SAFOSO

Animal Health Matters.
For Safe Food Solutions.

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Economic Affairs,
Education and Research EAER
State Secretariat for Economic Affairs SECO

Epidemiological Aspects of Laboratory Investigations

Marco De Nardi

Overview:

- Diagnosis and importance of uncertainty in diagnostic tests
- Tests characteristics (se-sp)
- Predictive values (ppv-npv)
- Example

Diagnosis and uncertainty

- DIAGNOSIS:

- attempt to determine the **health status** of an animal, herd, flock (*Healthy or Diseased*?);

- art of identifying the **nature** of patient's disease (Brucellosis? Tubercolosis?);

- it is the **basis for a decision**!

(to treat a patient,… to implement a control program,…to investigate further…to do nothing….)

Diagnosis and uncertainty

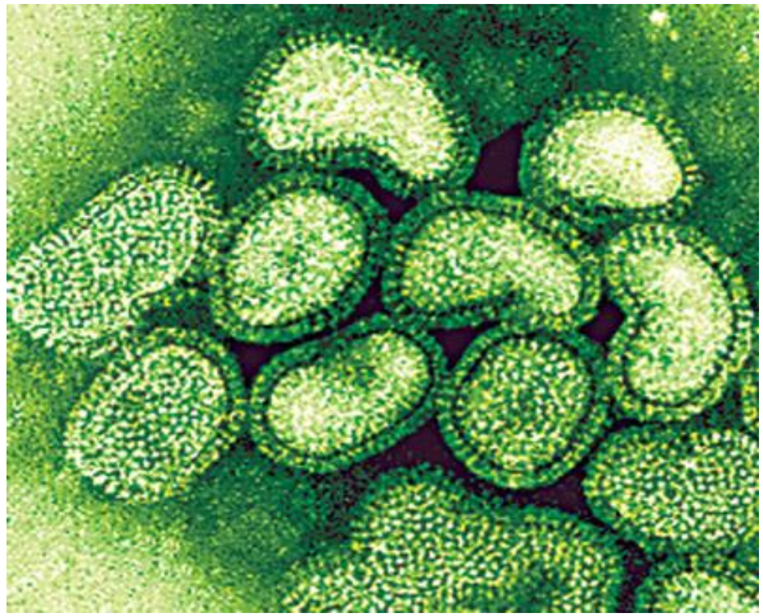
- Diagnostician does not work with certainties;

  - incomplete understanding of biological processes;
  - true biological variation;
  - diagnostic tests are not perfect *(sensitivity 95 % = 5 % F –; specificity 98 % = 2 % F +)*
  - systematic error (*information bias, selection bias*)
  - measurement error (*misclassification*);
  - random error (*chance*);

- Medicine as a stochastic art (versus deterministic paradigm): outcome not certain but probabilistic.

