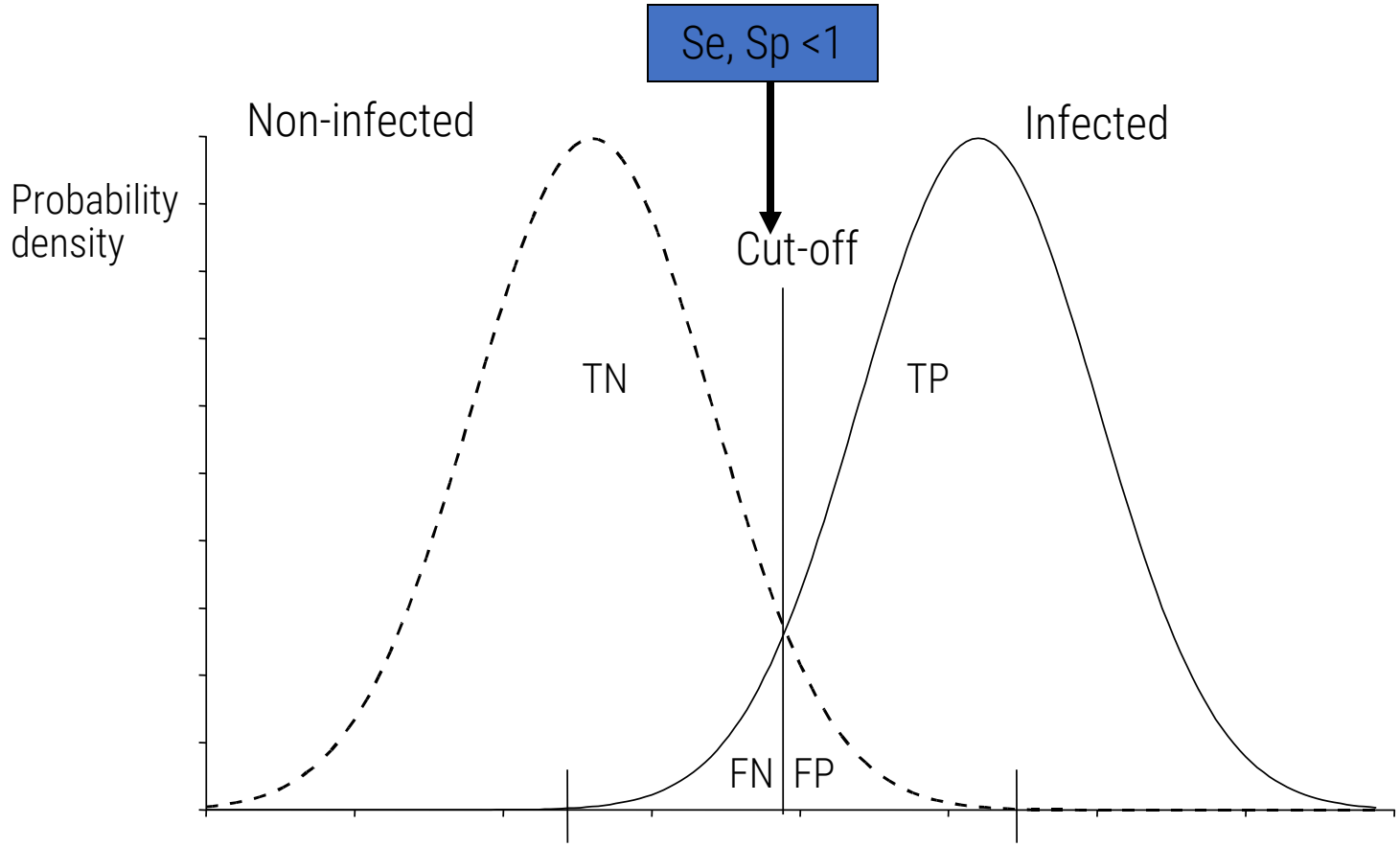
and negatives

- If false +ves (100% Sp) are not allowed: increase cutoff to 1:10,240
- If false negatives (100% Se) are not allowed: decrease cutoff to 1:160
- Changing the cutoff shows the **inverse relationship** between sensitivity and specificity.

