Better Training for Safer Food
BTSF

The role of reference laboratories

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Needs for reference laboratories

Reference lab <> regional lab

Standardisation of diagnostic procedures

- **=> make results comparable**

Quality assurance

- **Proficiency tests**
- **Production and distribution of proficiency samples**

=> Accurate and precise diagnosis

=> Improved disease surveillance

Diagnostic test

*A **test** is a procedure for critical evaluation; a means of determining the presence, quality, or truth of something*

*A **diagnostic test** is a procedure which gives a rapid, convenient and/or inexpensive indication of whether a patient has a certain disease*

Properties of a useful diagnostic test

*Test **methodology** has been described in detail and can be accurately and reliably **reproduced***

*Test **accuracy** and **precision** are known*

*The **reference range** has been established (expected range in healthy patients) – **cut-off***

Sensitivity** and **specificity** have been reliably established by comparison with a **gold standard

Test accuracy

The accuracy of a laboratory test is its correspondence with the true value

Accuracy = f(1/systematic error)

Accuracy is maximised by calibrating the required laboratory equipment with reference material and participating in external quality control programmes

Test precision

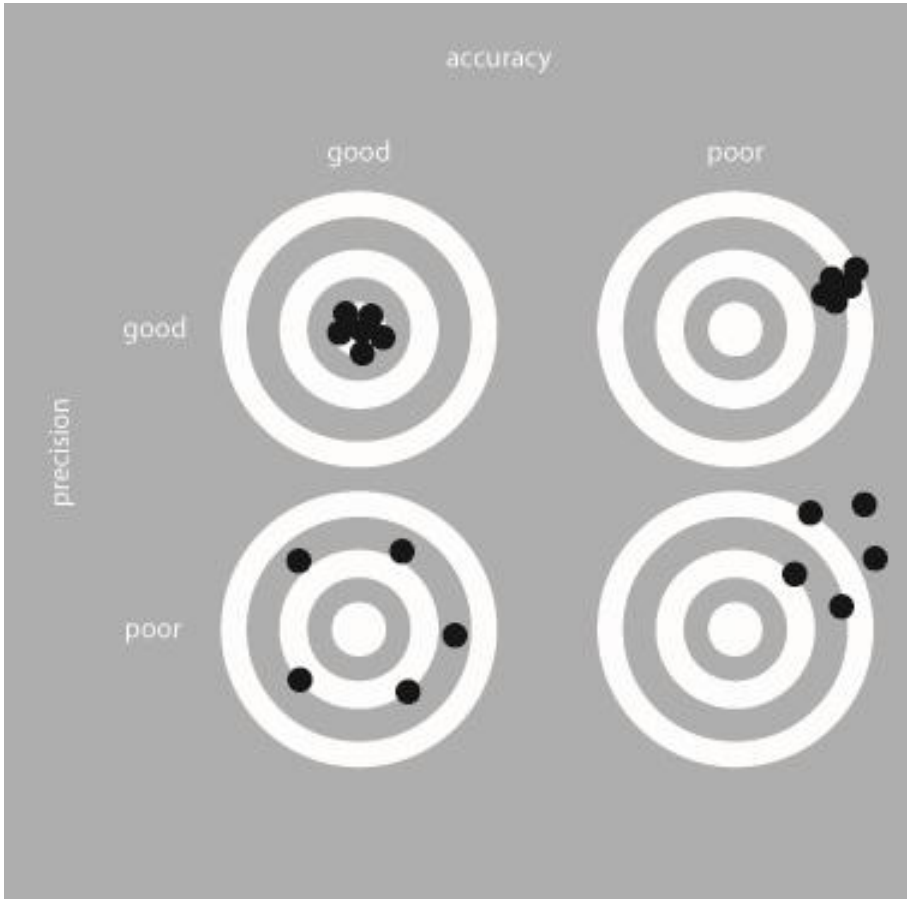
Test precision is a measure of a test's reproducibility when repeated on the same sample

Precision = f(1/random error)

Precision is monitored by using test control material

Precision must be good enough to distinguish relevant changes in patient's status from analytic variability of test

Accuracy and Precision



Test sensitivity

Test sensitivity is the likelihood that a diseased patient has a positive test

Sensitivity is a conditional probability (definition of 'diseased')

Sensitivity is determined in a population consisting exclusively of diseased individuals, which are a reflection of the diseased population in which the test will be used (to avoid spectrum bias)

Determination of sensitivity requires a gold standard

Test specificity

Test specificity is the likelihood that a healthy patient has a negative test

Specificity is a conditional probability (definition of 'healthy')

Specificity is determined in a population consisting exclusively of healthy individuals, which are a reflection of the healthy population in which the test will be used (to avoid spectrum bias)

Determination of specificity requires a gold standard

	Test positive	Test negative
Diseased	?	?
Healthy	?	?

	Test positive	Test negative
Diseased	True positive (TP)	
Healthy	?	

	Test positive	Test negative
Diseased	True positive (TP)	?
Healthy	False positive (FP)	

	Test positive	Test negative
Diseased	True positive (TP)	False negative (FN)
Healthy	False positive (FP)	?