Outcome of diagnostic tests

- **Dichotomous**: *presence or absence of a pathogen;*



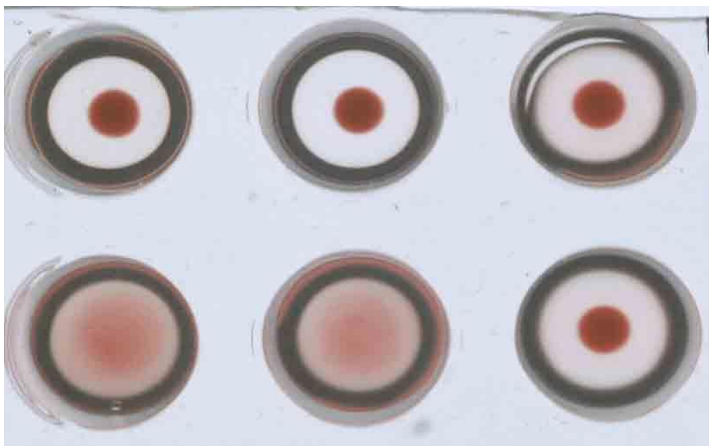
↓

Interpretation is often straightforward

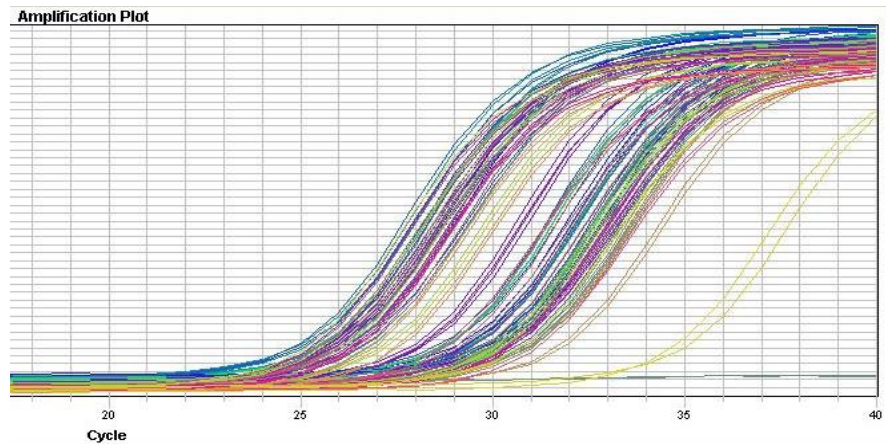
Outcome of diagnostic tests

- **Continuous scale**:

HI

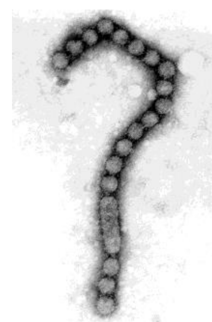


rt-PCR



↓

Interpretation:



Cut-off value

- Measurement on continuous scale: need of a cut-off value to interpret results expressed in a continuos scale as a dichotomous variable (healthy-diseased).

REGULATION (EC) No 853/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 29 April 2004

laying down specific hygiene rules for on the hygiene of foodstuffs

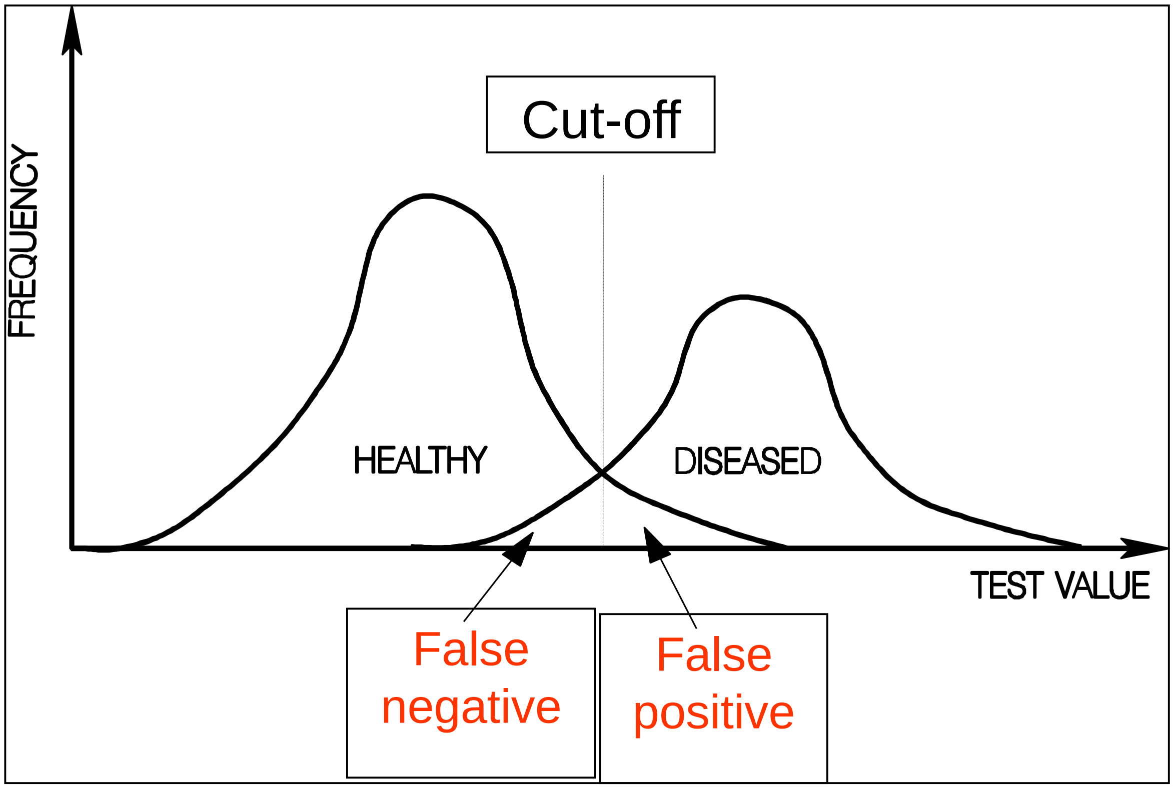
Food business operators must initiate procedures to ensure that raw milk meets the following criteria:

