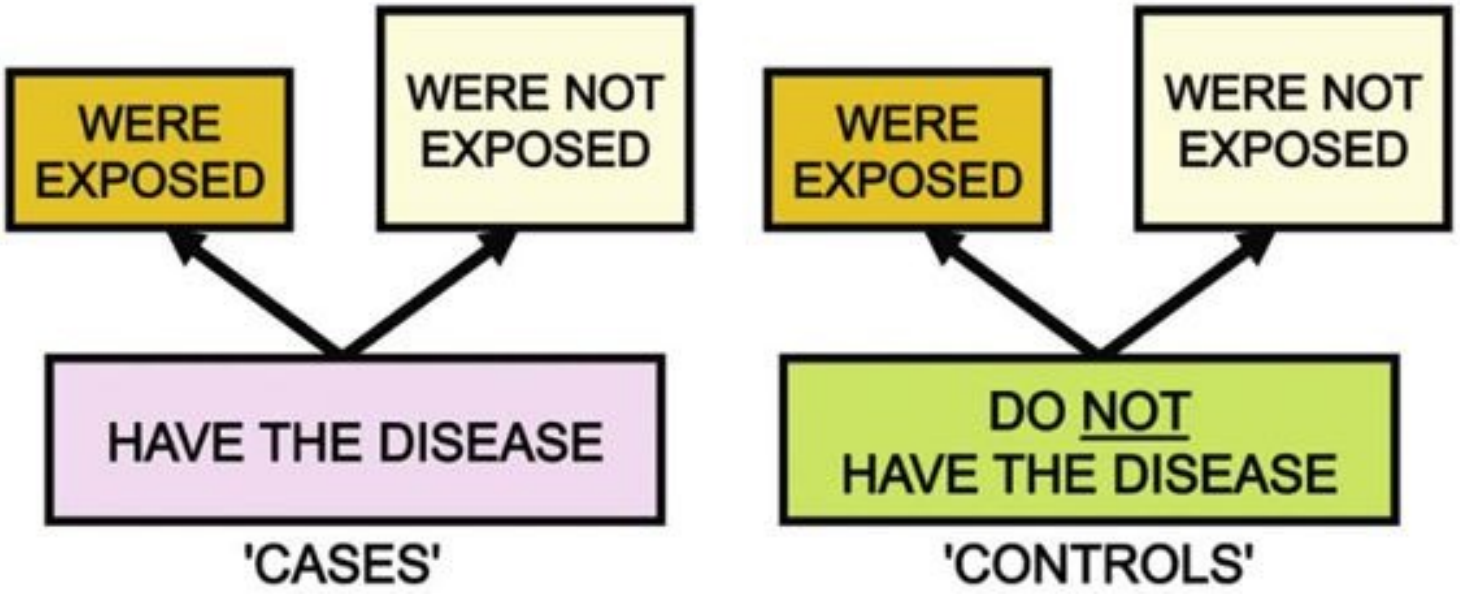
Lecture 9: Diagnostic and Screening Studies

Lecture prepared by Dr. Hailey Banack, PhD

Review:
Case Control Study Design

Step 2

Step 1



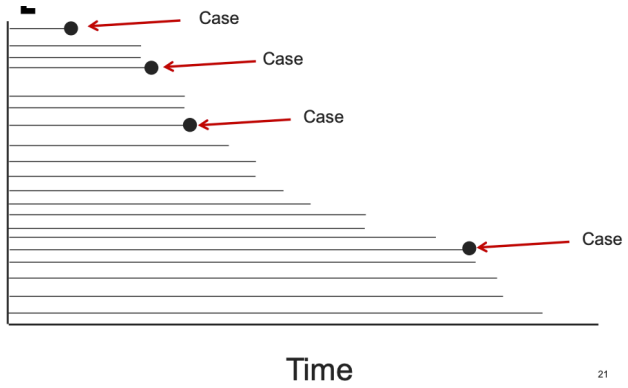
Types of case control study

1) Cumulative sampling (i.e. traditional case-control design): from those who do not develop the outcome until the end of the study period (i.e. from the "survivors" or prevalent cases)

2) Case-cohort design (case-base; case-referent) sampling: from the entire cohort at baseline (start of the follow-up period; when cohort is established)

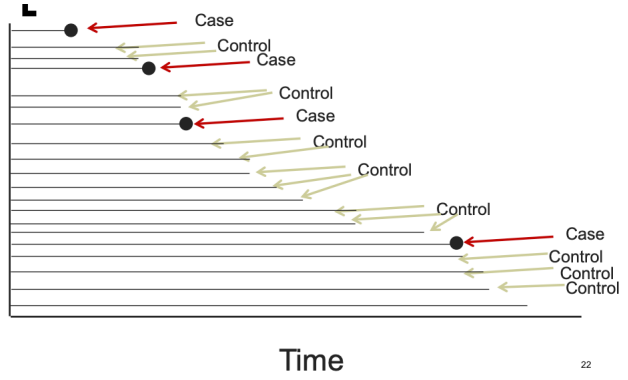
3) Incidence density case control design (risk-set sampling): throughout the course of the study, from individuals at risk ("risk-set") at the time each case is diagnosed

Cases



Courtesy: Kris Filion

Controls



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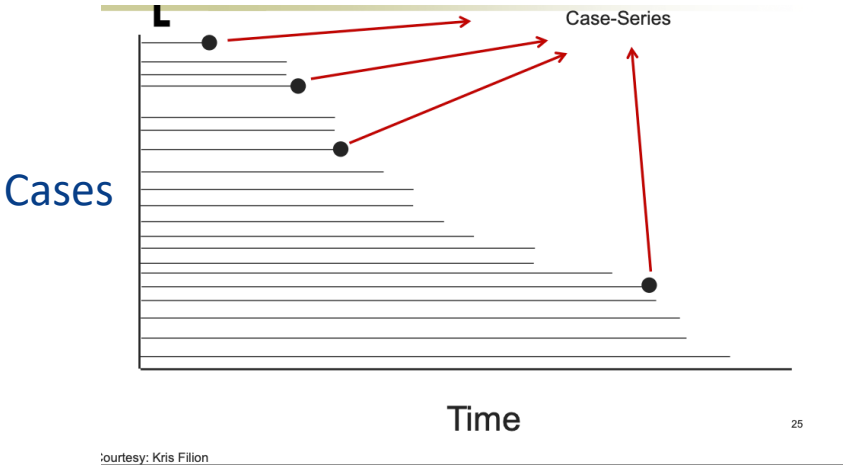