	Test positive	Test negative
Diseased	True positive (TP)	False negative (FN)
Healthy	False positive (FP)	True negative (TN)

Test sensitivity is the likelihood that a diseased patient has a positive test

Test specificity is the likelihood that a healthy patient has a negative test

	Test +	Test -	Total
Diseased	180	20	200
Healthy	40	760	800
Total	220	780	1000

Test sensitivity is the likelihood that a diseased patient has a positive test

Test specificity is the likelihood that a healthy patient has a negative test

Se = 180/200 = 0.9 **Sp = 760/800 = 0.95**

Sensitivity and Specificity

Example: a serological test

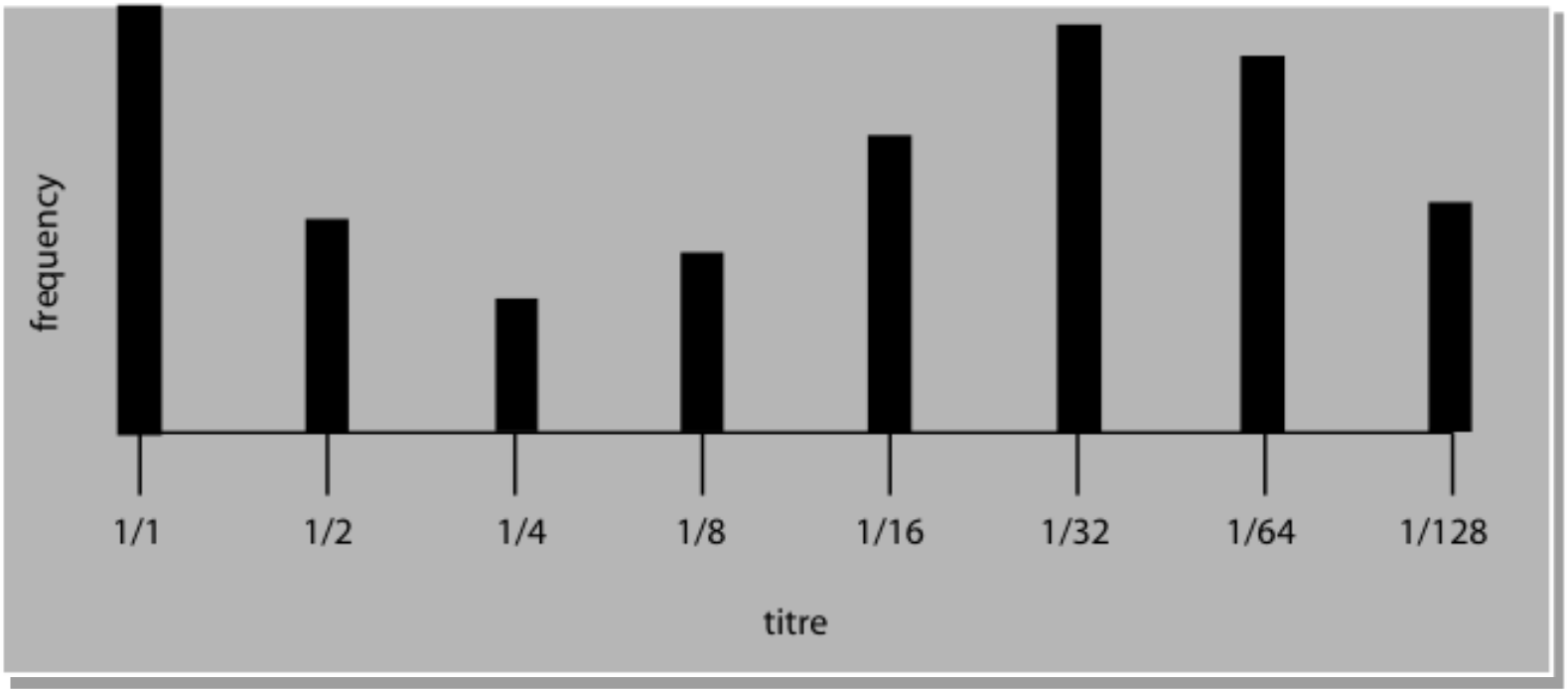
Results are function of the antibody concentration