(i) for raw cows' milk:

Plate count at 30 °C (per ml)	≤ 100 000 (*)
Somatic cell count (per ml)	≤ 400 000 (**)

Cut-off

Limitation: likely to result in overlap between healthy and diseased → uncertainty



Performance of a diagnostic tests

- The evaluation of diagnostic tests needs the use of a **“gold standard”**.
- G.S.: it is a mean by which we can assess whether a disease, or any other outcome of interest, is truly present or not.
- The definition of the “gold standard” is not always straightforward!

Tests characteristics

		Disease status Gold Standard		
		+	-	TOTAL
Test result	+	a true +	b false +	$a+b$
	-	c false -	d true -	$c+d$
	TOTAL	$a+c$	$b+d$	N

Tests characteristics

- The performance of a diagnostic test, relative to the gold standard, are quantified by two parameters indicators of the validity (accuracy) of diagnostic tests:

- **Sensitivity (Se):**

- ability of a test to correctly identify **diseased** animals
- the proportion of **true +** detected by the test
- indication of how many **false –** are expected (Se=95% → F- = 5%)

- **Specificity (Sp):**

- ability of a test to correctly identify **non-diseased** animals
- the proportion of **true –** detected by the test
- indication of how many **false +** are expected (Sp=95% → F+ = 5%)

Tests characteristics

		Disease status		
		+	**-**	**TOTAL**
Test result	**+**	*a*	*b*	*a+b*
	-	*c*	*d*	*c+d*
	TOTAL	*a+c*	*b+d*	N

$$SENSITIVITY = \frac{a}{a+c}$$

Tests characteristics

		Disease status		
		+	-	TOTAL
Test result	+	*a*	*b*	*a+b*
	-	*c*	*d*	*c+d*
	TOTAL	*a+c*	*b+d*	N

$$SPECIFICITY = \frac{d}{b+d}$$

Tests characteristics

		Disease status		
		+	**-**	**TOTAL**
Test result	**+**	*79* T+	*7* F+	*86*
	-	*12* F -	*110* T-	*122*
	TOTAL	*91*	*117*	208

Se = 79/91 = 87% **F- = 13% (12/91=0.13)**
Sp = 110/117 = 94% **F+ = 6% (7/117=0.06)**

True and apparent Prevalence

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

- **True Prevalence**: **Based on the true disease status of the individuals**

True prevalence = (a+c)/(a+b+c+d)

- **Apparent prevalence:** **Estimate of the prevalence based on the means used to identify disease**

Apparent prevalence = (a+b)/(a+b+c+d)

$$\text{true prevalence} = \frac{\text{apparent prevalence} + (\text{specificity} - 1)}{\text{specificity} + (\text{sensitivity} - 1)}$$

Tests characteristics

- Se-Sp are intrinsic characteristics of a test (do not depend on the prevalence of the disease);

- Se-Sp are not solid…can be changed accordingly;

- Inversely related (decrease F-, increase F+ or viceversa).