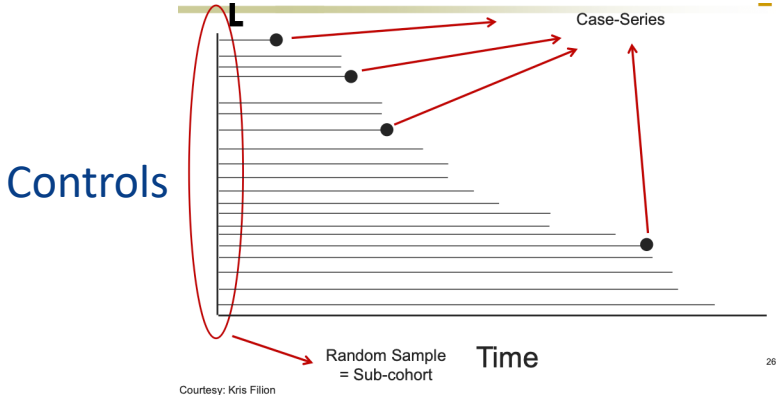

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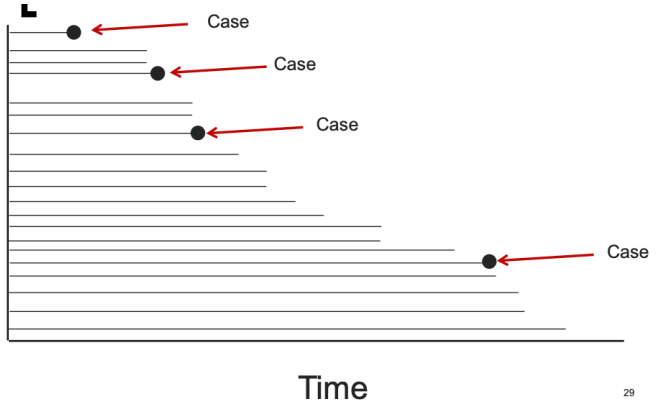

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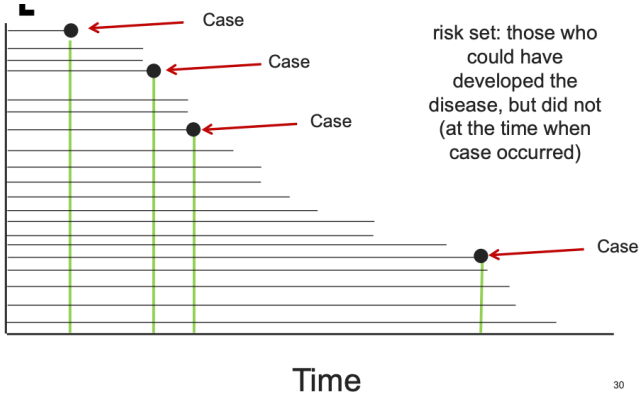

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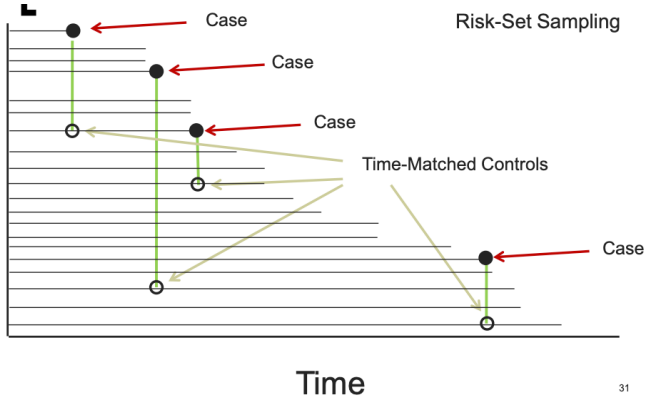


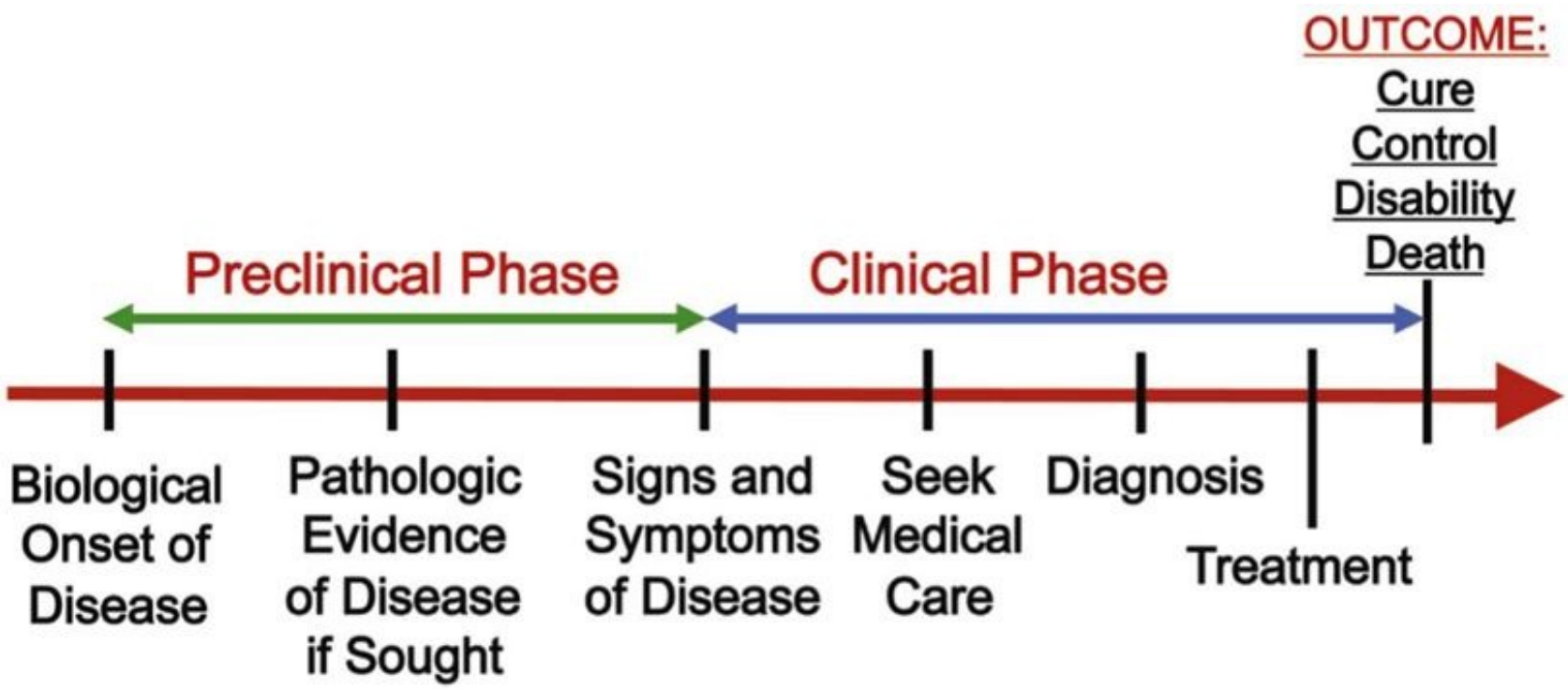
Courtesy: Kris Filion

Challenges of Case Control Studies

- Do the controls come from the same study base as the cases? Do they represent the exposure distribution in the source population (study base)?
- Recall bias vs. poor recall
  - Do cases and controls recall their exposures differently?
  - Or, is it just hard to recall past exposures (non-differential)