Elisa: OD, PP…

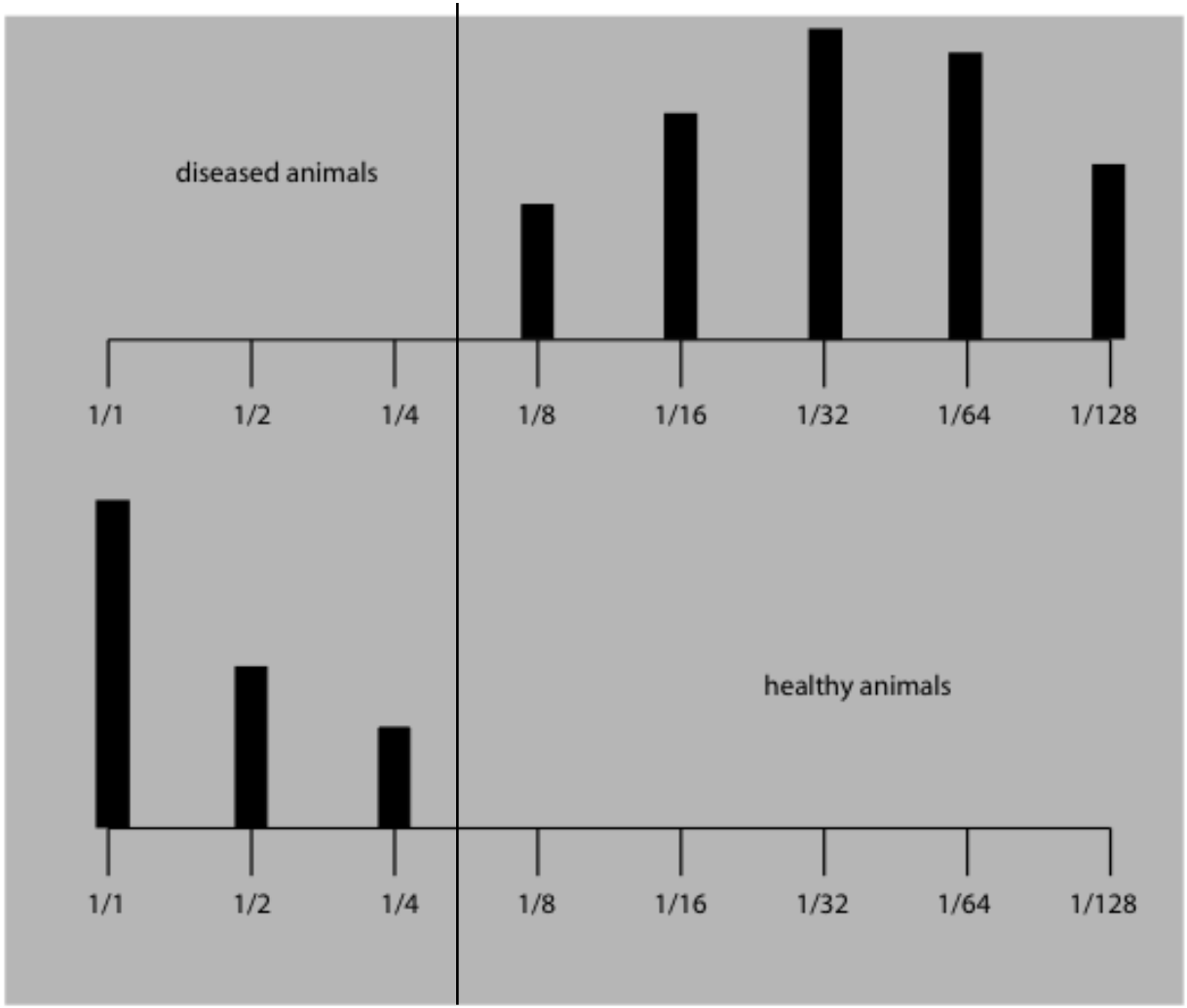
IFAT titre: maximum dilution at which the test is positive

