Diagnostic & Screening Tests

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Diagnostic Test

- Clinical test applied to diseased individuals for the purpose of confirming or ruling out a specific diagnosis
- Indicates that individual has or does not have the disease
- Gold Standard: the definitive diagnosis of the disease
  - Necropsy
  - Biopsy
  - Surgery
  - Long-term follow-up

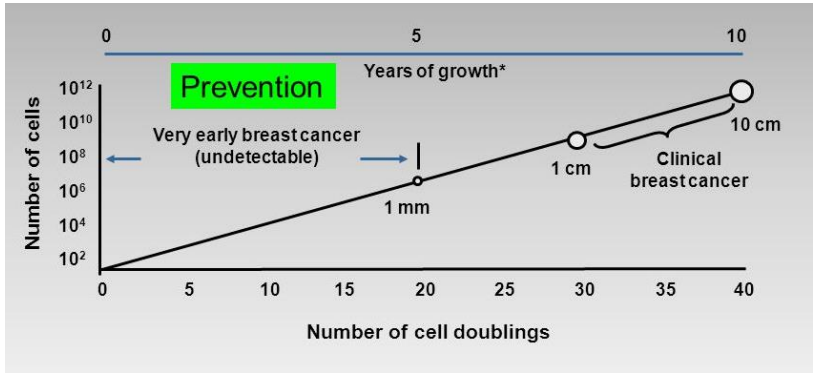
Screening Test

- Clinical test applied to apparently healthy individuals for the purpose of early diagnosis of disease
- Screening attempts to classify asymptomatic individuals as likely or unlikely to have disease or condition
- Ultimate goal: to reduce morbidity & mortality from disease
- Usually not diagnostic

"I'd have been here sooner if it hadn't been for early detection."

Requirements for effective screening test

1. Suitable Disease
   - Sufficient burden of suffering to warrant attention
     - Consequences if left undetected or untreated are severe
     - What about rare disease?
   - Suitable "window of opportunity"
     - Detectable preclinical period of sufficient length to allow early detection

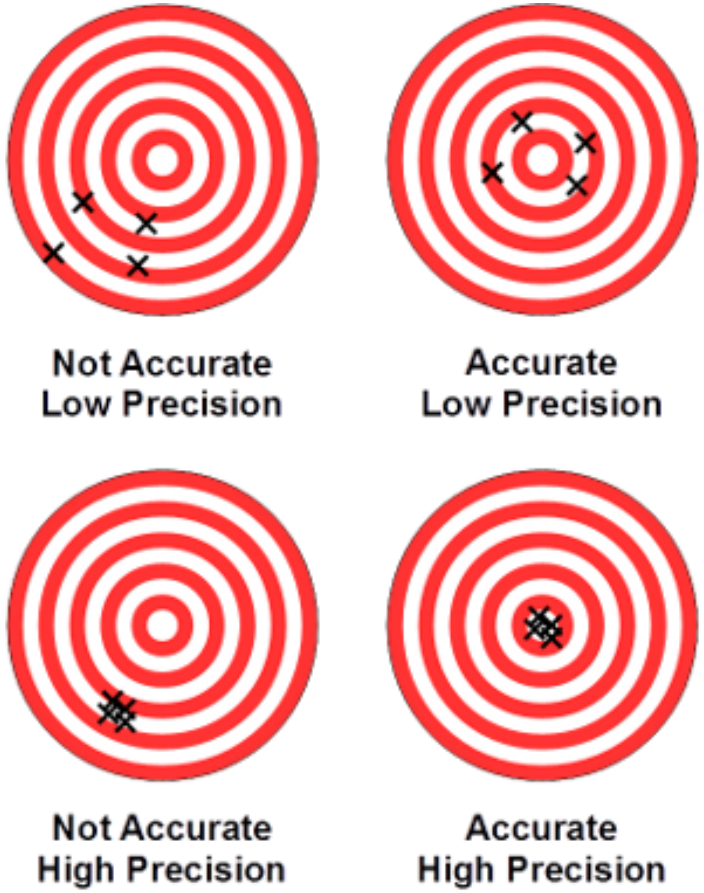


Breast cancer

Requirements for effective screening test

2. Accurate & Precise Test

- Reliable or precise (Repeatable)
  - Same result each time
- Valid or accurate (Correct)
  - Sensitive
  - Specific