Screening and Diagnostic Tests

Natural History of Disease



Importance of screening and diagnostic testing

Want to distinguish individuals in the population who have/don't have disease

Important for:

- Understanding disease etiology
- Disease prevention
- Disease surveillance and detection
- Treatment and elimination of disease

Also causes:

- Chances of misinformation, loss of trust in practitioners
- Stress, unproductive worry, behavior changes
- Overtreatment, other differential treatment

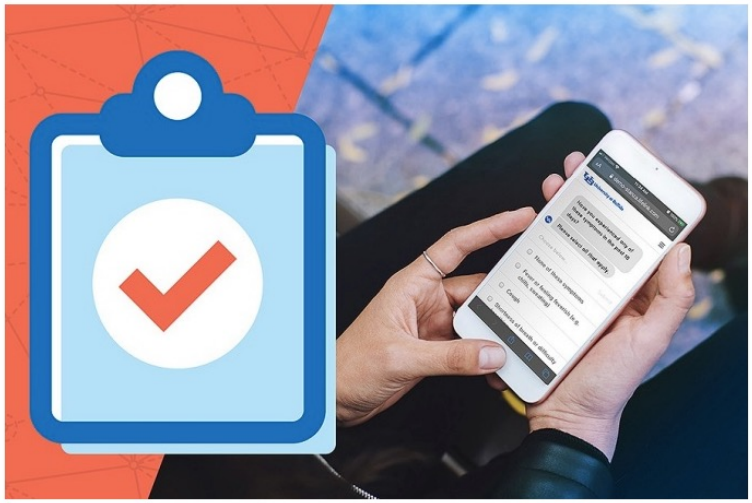
Screening vs. Diagnostic Tests

- Screening tests are usually done on asymptomatic, apparently healthy individuals
  - Application of a test to detect a potential disease in a person who has no symptoms
  - Useful for: detecting disease early, detecting people at high risk of developing disease for targeted intervention
- Diagnostic tests are usually done on individuals with specific symptoms

Examples of Screening Programs

- Mammograms for breast cancer
- Colonoscopy for colorectal cancer
- PKU blood testing in newborns
- PSA for prostate cancer

UB deploys mandatory daily health screening tool



The Daily Health Check is a virtual screener that uses advanced chatbot technology to detect potential cases of COVID-19 infection early and provide users with need-to-know information tailored to their situation. Image: Bob Wilder

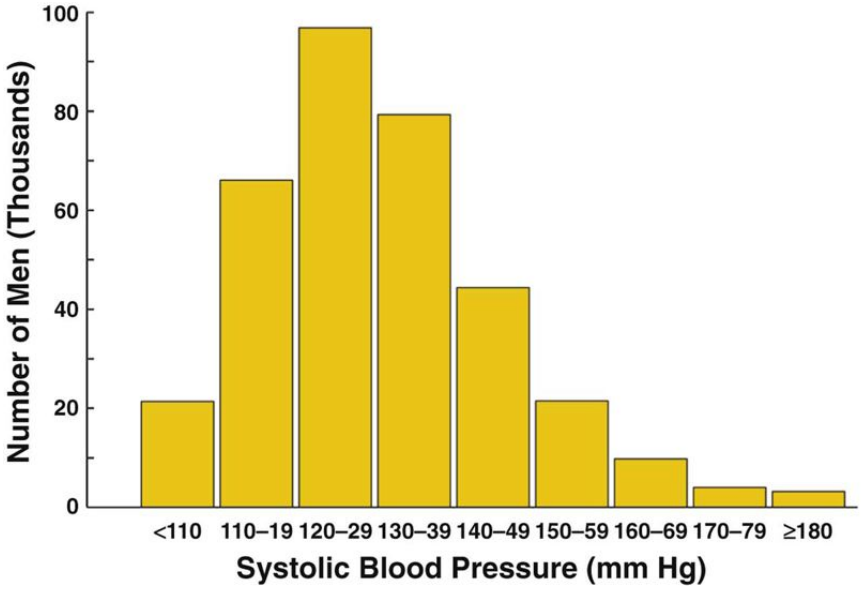
Validity of Tests

- Validity= ability to distinguish between who has a disease and who does not
  - Must have referent point to determine what is normal vs abnormal

- Validity can vary as a function of:
  - Individual biology
  - Test procedures (e.g., properties of instrument)
  - Population characteristics (e.g., prevalence of disease)

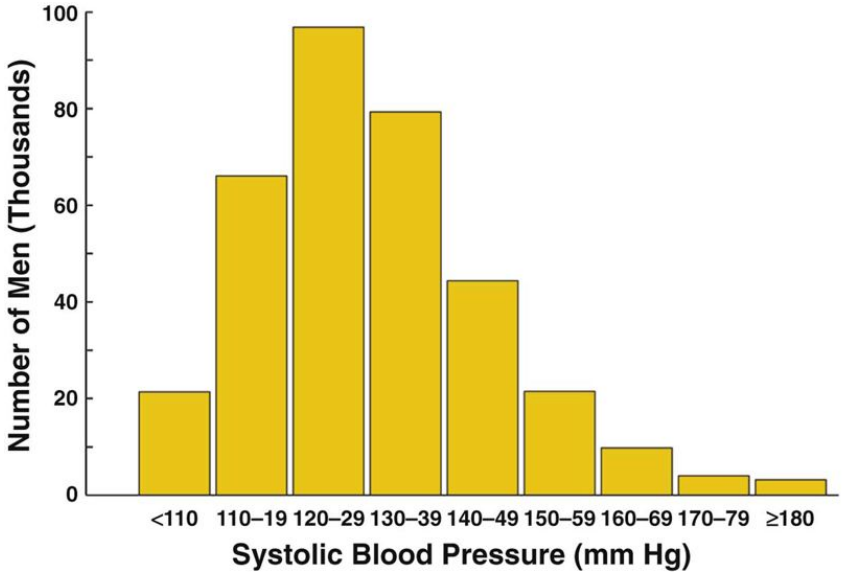
Biologic Variation

- Biologic variation in a population is normal
- We expect biologic variation in a population
- Important to remember this when assessing the results of a screening test and determining what is normal/abnormal



The importance of cut points

- When deciding what is a normal result versus an abnormal result a cutoff level must be established
- Ideally a cut point will be established based on biologic criteria (e.g., past this point, people are at an increased risk of disease)



Sensitivity and Specificity

Sensitivity: Ability of test to correctly identify who has the disease

-proportion of D+ people who were correctly identified as positive by the test

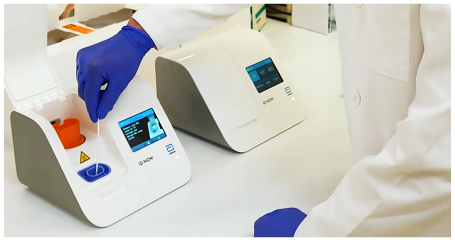
Specificity: Ability of the test to correctly identify who does not have the disease

-proportion of D- people who are correctly identified as negative by the test

A “perfect” test with 100% sensitivity and specificity would be (+) for everyone with the disease and (-) for everyone without the disease

Compared to what?