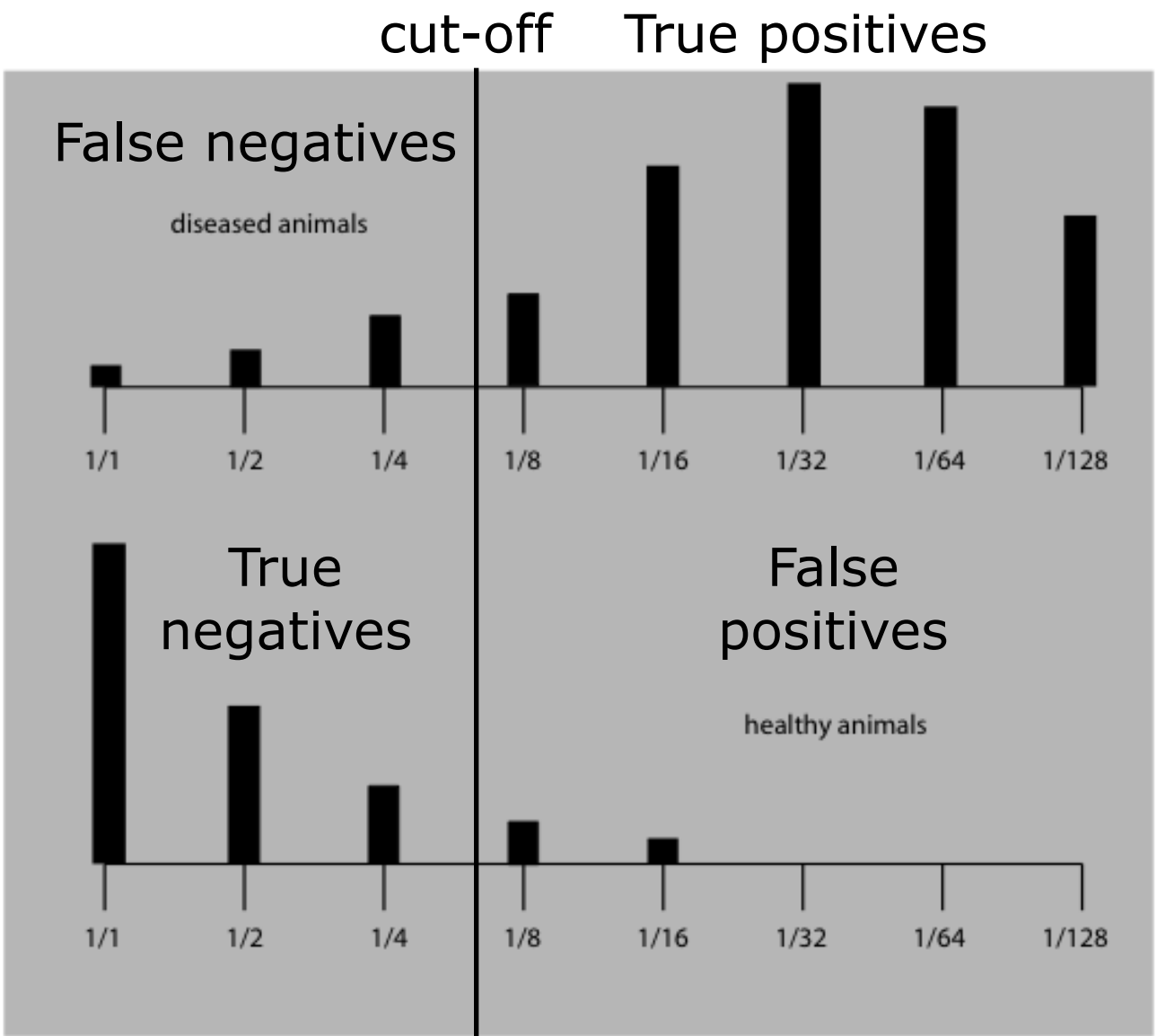
Continuous or ordinal results are transformed in binary results (positive or negative) using a cut-off