Requirements for effective screening test

Valid tests are both:

- Sensitive
  - Ability of test to designate individuals with preclinical disease as positive (likely to have disease)
  - Sufficiently sensitive to detect disease that could benefit from earlier treatment
- Specific
  - Ability of test to designate individuals who are free of preclinical disease as negative (unlikely to have disease)
  - Do not want consequences of false positive tests to overwhelm the true positives

Requirements for effective screening test

3. Effective Treatment

- There must be treatment for disease that:
  - can favorably alter the disease's natural progression
  - is more effective when applied to screen-detected disease than clinically-detected disease
  - is available to those screenees who are found to have the disease

Requirements for effective screening test

4. Overall benefits must outweigh overall harms

Benefits	Harms
○ Late manifestation of disease ○ Successful treatment of disease ○ Achieve better quality of life	○ Adverse events from invasive diagnostic procedures ○ Adverse effects of treatment ○ Psychological effects of false positives

Requirements for effective screening test

5. Reasonable Costs

- Screening
- Diagnosis
- Treatment

Validity of Screening Tests

Screening Test Result	Disease Status (Truth): D +	Disease Status (Truth): D -
T+	a (True Positive; TP)	b (False Positive; FP)
T-	c (False Negative; FN)	d (True Negative; TN)

Sensitivity (Se) = a/a+c

Specificity (Sp) = d/d+b

Validity of Screening Tests

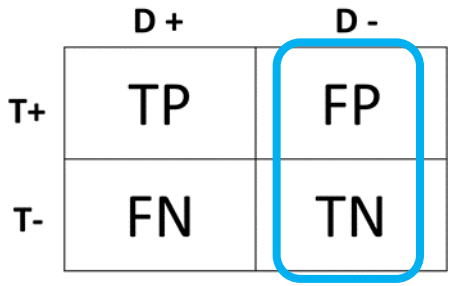
	D +	D -
T+	TP	FP
T-	FN	TN

Sensitivity (Se)

- Proportion of truly diseased individuals who test positive for preclinical disease
- Probability that you test positive for the disease, given that you have the disease → P(T+|D+)
- Enables to correctly 'rule out'
- Percentage of true positives among all cases

$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

Validity of Screening Tests



Specificity (Sp)

- Proportion of truly disease free individuals who test negative for preclinical disease
- Probability that you test negative for the disease given that you do not have the disease → P(T-|D-)
- Enables to correctly 'rule in'
- Percentage of true negatives among all non-cases

$$\text{Specificity} = \frac{TN}{TN+FP}$$

Validity of Screening Tests

Example: Breast Cancer Screening Program

- Women assigned to screening or usual care
- Screening consisted of yearly mammogram and physical exam
- Five years of follow-up produced these results:

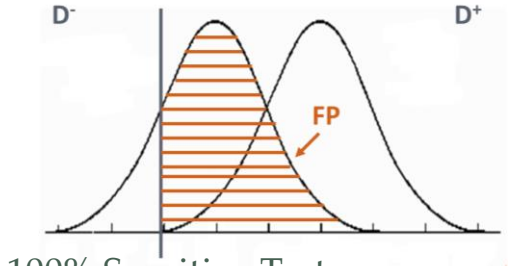
		Breast Cancer		
		Confirmed	**Not confirmed**	**Total**
Screening Test Result	**Positive**	132	983	1,115
	Negative	45	63,650	63,695
	Total	177	64,633	64,810

What are sensitivity and specificity ?