- To calculate sensitivity and specificity, we must know who truly has the disease according to a gold standard
  - Gold standard= referent test
- Compare results from index text to the gold standard test
- Gold standard test:
  - Best test available (but more often invasive or expensive)
  - Well-accepted
  - If gold standard says D+ we assume true D+, if gold standard says D- we assume D-



2x2 table in for dichotomous test results

	TRUE CHARACTERISTICS IN THE POPULATION	
Test Results	**Have the Disease**	**Do Not Have the Disease**
Positive	**True Positive (TP):** Have the disease and test positive	**False Positive (FP):** Do not have the disease but test positive
Negative	**False Negative (FN):** Have the disease but test negative	**True Negative (TN):** Do not have the disease and test negative

$$\text{Sensitivity} = \frac{TP}{TP + FN} \qquad \text{Specificity} = \frac{TN}{TN + FP}$$

Example: Assume a population of 1,000 people, of whom 100 have the disease and 900 do not have the disease. A screening test is used to identify the 100 people who have the disease.

	TRUE CHARACTERISTICS IN THE POPULATION		
Results of Screening	**Have the Disease**	**Do Not Have the Disease**	**Totals**
Positive	80	100	180
Negative	20	800	820
Totals	100	900	1,000

Sensitivity: $\frac{80}{100} = 80\%$

Specificity: $\frac{800}{900} = 89\%$

False Results

False Negatives (poor sensitivity):

- Individual consequences
- Public health/population-level consequences
- Delayed treatment, worse prognosis, spread of disease
- **False negative probability** = 1- sensitivity

False Positives (poor specificity):

- Costly/invasive confirmatory testing
- Anxiety and psychosocial stress
- Discrimination
- **False positive probability**= 1- specificity

The effect of cut-points

- Values of sensitivity and specificity are dependent on the cut-off level used to define diseased/not diseased

- Assessing sensitivity and specificity of a continuous biologic characteristic is somewhat arbitrary

Example: Type II Diabetes

Example: Type II diabetes

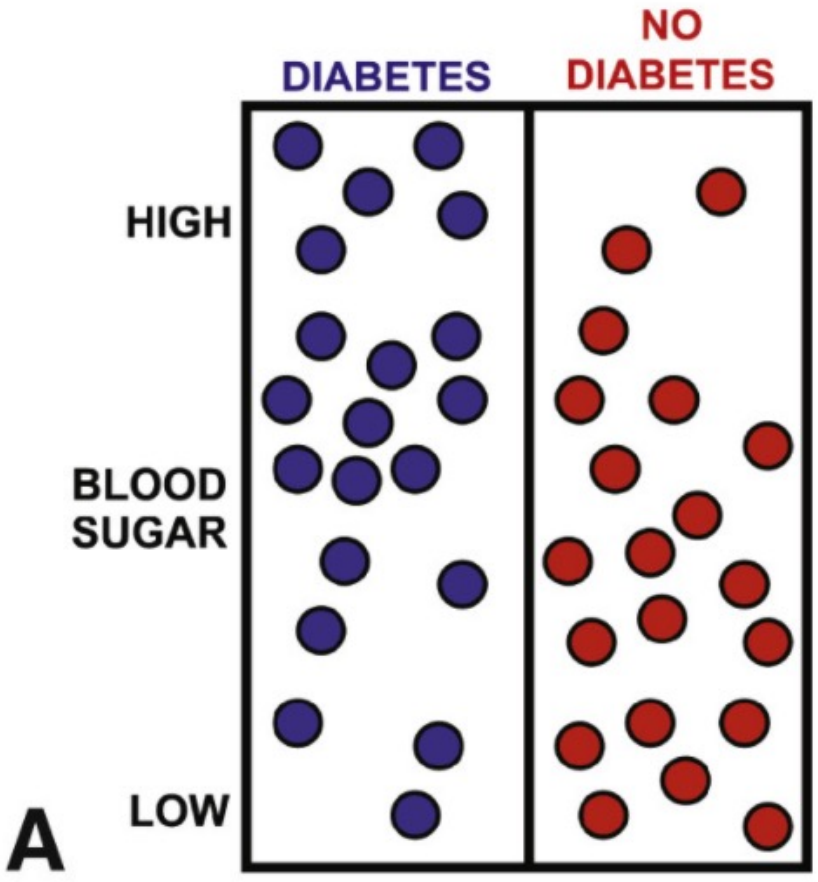
-Highly prevalent in US population

-Gold standard= oral glucose tolerance test

- Drink glucose solution, blood tests at specific intervals
- Can take up to 4 hrs

-Fasting plasma glucose = screening test

- Fast 8-10hr, blood test
- Easier, faster, more convenient, less expensive

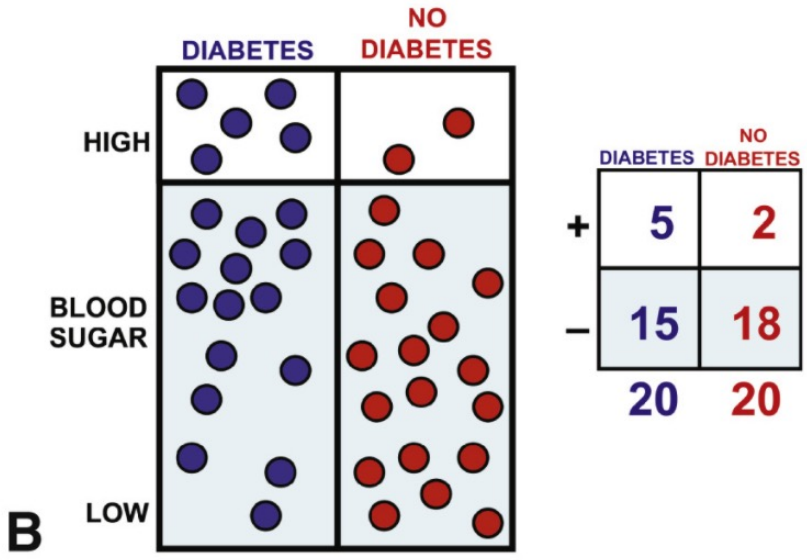


Population of 40 individuals, 20 with diabetes and 20 without diabetes

Blood sugar test (high→ low) does not have any obvious cut point

How do we select a cut-point?