Example: Non-cumulative frequencies of IFAT titres

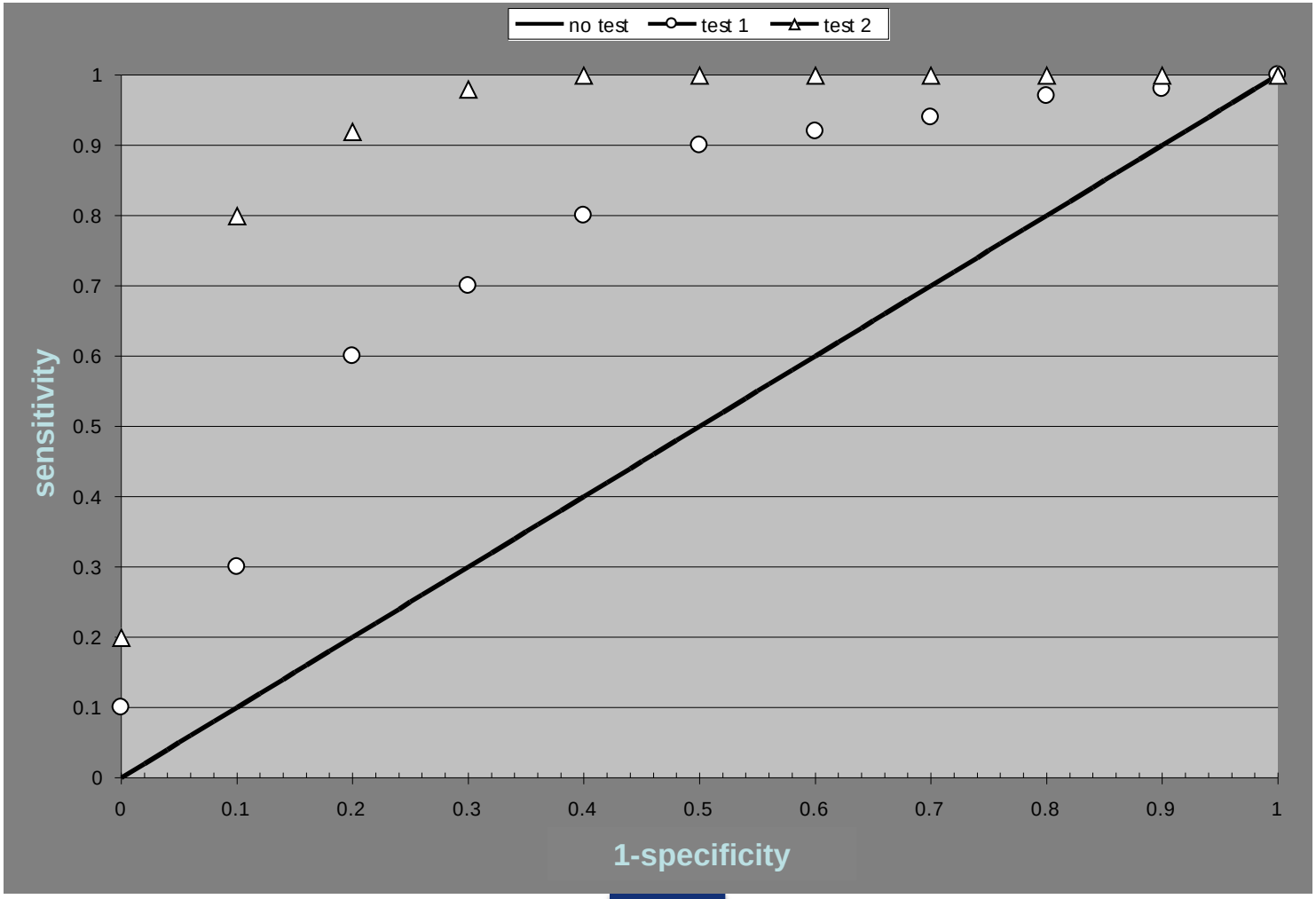


Gold standard




cut-off
True positives
False negatives
diseased animals
1/1
1/2
1/4
1/8
1/16
1/32
1/64
1/128
True negatives
False positives
healthy animals
1/1
1/2
1/4
1/8
1/16
1/32
1/64
1/128

Receiver operator characteristic (ROC)



Predictive values

The predictive value of a test result is the proportion of patients who are correctly diagnosed.

The predictive value of a positive result is the probability that a positive test reflects the underlying condition being tested for.

The predictive value of a negative result is the probability that a negative test reflects the underlying healthy condition being tested for.

Predictive values are conditional probabilities (definition of 'positive or negative')

	Test +	Test -	Total
Diseased	18 (TP)	2 (FN)	20
Healthy	5 (FP)	75 (TN)	80
Total	23	77	100