Trade-off between Sensitivity & Specificity

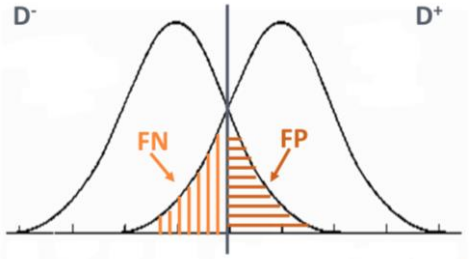
- Not all screening tests result in a positive or negative result
- Many tests are of continuous variables (e.g., prostate specific antigen: PSA; blood glucose; enzyme level) where cut-points are used to distinguish between likely “normal” and “diseased” individual
- The value of cut-point determines the sensitivity and specificity of the test
- As one increases, the other decreases

Cut-points of Disease Classification



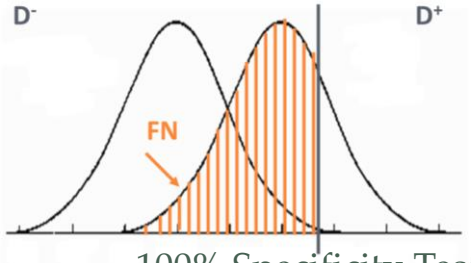
100% Sensitive Test

Low cutoff → High sensitivity
→ Low specificity
→ False positive



Minimal Error

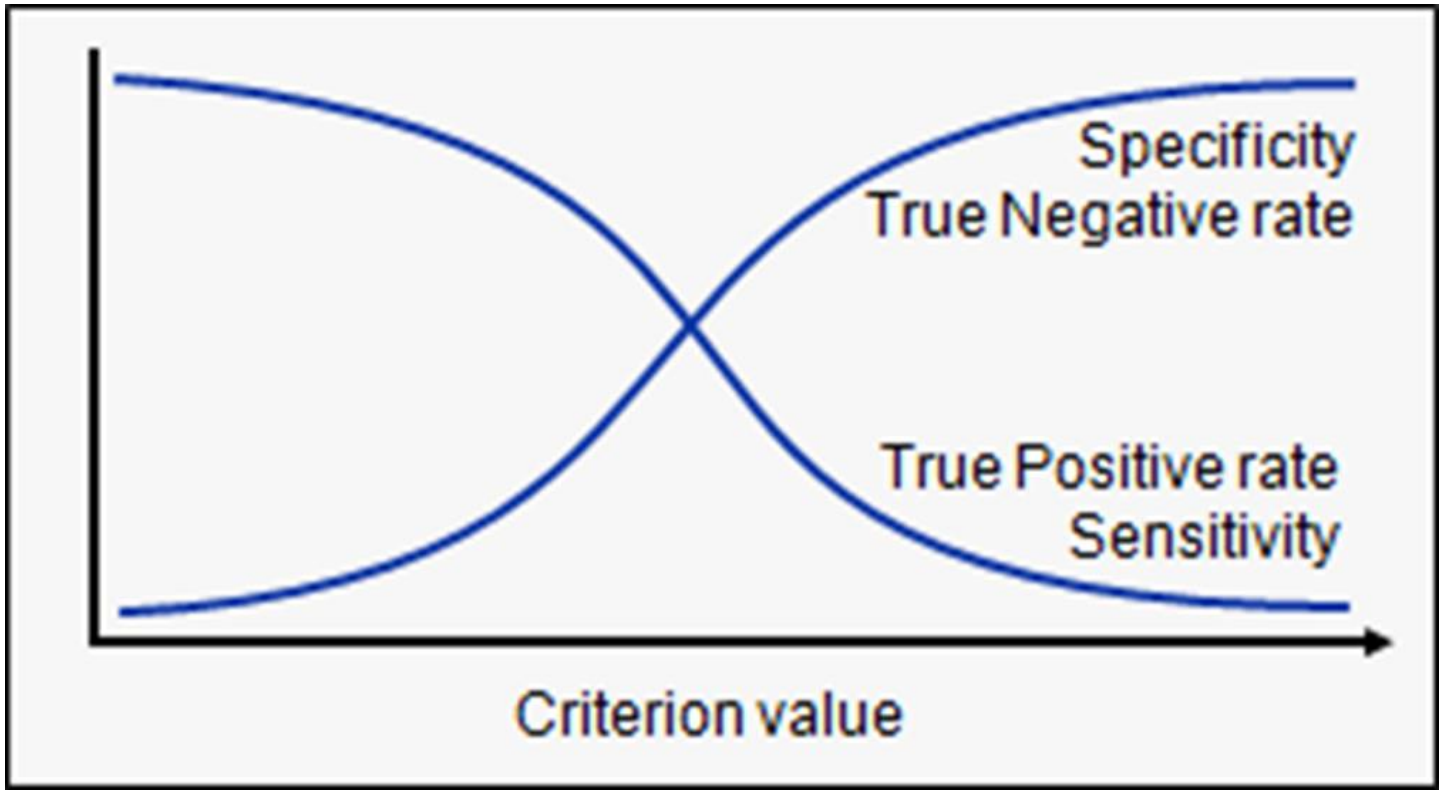
Moderate cutoff → balance



100% Specificity Test

High cutoff → Low sensitivity
→ High specificity
→ False negative

Sensitivity-Specificity plot



Rules for selecting tests

	D +	D -
T+	TP	FP
T-	FN	TN

SpIN: Highly specific test rules

- Have confidence in a positive result
- "Rule In" or confirm a diagnosis e.g. prior to major treatment
- When a FP is dangerous e.g. when treatment involves risk

SnOUT: Highly sensitive test rules

- Have confidence in a negative result
- "Rule out" disease in early stages of workup
- When a FN is dangerous e.g. screening for a FAD

Multiple Testing

Sequential testing/two-stage testing

- Involves applying two screening tests sequentially
- Individuals who are "positive" on the first test go on to have a second test
- The first test is cheaper and easier to administer

  The second test is more expensive or invasive
- Net loss in sensitivity

  Net gain in specificity

- Example: Two-stage screening for TB