Choosing a high cutpoint



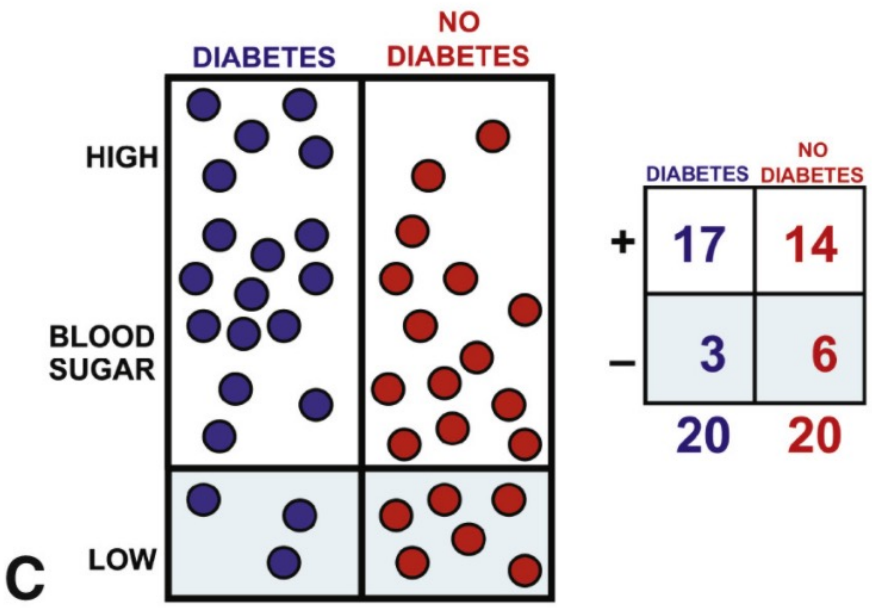
	DIABETES	NO DIABETES
+	5	2
–	15	18
	20	20

-Many individuals with diabetes will be incorrectly identified as negative

Sensitivity=5/20=25%
Specificity= 18/20=90%

-Most of the diabetics will incorrectly classified as non-diabetic, but most of the nondiabetics will be correctly classified as nondiabetic

Choosing a low cutpoint



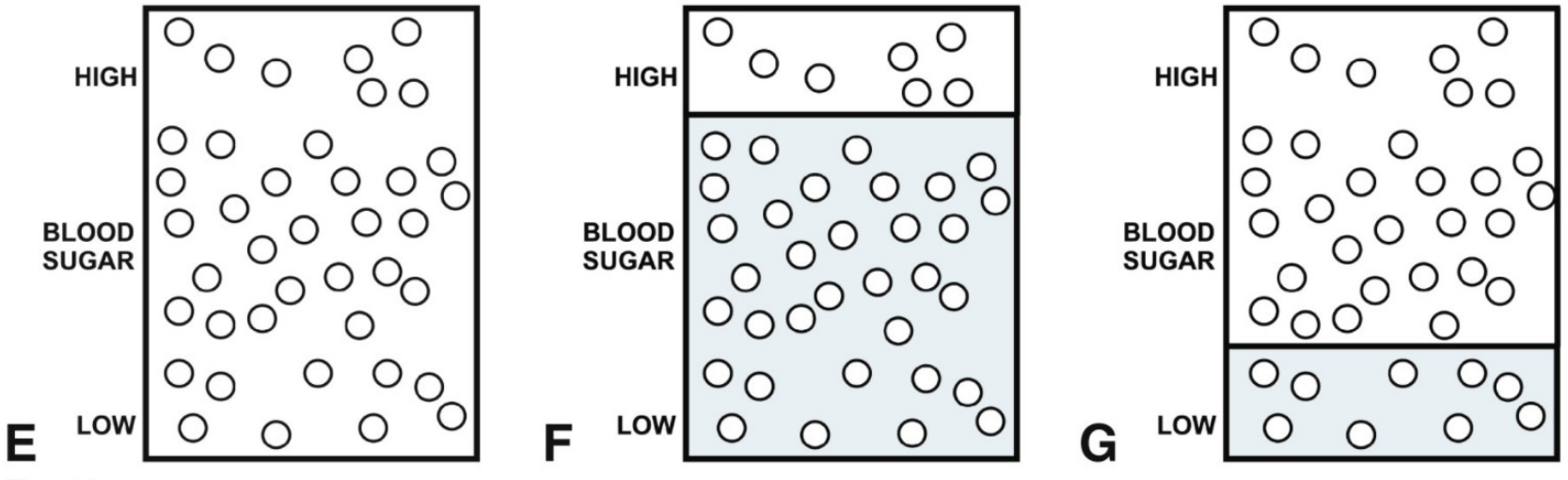
	DIABETES	NO DIABETES
+	17	14
–	3	6
	20	20

-Fewer individuals with diabetes will be misdiagnosed, many individuals without diabetes will be incorrectly classified as diabetic

Sensitivity=17/20=85%
Specificity=6/20=30%

-Most of the diabetics will incorrectly classified as non-diabetic, but most of the nondiabetics will be correctly classified as nondiabetic

Real world scenario



Potentially missing cases

Potentially unnecessary follow up testing

Trade-Offs

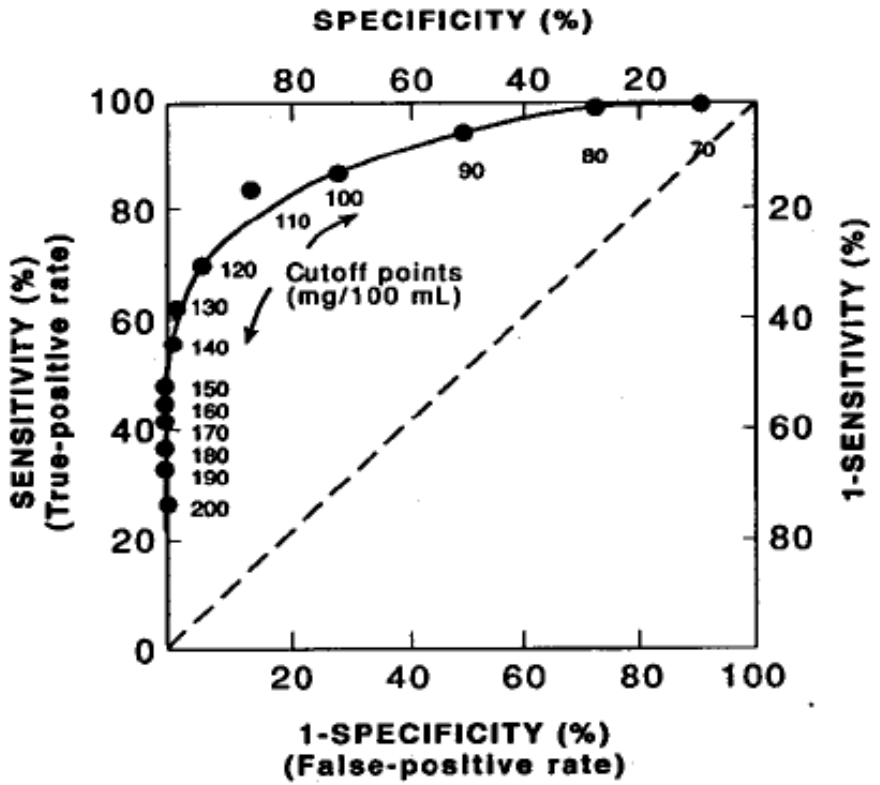
Trade off between sensitivity and specificity:

- Increase sensitivity by lowering the cutoff level, we decrease the specificity
  - Lower threshold: to “catch everyone”
  - Increases sensitivity, decreases false negatives
  - Decreases specificity, increases false positives
  - E.g., Airport screening
- Increase the specificity by raising the cutoff level, we decrease the sensitivity
  - Higher threshold: to “rule out more”
  - Increases specificity, decreases false positives
  - Decreases sensitivity, increases false negatives
  - E.g., Invasive biopsy req’d as follow-up