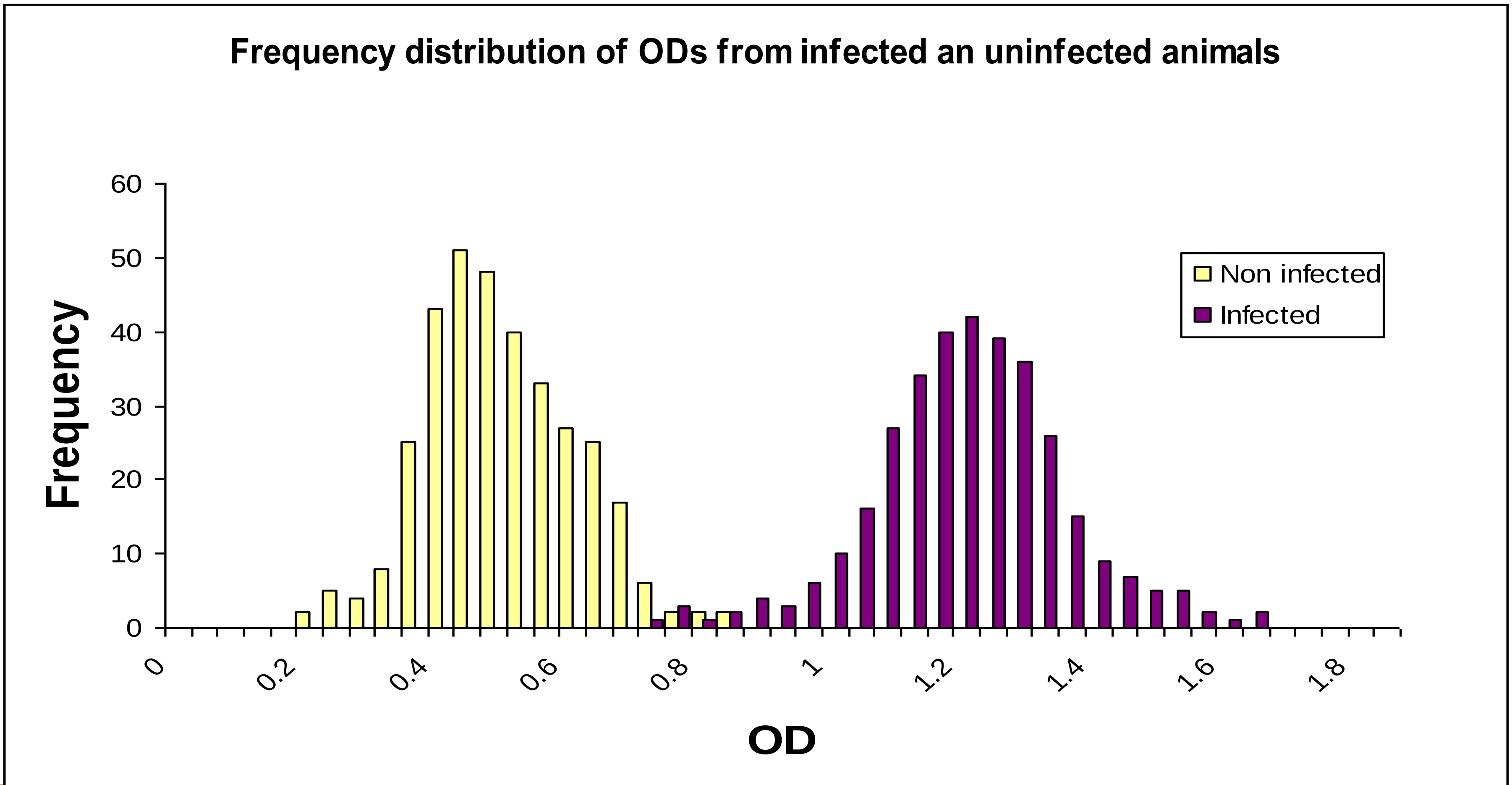
Selection of the cut-off

- Changing the cut-off value (in case of results expressed in a continuous scale) will vary both Se and Sp.