Sensitivity = TP/(TP+FN) = 18/20 = 0.9

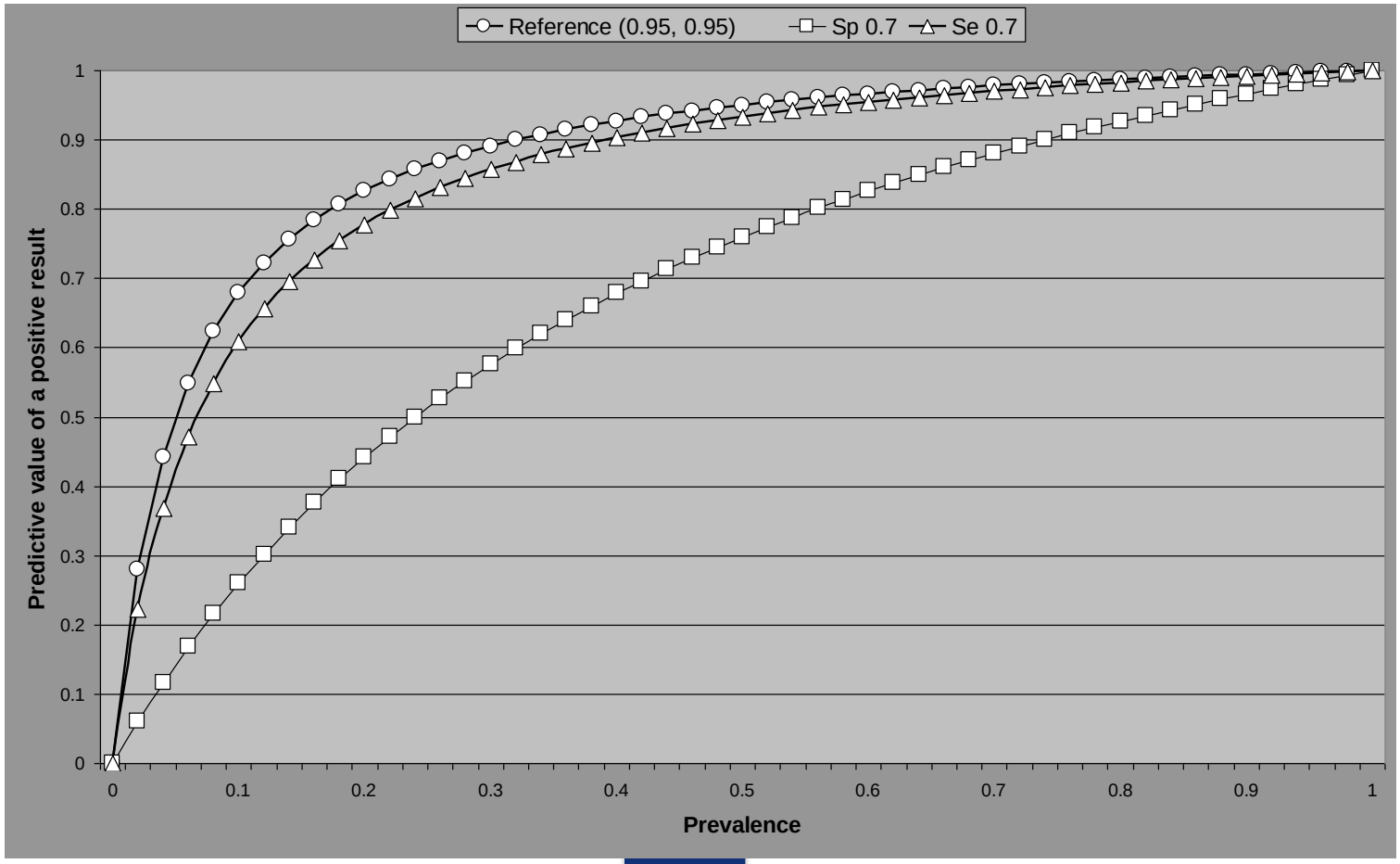
Specificity = TN/(TN+FP) = 75/80 = 0.94

Prevalence = (TP+FN)/(TP+FN+TN+FP) = 20/100

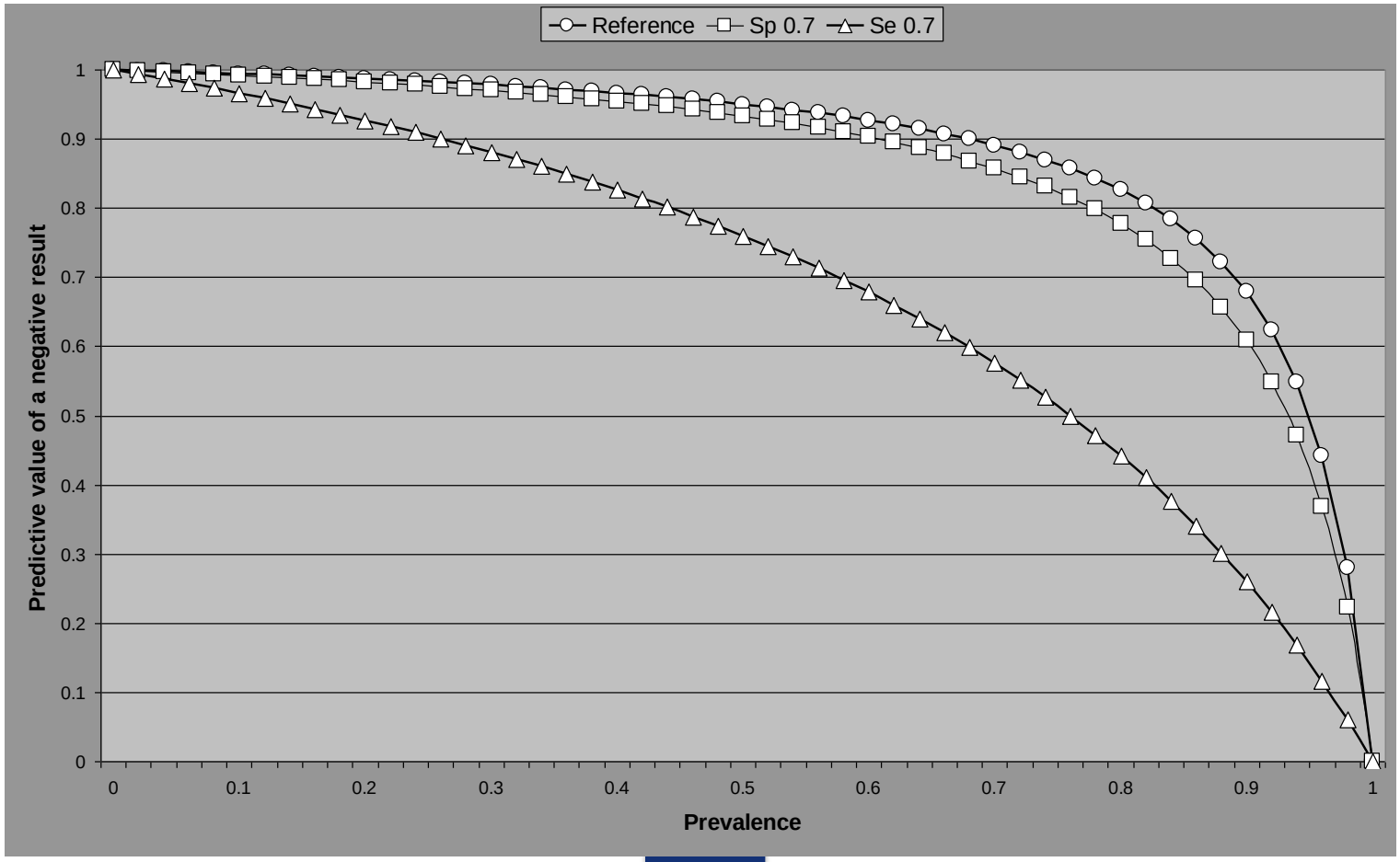
Pred value + = TP/(TP+FP) = 18/23 = 0.78

Pred value - = TN/(TN+FN) = 75/77 = 0.97

Predictive value of positive results in function of the prevalence



Predictive value of negative results in function of the prevalence



Diagnostic test characteristics

Repeatability: in identical conditions

Reproducibility: in different conditions

Accuracy: calibration

Precision: instruments & number of observations

Cut-off: effect on sensitivity and specificity

=> Need for validated standard protocols

=> Need for proficiency testing

Quality assurance of test results

Quality assurance = Quality Control + Credibility

Quality Control = Proficiency testing + Corrective actions

Use of existing standards

- **To make activities comparable to others'**
- **To make activities verifiable (quality assurance)**
- **To benefit from quality assured by suppliers**

Proficiency testing

Why?