How to choose a cut point

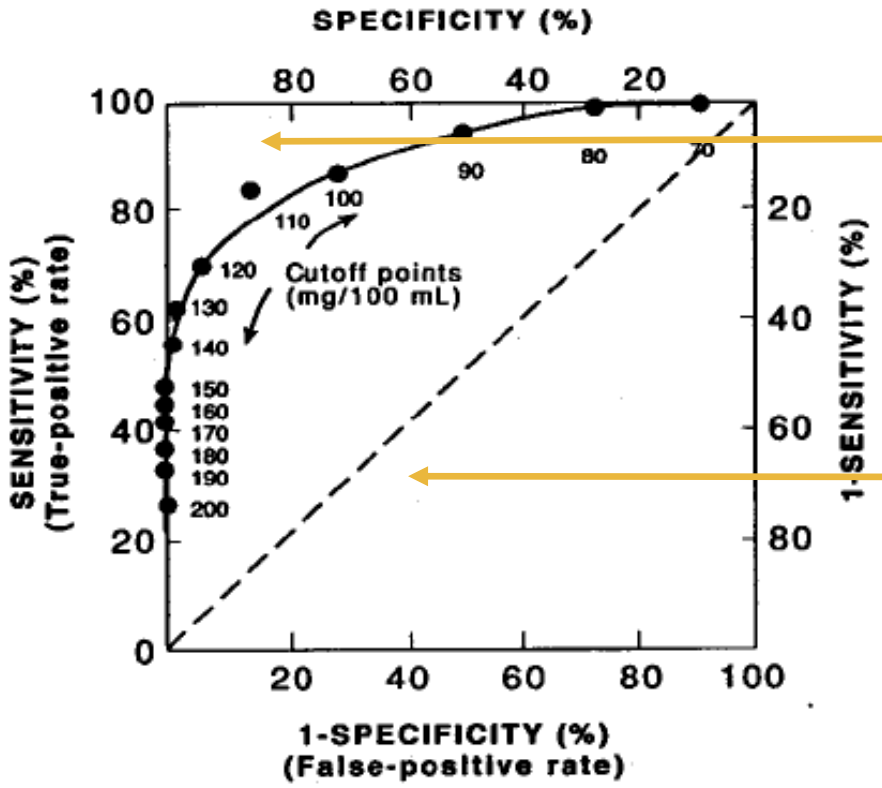
Scenario	If the confirmatory test (gold standard) test is expensive or invasive	If the penalty for missing a case is high
Priority	Minimize false positives	Maximizes true positives
Action	Use a cut point with high specificity	Use a cut point with high sensitivity

ROC curves (Receiver Operating Characteristic)



A ROC curve. The accuracy of 2-hr postprandial blood sugar as a diagnostic test for diabetes mellitus.

ROC curves assess the performance of a diagnostic test over a range of possible cut-point values for a for the index test



A ROC curve. The accuracy of 2-hr postprandial blood sugar as a diagnostic test for diabetes mellitus.

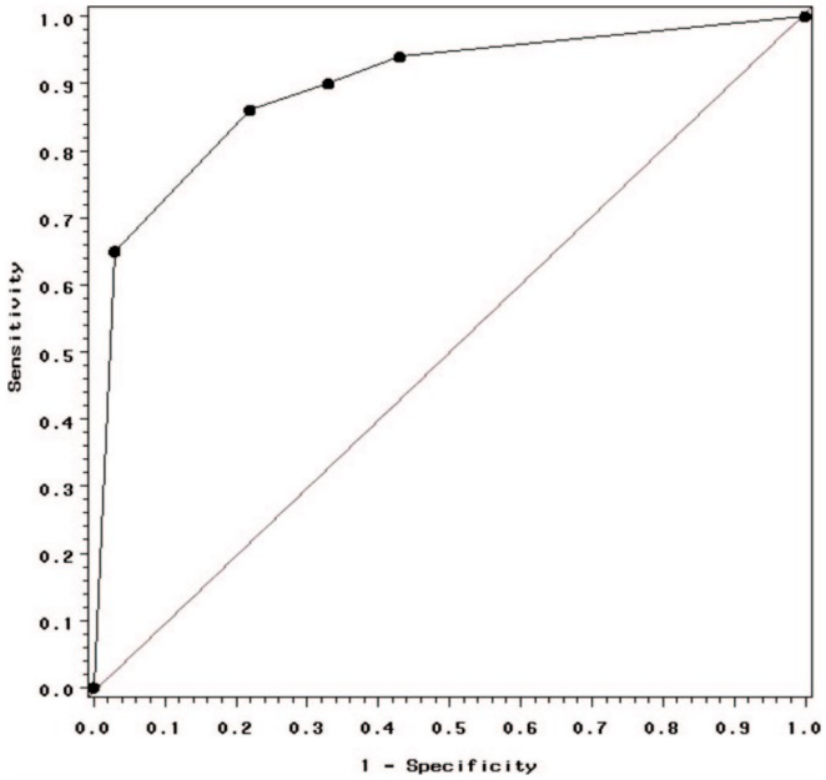
Better tests rise steeply: Close to the top left corner, where both sensitivity and specificity are 1

Useless tests lie near the diagonal

Area under the curve (AUC): summary of accuracy of diagnostic test (0= useless test, 1= perfect test)

TABLE 1. True Disease Status by Image Ratings

True Disease Status	Image Ratings: 1 = Definitely Normal	2 = Probably Normal	3 = Unsure	4 = Probably Abnormal	5 = Definitely Abnormal	Total
Normal	33	6	6	11	2	58
Abnormal	3	2	2	11	33	51
Total	36	8	8	22	35	109



AUC = 0.89

89% chance that the radiologist reading the image will correctly distinguish a normal from an abnormal patient based on the image ratings

Sequential and Simultaneous Testing

The decision to do multiple diagnostic tests

Use of Multiple Tests

- Commonly done in medical practice
- Choices depend on cost, invasiveness, volume of test, presence and capability of lab infrastructure, urgency, etc.
- Tests can be done **sequentially** or **simultaneously**

Sequential Testing

- Two stage testing
- After the first (screening) test was conducted, those who tested **positive** were brought back for the second test to further reduce false positives
- Those who test positive on both are presumed to have the disease
- This process will **increase specificity**

Example: Blood sugar test and OGTT

Sequential Testing
Step 1: Blood sugar measurement

Assume: Disease Prevalence = 5%,
Population = 10,000

TEST 1 (Blood Sugar)

Sensitivity = 70%
Specificity = 80%

TEST RESULTS	DIABETES +	DIABETES -	
+	350	1900	2250
-	150	7600	7750
	500	9500	10,000

Specificity: 80% of non-diabetics will correctly test negative

Sensitivity: 70% of diabetics will correctly test positive

Step 2: Glucose Tolerance Test

Assume: Disease Prevalence = 5%, Population = 10,000

TEST 1 (Blood Sugar) Sensitivity = 70%, Specificity = 80%

TEST RESULTS	DIABETES +	DIABETES -	
+	350	1900	2250
-	150	7600	7750
	500	9500	10,000

TEST 2 (Glucose Tolerance Test) Sensitivity = 90%
Specificity = 90%

TEST RESULTS	DIABETES +	DIABETES -	
+	315	190	505
-	35	1710	1745
	350	1900	2250

Specificity: 90% of non-diabetics will correctly test negative

Sensitivity: 90% of diabetics will correctly test positive

dis: Epidemiology, 4th Edition.