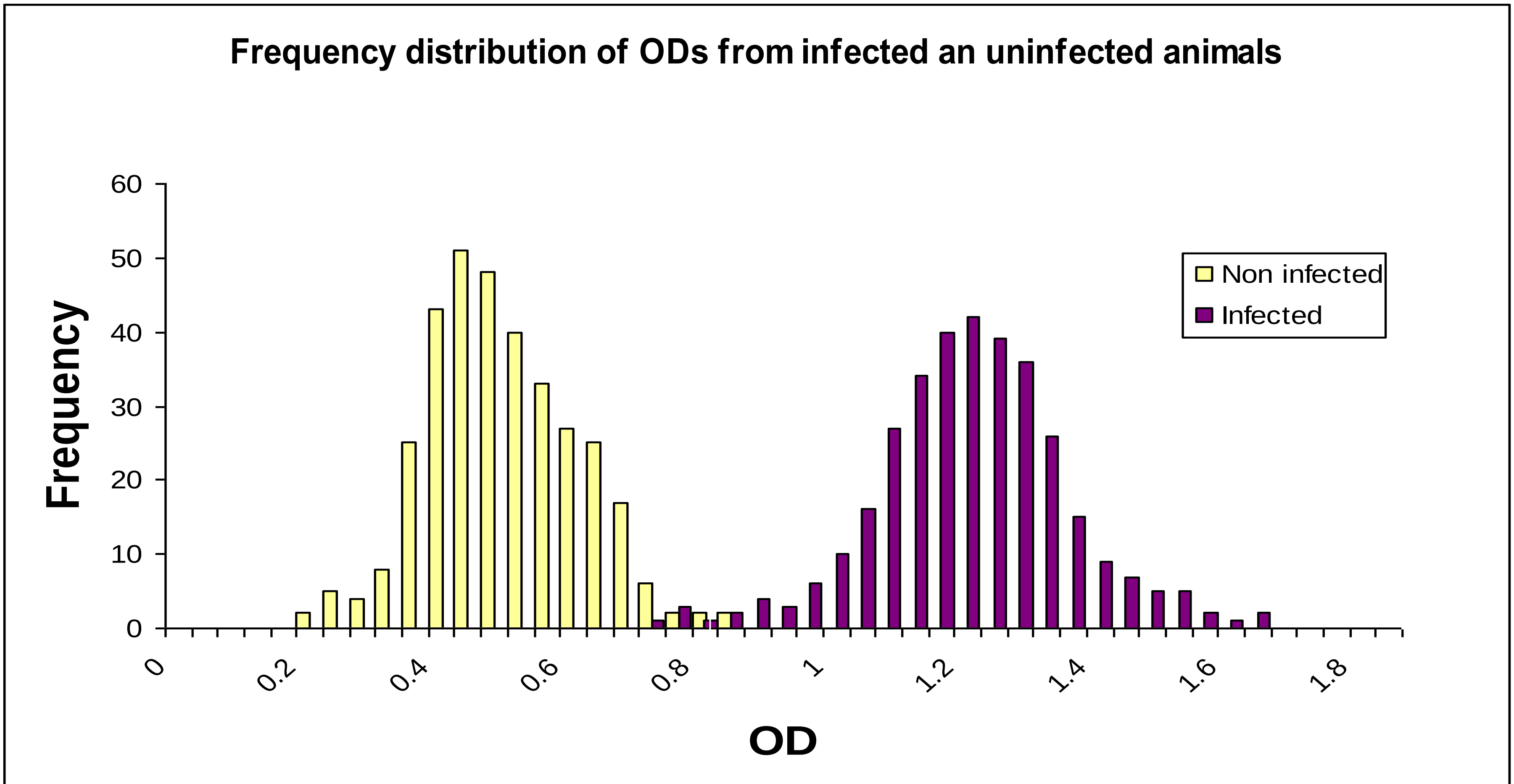
There are sophisticated methods for optimum selection of the cut-off point.