  Symptoms screening first. If positive for any symptom of any duration, move to chest x-ray

Multiple Testing

Simultaneous testing/parallel testing

- Involves applying several tests at one time
- Positive result: if positive on at least one test
- Negative result: must be negative on all tests
- Net gain in sensitivity

  Net loss in specificity

- Example: Testing for Down Syndrome among pregnant women

All women receive the following:

- First trimester: serum screening for two markers plus ultrasound measurements of the neck area of the baby
- Second trimester: four serum markers plus maternal age

Test Performance

- The probability that an individual test result reflects the true disease status of the animal
  - Positive Predictive Value (PPV)
  - Negative Predictive Value (NPV)

- Sensitivity and specificity are used to evaluate and apply tests
  - Use before you test

- Predictive values are used to evaluate and interpret test results
  - Use after you have a test result

Evaluate test performance

Screening Test Result	Disease Status (Truth): D +	Disease Status (Truth): D -	
T+	a (True Positive; TP)	b (False Positive; FP)	PPV = a/a+b
T-	c (False Negative; FN)	d (True Negative; TN)	NPV = d/d+c

Test Performance

	D +	D -
T+	TP	FP
T-	FN	TN

Positive Predictive Value (PPV)

- Proportion of individuals with positive screening test result who truly have disease
- Probability that individuals have preclinical disease, given that you tested positive for preclinical disease
- P(D+|T+)

$$PPV = \frac{TP}{TP+FP}$$

Test Performance

	D +	D -
T+	TP	FP
T-	FN	TN

Negative Predictive Value (PPV)