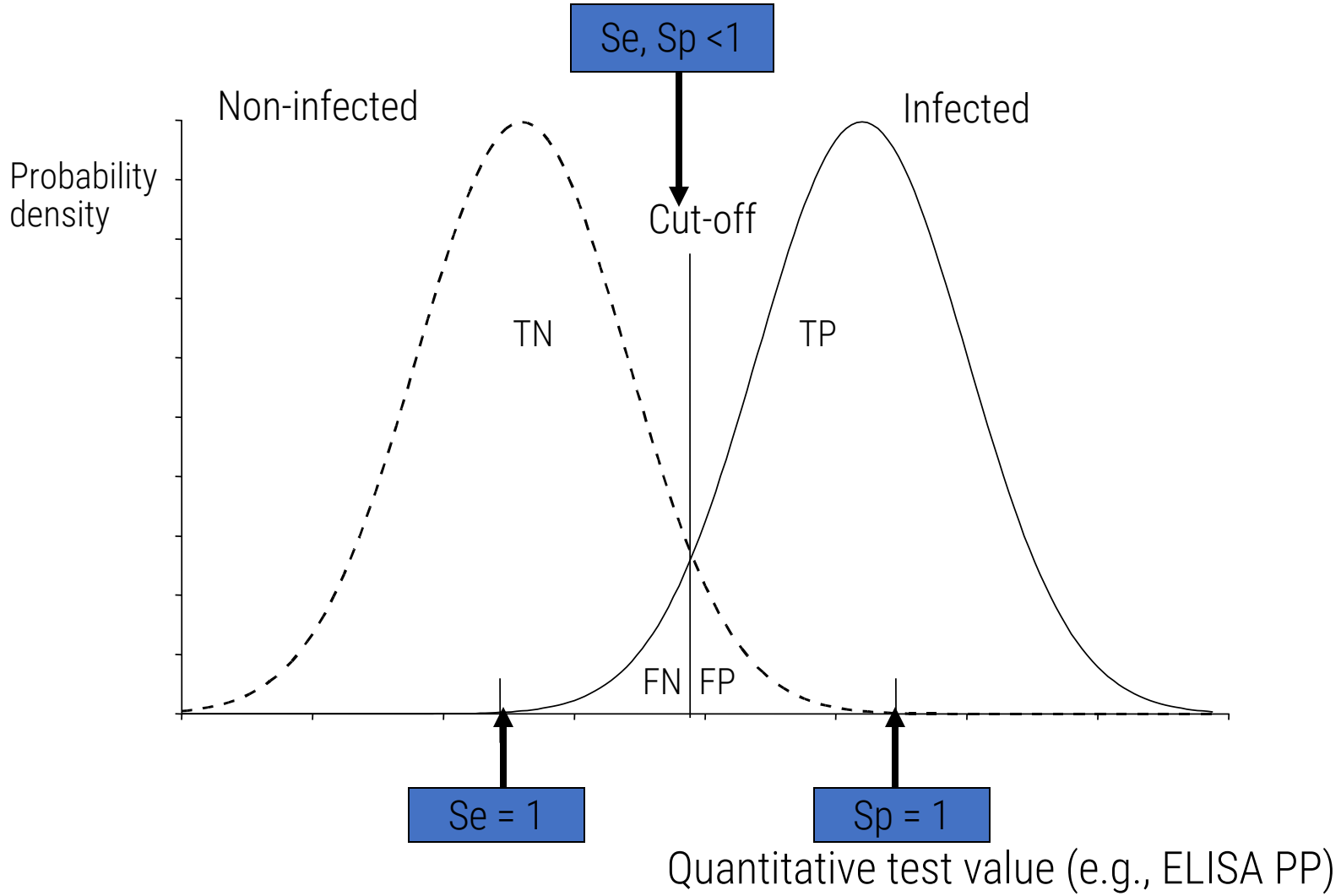
P. salmonis in farmed salmon



Cut-off selection



Cut-off selection



Selection of test cutoff-value

Depends on a number of factors including:

- Purpose of testing
- Relative cost of false +ve’s and false –ve’s
- Likely prevalence of infection
- Availability of confirmatory tests

Selection of test cutoff-value

- Reasons for cut-off selection are not always well justified
- Diagnostic laboratories often choose a cutoff that has good sensitivity and specificity
- Cut-off values should not be considered to be “fixed” - different cutoffs are appropriate given different circumstances for testing

Example: Calculation of PVP/PVN for IFAT test for *P. salmonis* assuming P = 50%.

Two methods: first using Bayes' Theorem formula or second by construction of a 2x2 table (Se = 0.964; Sp = 0.592)

Method 1:

PVP = PSe / [PSe + (1-P)(1-Sp)]

= 0.5 x 0.964 /[0.5 x 0.964 + 0.5 x 0.408]

= 0.703

PVN =(1-P)Sp / [(1-P)Sp + P(1-Se)]

= 0.5 x 0.592 /[0.5 x 0.592 + 0.5 x 0.036]

= 0.943

Method 2: Using a hypothetical population of animals to generate the appropriate 2 x 2 table:

		Infected	Non-infected	
Test results	+	964	408	1372
	-	36	592	628
		1000	1000	2000

Predictive value positive = 964/1372 = 0.703
Predictive value negative = 592/628 = 0.943

Strategies to improve PVP

Especially when disease is rare:

- Testing "high risk" groups - those with clinical signs rather than apparently healthy individuals
- Increasing the cutoff titer for the test to increase specificity or using a new test of higher specificity
- Retesting positives on the first test with a test of specificity close to 1 - only those individuals that are also positive on the second test are considered positive. Negative on test 1 is negative.

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Epidemiology Session
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