- **Legitimation of laboratory activities**
- **Determination of laboratory accuracy**

How?

- **Analytes**
- **Split samples**
- **Reference samples**

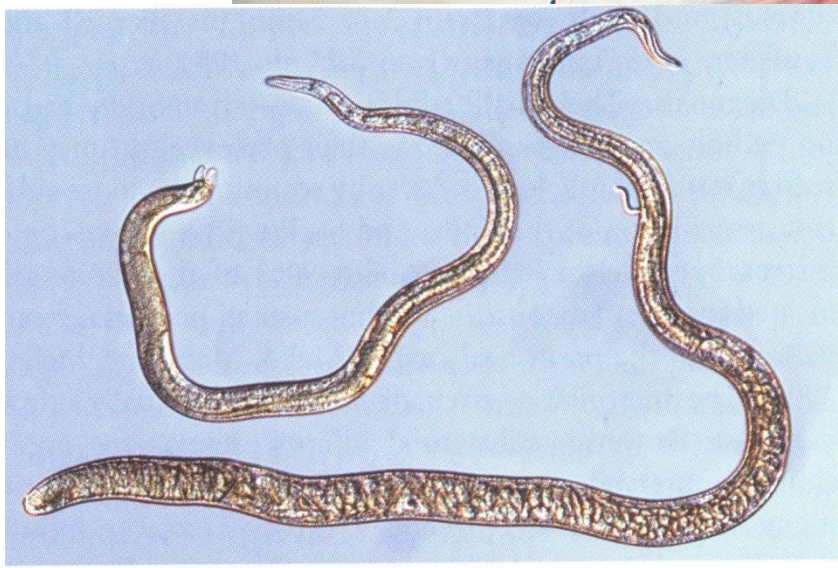
Usual working conditions

Statistical analysis and interpretation

Proficiency testing: the *Trichinella* example

Trichinella spiralis*:*

- **Parasitic and zoonotic nematod**
- **transmitted from prey to predator**
  e.g.: Rat – pig – rat
- **Adults live in the gut**
- **Larvae are encysted in the muscles of the same host**
- **Surveillance in pig and horse meat**