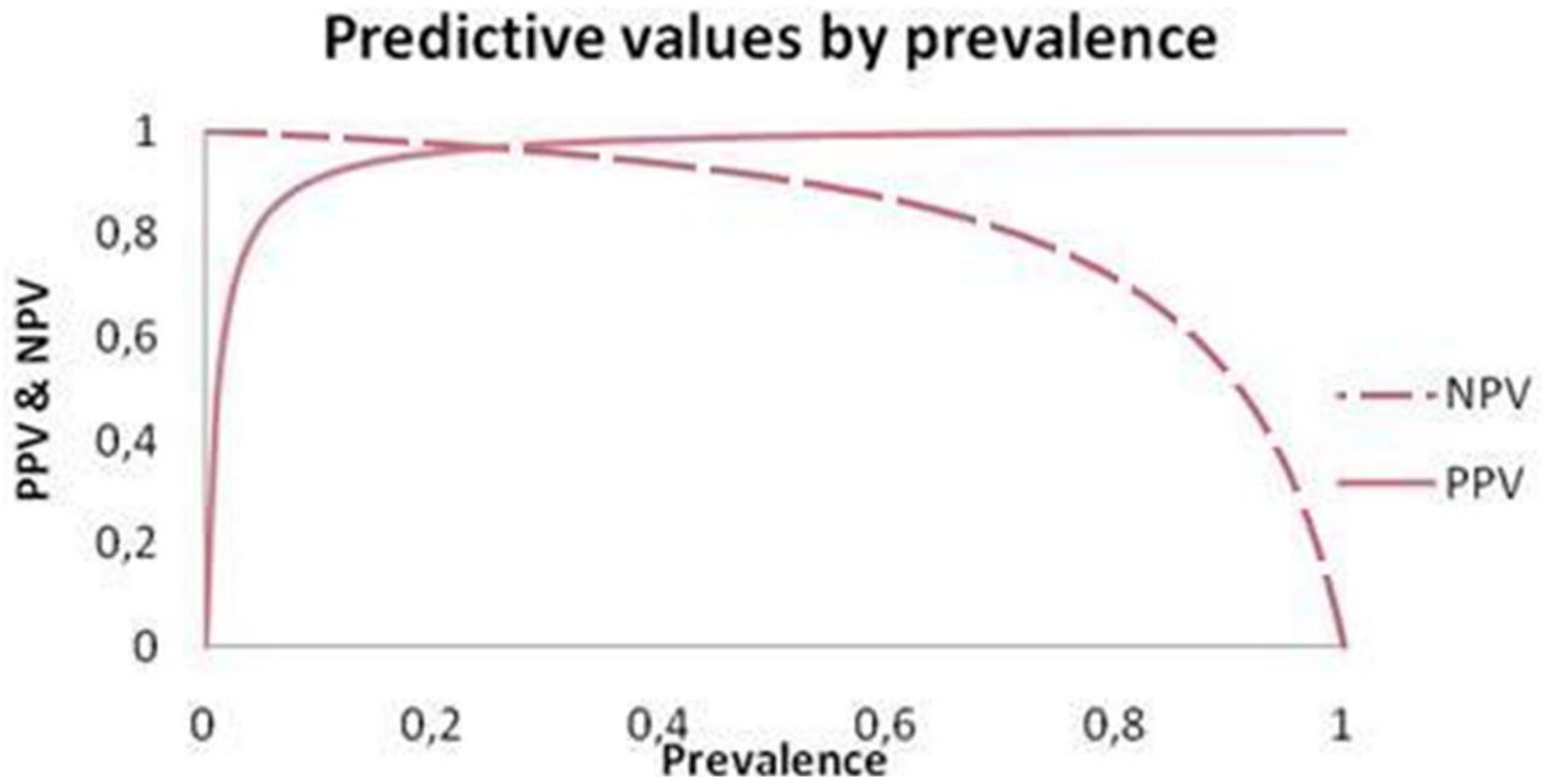
- Proportion of individuals with negative screening test result who truly do not have disease
- Probability that individuals do not have preclinical disease, given that you tested negative for preclinical disease
- P(D-|T-)

$$NPV = \frac{TN}{TN+FN}$$

Predictive Value

The relationship of predictive value and prevalence

- PPV: dependent on the prevalence of disease
  - Increases as prevalence increases
- NPV: dependent on the prevalence of disease
  - Decreases as prevalence increases
- The prevalence of disease increases in a population, so does the positive predictive value (holding sensitivity and specificity constant)
- This is why it is important to screen high-risk populations


Predictive values by prevalence
PPV & NPV
1
0,8
0,6
0,4
0,2
0
0
0,2
0,4
0,6
0,8
1
Prevalence
NPV
PPV

Example: The total of 1000 cows were tested for BVD using ELISA and then confirmed with the diagnosis of bovine fetuses persistently infected (PI)

The results are showed below:

	PI+	**PI-**	**Total**
Test+	382	216	598
Test-	118	284	402
Total	500	500	1000

What are the PPV and NPV ?