Net Sensitivity & Specificity

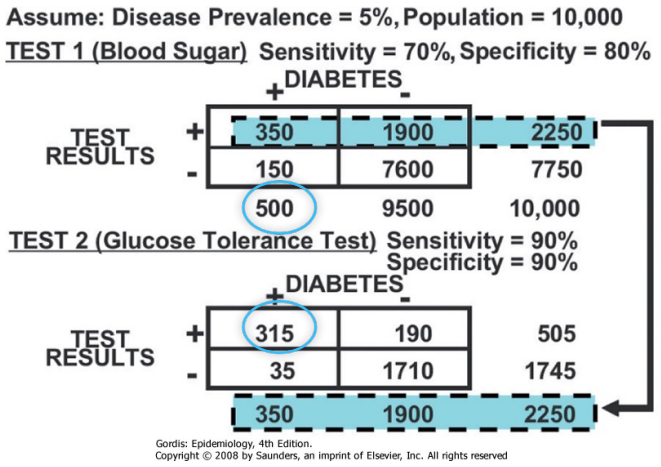
Two ways of calculating net sensitivity:

1. People who test positive on <u>both</u> tests / true diabetics

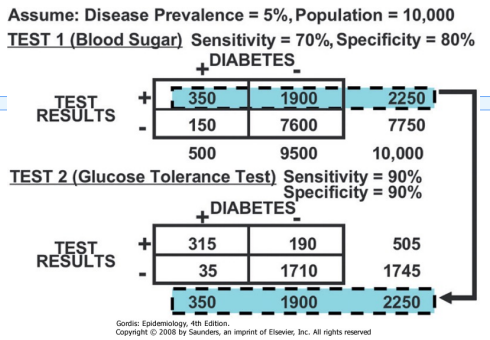
=315/500= 0.63

*2. $Sensitivity_{Test\ 1}$ * $Sensitivity_{Test\ 2}$*

= 0.70 * 0.90 = 0.63



Net Specificity of Sequential Tests



Two ways of calculating net specificity:

= Correctly identified as negative on either test / true negatives

= (7600 + 1710) / (9500) =0.98

$= Specificity_{Test\ 1} + Specificity_{Test\ 2} - (Specificity_{Test\ 1} * Specificity_{Test\ 2})$

= 0.8 + 0.9 – (0.8*0.9) = 0.8 + 0.9 – 0.72 = 0.98

Net sensitivity & specificity (sequential testing)

- Net sensitivity is worse than either test independently because at both points there are some people with disease that tested negative (two opportunities for false negatives)

- Net specificity is better than either test independently because sequential testing results in fewer false positives

Simultaneous Testing

- When two (or more) tests are conducted at the same time
- The goal is to maximize the probability that subjects with the disease (true positives) are identified (increase sensitivity)
  - Improve sensitivity by “adding on” positive tests
- Consequently, more false positives are also identified (decrease specificity)
  - When sensitivity is raised, specificity is lowered (twice the chance for a non-diabetic to test positive which = greater false positives)

Simultaneous Testing Example

- Population of 1000 people, prevalence of disease is 20%
  - 200 people have disease (=20/1000)
- Use two tests (at the same time)
  - Positive --> positive on both A and B
  - Negative --> negative on both A and B

Test A	Test B
Sensitivity = 80%	Sensitivity = 90%
Specificity = 60%	Specificity = 90%

Sensitivity of test A and B

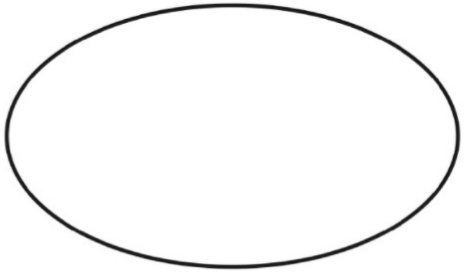
Test A

Results of Screening	POPULATION: Disease	POPULATION: No Disease
Positive	**160**	320
Negative	40	480
Totals	200	800
	Sensitivity = 80%	Specificity = 60%

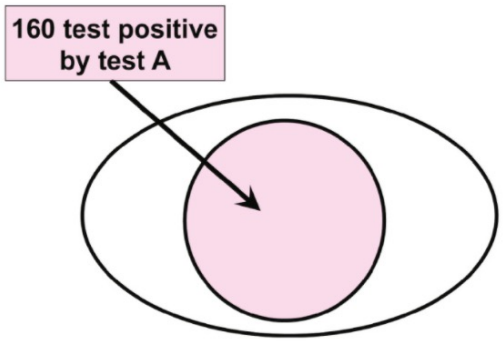
Test B

Results of Screening	POPULATION: Disease	POPULATION: No Disease
Positive	**180**	80
Negative	20	720
Totals	200	800
	Sensitivity = 90%	Specificity = 90%

THIS OVAL REPRESENTS THE 200 PEOPLE WHO HAVE THE DISEASE



OF THE 200 PEOPLE WHO HAVE THE DISEASE



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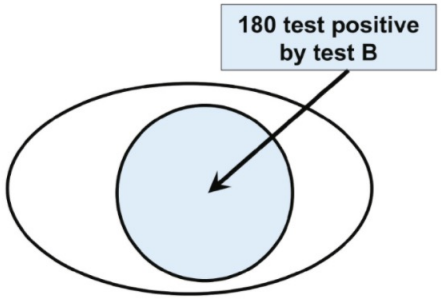
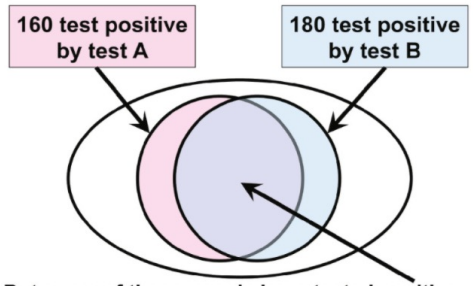


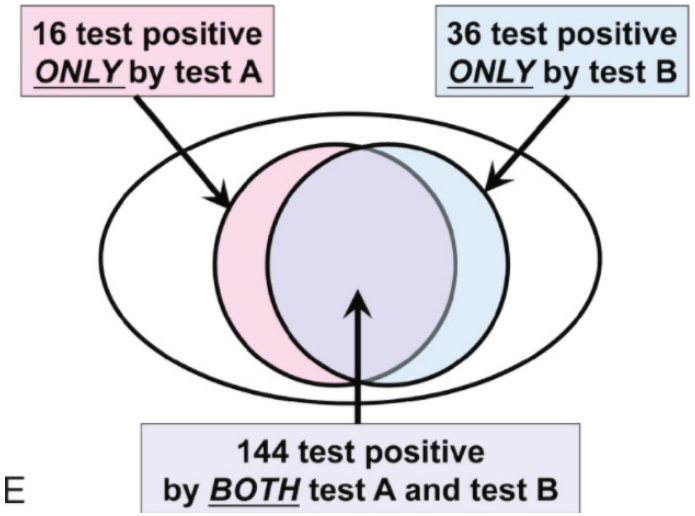
OF THE 200 PEOPLE WHO HAVE THE DISEASE



How do we determine how many people tested positive on both tests?