- ROC curve, likelihood ratios etc….

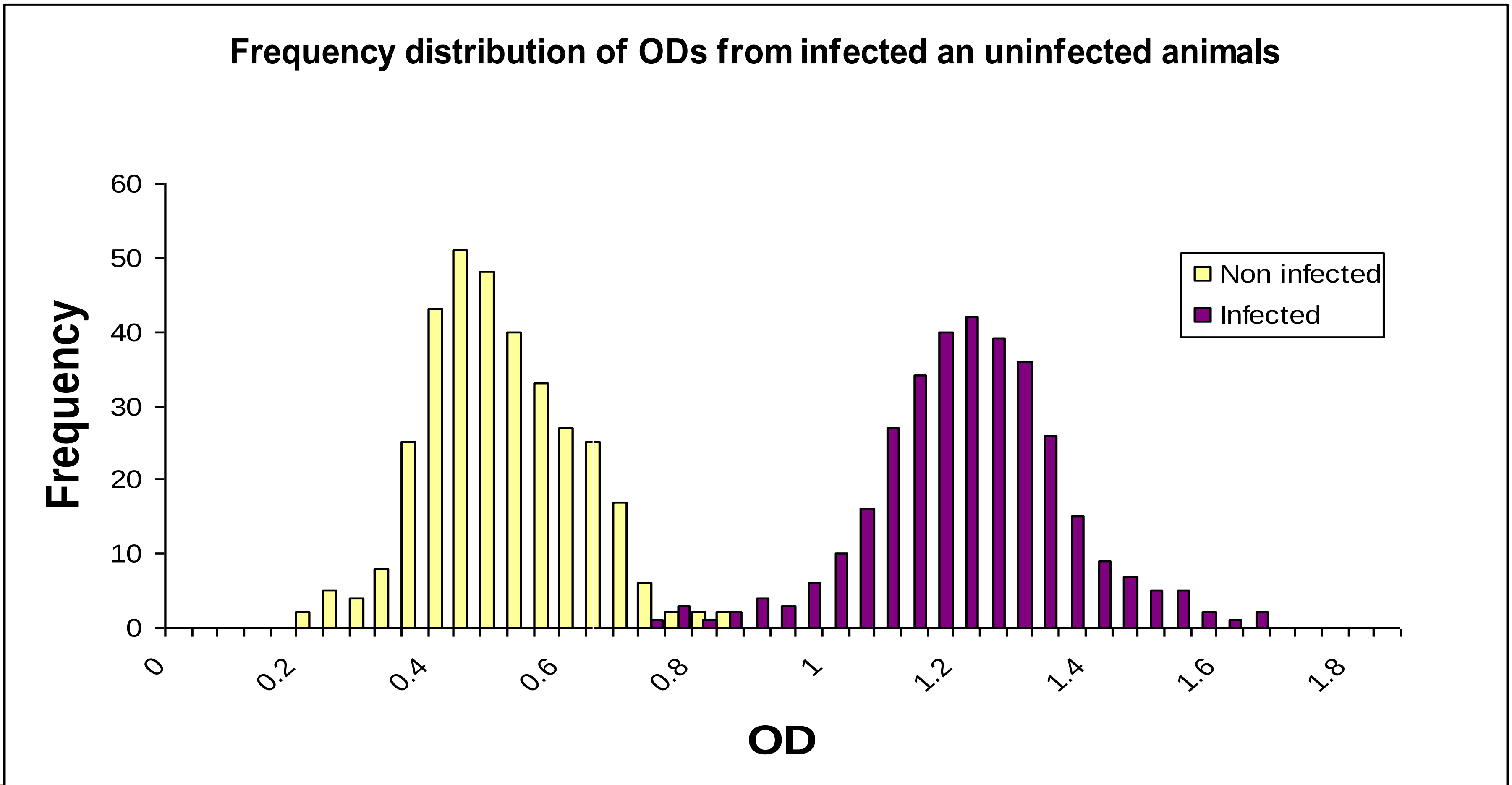
Selection of the Cut-off



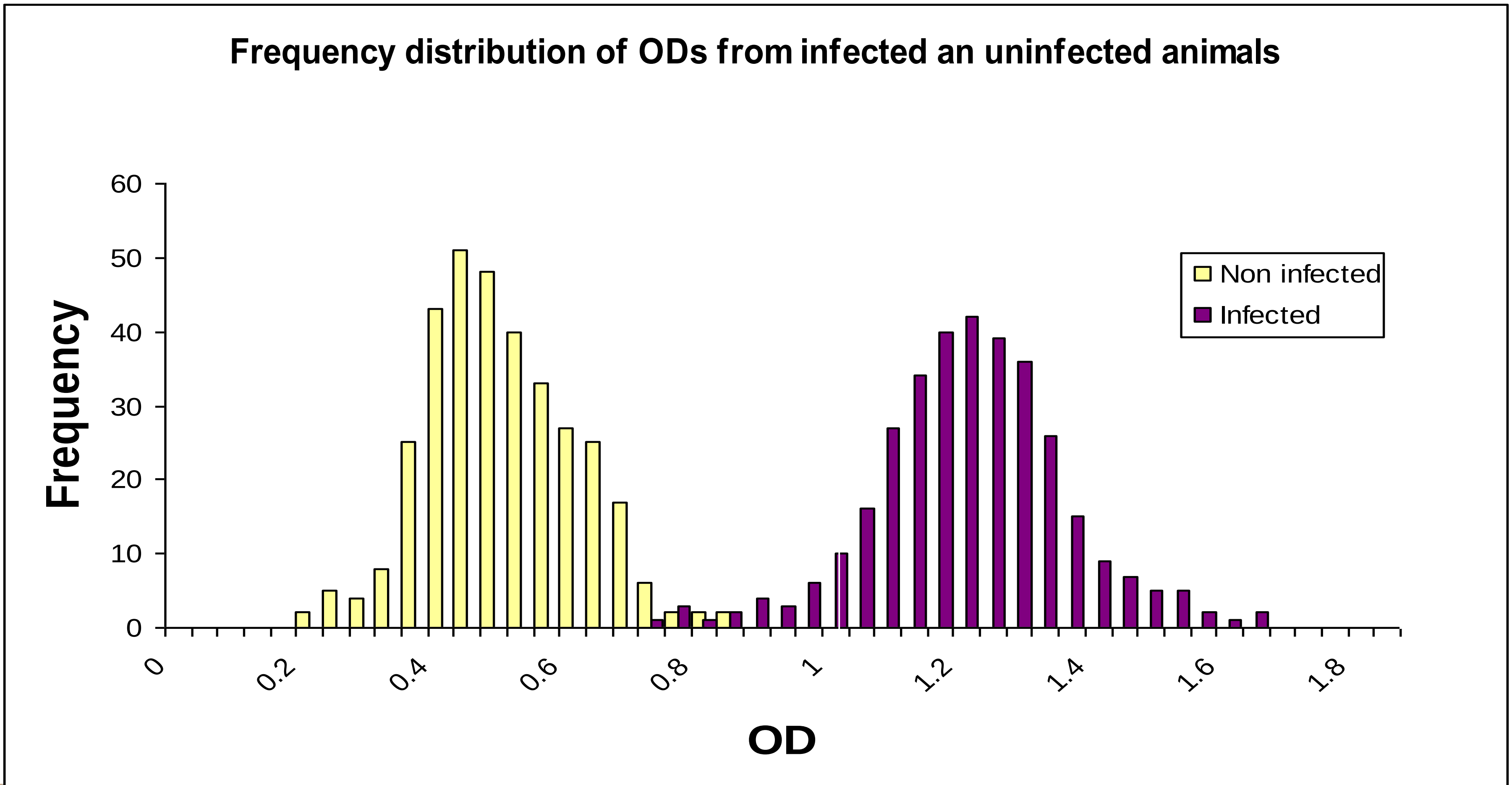
Selection of the Cut-off



To ↑ Se → cut-off shifted to the left



To ↑ Sp → cut-off shifted to the right



Predictive values

- Positive predictive value?
- Negative predictive value?