Precision of Screening Tests

Types of precision errors:

- Method variability
  - Has to do with the test itself
  - Is the test inconsistent under similar circumstances?
- Subject variability
  - Occur due to physiologic changes taking place in the subject tested
- Observer variability
  - Intraobserver variability
  - Interobserver variability

Interobserver/Intraobserver variability

- Different observers of the same test result come to different conclusions about disease status
- Evaluated using "**kappa**"
- The closer kappa is to 1, the better the agreement between observers & lower the interobserver variability

kappa > 0.75 is considered excellent

kappa < 0.40 is considered poor

kappa 0.40-0.75 is considered moderate to good

Kappa

Results from Observer 2	Results from Observer 1: Positive	Results from Observer 1: Negative	Total
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	a+b+c+d

$$\mathbf{K = (P_O - P_E) / (1- P_E)}$$

P_O= the proportion of times agreement occurs between the observers

P_E = the proportion of times agreement is expected between the observers due to chance

$P_O = [(a+d) / (a+b+c+d)]$

$P_E = [(a+b)(a+c) + (b+d)(c+d)] / (a+b+c+d)^2$

Example: In a study of intraobserver variability in assessing cervical smears 3,325 slides were screened for the presence or absence of abnormal squamous cells. Each slide was screened by a particular observer and then re-screened six months later by the same observer. The results of this study are shown below:

1st screening	2nd screening		
	Present	**Absent**	**Total**
Present	1763	489	2252
Absent	403	670	1073
Total	2166	1159	3325

How reproducible are this observer's screenings?

Practice!!!

SNAP 4Dx Plus Test

Test accuracy

The SNAP® 4Dx® Plus Test uses highly purified reagents on the ELISA platform.

The SNAP® Test's peptide-based technology allows for the evaluation of only highly specific antibodies for *Anaplasma* spp., Lyme disease, and *Ehrlichia* spp., which helps to eliminate false positives.

Analyte	Gold standard	Device result +	Device result –	Total	Sensitivity (95% CL) / Specificity (95% CL)
Heartworm[1,2]	+	95	1	96	99.0% (94.3%–99.9%)
	–	2	269	271	99.3% (97.4%–99.9%)
Anaplasma[3]	+	214	23	237	90.3% (85.8%–93.7%)
	–	15	246	261	94.3% (90.7%–96.7%)
Ehrlichia[4]	+	231	7	238	97.1% (94.0%–98.8%)
	–	18	366	384	95.3% (92.7%–97.2%)
B. burgdorferi[5]	+	112	7	119	94.1% (88.3%–97.6%)
	–	9	229	238	96.2% (92.9%–98.3%)

Note: Male-only heartworm infections typically produce antigen levels that are below the detection capability of antigen tests.

Gold Standards:

1. Heartworm positives: Necropsy
2. Heartworm negatives: PetChek® Heartworm PF Antigen Test (5018.02)
3. *Anaplasma*: IFA
4. *Ehrlichia*: *E. canis* IFA; *E. ewingii* ELISA
5. *B. burgdorferi*: IFA

SNAP 4Dx

1. Disease_____
2. How about 2*2 table?
3. What is the confirmation test?
4. Sensitivity? / Specificity?
5. How is this test kit? Appropriate or not?

		Disease Status (Truth)	
		D +	D -
Screening Test Result	T+		
	T-		

Assignment

Assignment for R-FETPV 1st Module 2021 "Diagnostic & Screening Tests"

Brucellosis Screening in Cattle

Read the USDA "Facts about Brucellosis". Answer all of the following questions (NOTE: one item may contain several questions) using complete sentences. Responses that are not well developed or thorough will not receive full credit.

You may work in groups of 2-3 (3 is the max). Turn in ONE solution set to e-mail: suchawan_po@rmutto.ac.th
by the deadline listed on the syllabus. Be sure to include the names of all group members.