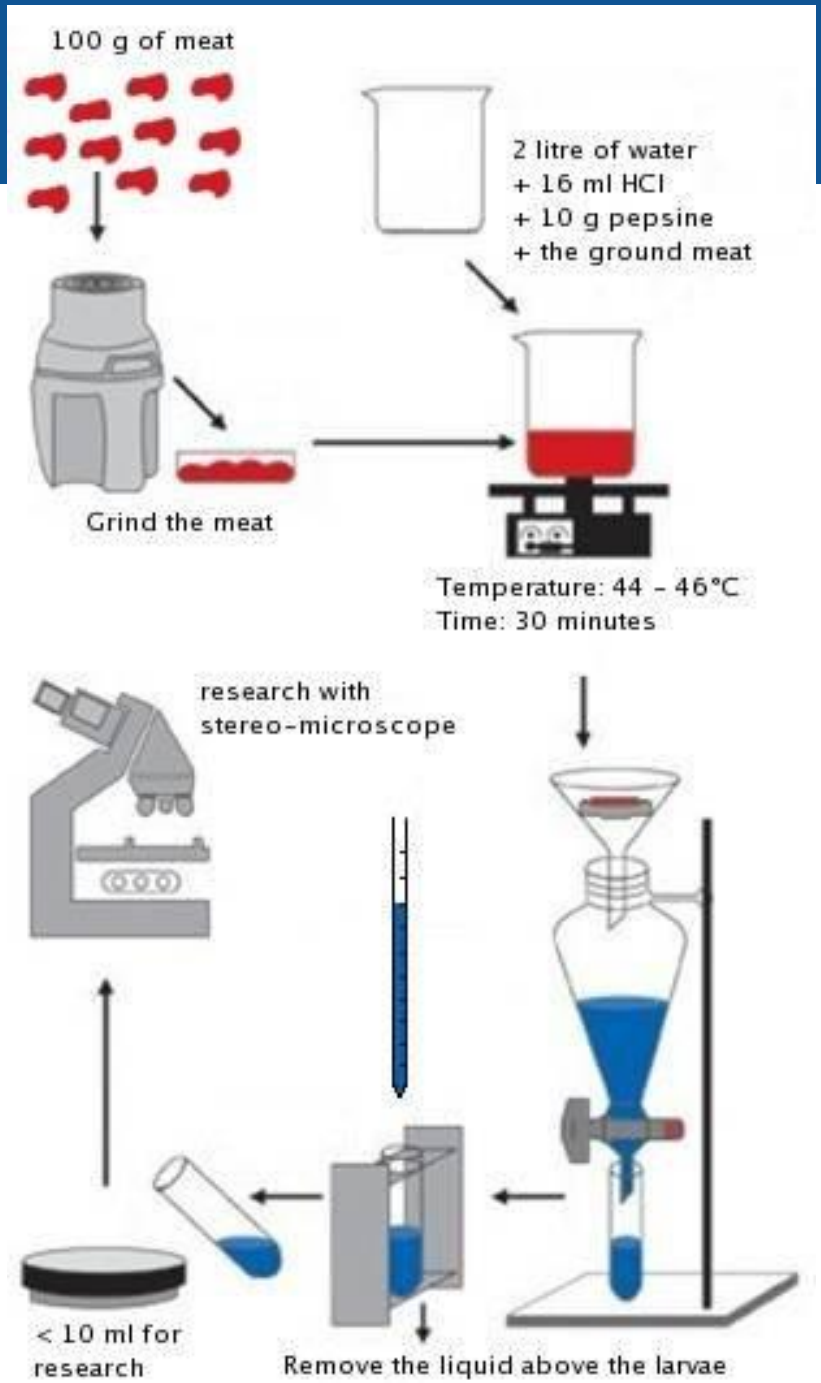


Diagnostic test: artificial digestion test

Artificial digestion of meat sample

Sedimentation of the larvae

Observation and counting using a stereoscopic microscope


100 g of meat
2 litre of water
+ 16 ml HCl
+ 10 g pepsine
+ the ground meat
Grind the meat
Temperature: 44 - 46°C
Time: 30 minutes
research with
stereo-microscope
< 10 ml for
research
Remove the liquid above the larvae

Trichinella proficiency test

Preparation of proficiency samples

Distribution of the samples to the participating laboratories (by courier)

Detection and counting of larvae

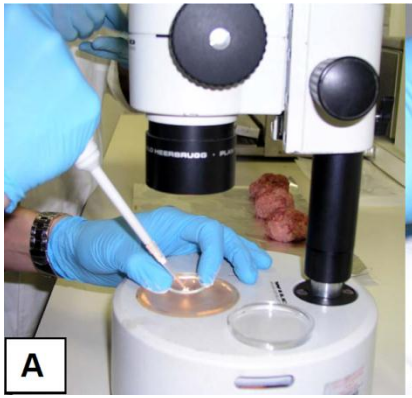
Report

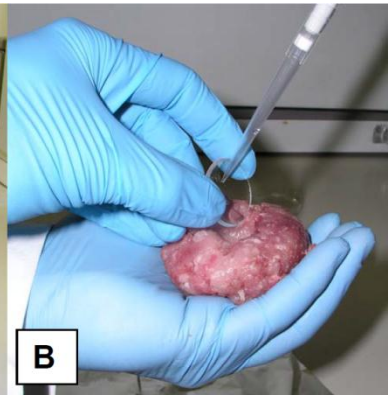
Analysis of the results

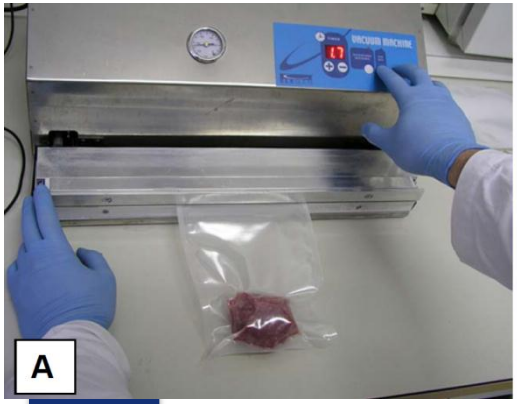
Corrective measures

Test material

- Pig or horse meat
- 35 or 100 g meat balls
- Count larvae in a watch glass
- Flush all larvae in the meat
- Pack, seal and send







Results (fictive)

Minimum detection level: 3 larvae

Minimum recovery: 40%

Laboratory	Sample code	Expected number of larvae	Number of larvae found	Binary result	Result evaluation
L1	S1	0	0	0	ok
L1	S2	5	4	1	ok
L1	S3	8	8	1	ok
L1	S4	0	0	0	ok
L1	S5	11	10	1	ok
L2	S6	6	0	0	false negative
L2	S7	0	0	0	ok
L2	S8	9	3	1	low analytical sensitivity
L2	S9	10	5	1	low analytical sensitivity
L2	S10	0	0	0	ok
L3	S11	0	2	1	false positive
L3	S12	3	3	1	ok
L3	S13	7	8	1	more larvae observed than expected
L3	S14	0	0	0	ok
L3	S15	9	8	1	ok

Reference diagnostic tests and laboratories (animal health)

***OIE**: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals + reference laboratories*

***FAO** Reference Labs provide consultations, assist in making diagnoses and develop diagnostic capability, maintain a reference collection of disease agents, produce and standardize reagents and assist in characterization of causative agents and in training activities*

***EU** and **national** reference laboratories*

***ISO**: International Organization for Standardization*