Predictive Values (PVs)

- **Positive Predictive Value (ppv):** probability that an animal positive according to the test, is actually truly positive
- **Negative Predictive Value (npv):** probability that an animal negative, according to the test, is actually truly negative
- **PVs depend on:**

- **Se, Sp** : given a reference population and a cut-off, Se and Sp are relatively stable;
- **Prevalence**: unstable

Predictive Values (PVs)

		Disease status		
		+	-	**TOTAL**
Test result	+	***a***	*b*	*a+b*
	-	*c*	***d***	*c+d*
	TOTAL	*a+c*	*b+d*	N

$$POSITIVE\ \ PREDICTIVE\ \ VALUE = \frac{\mathrm{a}}{\mathrm{a+b}}$$

Predictive Values (PVs)

		Disease status		
		+	**-**	**TOTAL**
Test result	**+**	***a***	*b*	*a+b*
	-	*c*	***d***	*c+d*
	TOTAL	*a+c*	*b+d*	N

$$NEGATIVE\ \ PREDICTIVE\ \ VALUE = \frac{\mathrm{d}}{\mathrm{c+d}}$$

Predictive Values (PVs)

Or….(using Prevalence (P), Se, Sp)

- PPV: $(P*Se)/((P*Se)+[(1-P)*(1-Sp)])$

- NPV: $(1-P)*Sp/\ ([(1-P)*Sp]+[P*(1-\ Se)])$

Predictive Values (PVs)

- Example:

  Tests: Se: 95%, Sp: 90%)

a) Prevalence: 30 %

- PPV: $(P*Se)/(P*Se)+[(1-P)*(1-Sp)]$ = **80%**
- NPV: $(1-P)*Sp/ [(1-P)*Sp] +[P*(1- Se)]$ = **98%**

b) Prevalence: 3 %

- PPV: $(P*Se)/(P*Se)+[(1-P)*(1-Sp)]$ = **23%**
- NPV: $(1-P)*Sp/ [(1-P)*Sp] +[P*(1- Se)]$ = **99,8%**

Example

Trop Anim Health Prod (2015) 47:79–86
DOI 10.1007/s11250-014-0688-0

REGULAR ARTICLES

Different screening tests and milk somatic cell count for the prevalence of subclinical bovine mastitis in Bangladesh

Md. Nazmul Hoque · Ziban Chandra Das · Anup Kumar Talukder · Mohammad Shah Alam · Abu Nasar Md. Aminoor Rahman

A total of 892 quarters milk samples from 228 lactating cows were screened by California mastitis test (**CMT**), White side test (**WST**), Surf field mastitis test (**SFMT**), and somatic cell count (**SCC**) to study the prevalence of bovine SCM in some selected areas of Bangladesh.

Table 4 Percentage accuracy, sensitivity, and specificity of various indirect tests used for the diagnosis of bovine subclinical mastitis. Data presented as number (%)

Tests	Samples examined	Positive samples	TP	FP	TN	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	*k*	*r* (%)
CMT	892	408 (45.7)	307 (78.3)	101 (24.7)	325 (67.1)	159 (32.8)	70.0	65.8	76.2	75.2	0.77	78.2
WST	892	388 (43.5)	271 (69.8)	117 (30.1)	307 (60.4)	197 (39.0)	64.8	57.9	72.4	69.8	0.53	68.3
SFMT	892	368 (41.2)	239 (64.9)	129 (35.0)	295 (56.2)	229 (43.7)	59.9	51.0	69.5	64.9	0.41	56.6
SCC	892	491 (55.0)	455 (92.6)	36 (7.3)	305 (76.0)	96 (23.9)	85.2	82.5	89.4	92.7	0.88	92.4

TP true positive, *FP* false positive, *TN* true negative, *FN* false negative, *PPV* positive predictive value, *k* Kappa index (% agreement), *r* Pearson's correlation

- Thanks