How many tested positive on both tests?

- Test A has a sensitivity of 80%
  - 160 people were positive with test A (80% of the 200 who have the disease)
- Test B has a sensitivity of 90%
  - Correctly identifies 90% of the same 160 people who were already tested as positive on test A
  - 0.9*160=144
- Alternate formula:
- = 200 * ($Sensitivity_A$ * $Sensitivity_B$) =144



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Net sensitivity

Net sensitivity = positive on either test A or test B / total

To calculate the number that tested positive on either (numerator)
= Number positive on A + number positive on B – number positive on both
= 160 + 180 – 144 = 196

Net sensitivity = positive on either / total = 196 / 200 = **0.98**

Net specificity

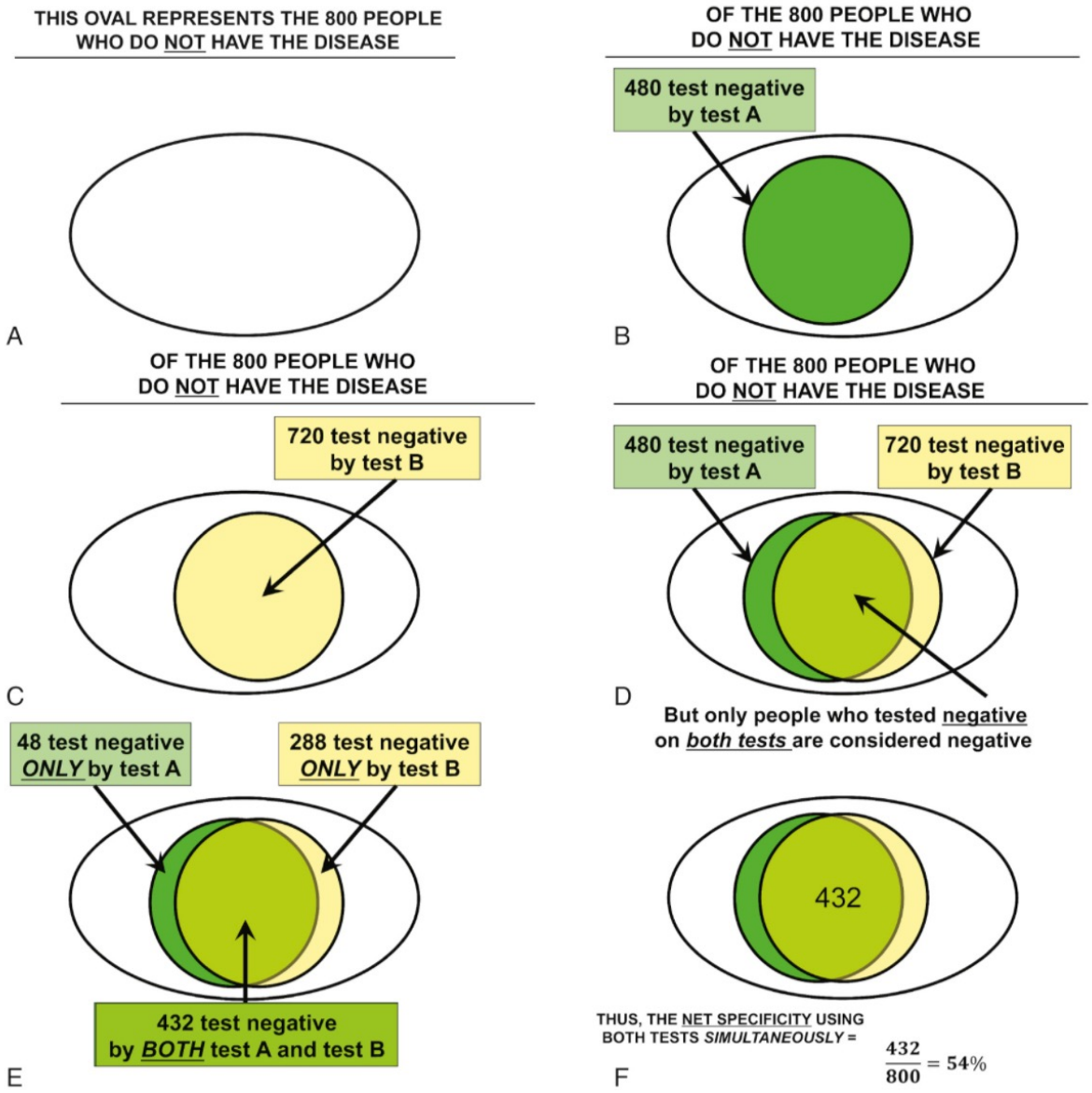
- Numerator for the net specificity calculation are individuals that test negative on BOTH tests and do not have the disease

Test A

Results of Screening	POPULATION: Disease	POPULATION: No Disease
Positive	160	320
Negative	40	**480**
Totals	200	800
	Sensitivity = 80%	Specificity = 60%

Test B

Results of Screening	POPULATION: Disease	POPULATION: No Disease
Positive	180	80
Negative	20	**720**
Totals	200	800
	Sensitivity = 90%	Specificity = 90%



The number that test negative on both:

= 800 * $Specificity_A$ * $Specificity_B$

= 432

Specificity of simultaneous tests

Test A:
Prevalence=20%; Sensitivity=80%; Specificity=60%

	True disease status		
Results of test	*Diabetic*	*Non-diabetic*	Total
Positive	160	320	480
Negative	40	480	520
Total	200	800	1000

Test B:
Prevalence=20%; Sensitivity=90%; Specificity=90%

	True disease status		
Results of test	*Diabetic*	*Non-diabetic*	Total
Positive	180	80	260
Negative	20	720	740
Total	200	800	1000

Net specificity = 432 / 800 = 0.54

or

Net specificity = $Specificity_A$ * $Specificity_B$ = 0.6 * 0.9 = **0.54**

Summary: Combination Testing

***Sequential testing*:**

- ↓ sensitivity (two opportunities for people to test negative falsely)
- ↑ specificity (have to test positive twice)

***Simultaneous testing*:**

- ↓ specificity (have to test negative twice; more likely to test positive falsely)
- ↑ sensitivity (two opportunities for people to test positive)

Predictive Value of Tests

Predictive Value of Tests

In a clinical setting, we don't ever know if patients truly have the disease or not (that's why we're testing)

With clinical testing, what are we interested in?

-If the test is positive, what is the probability that the patient really has the disease? (*Positive predictive value of the test, PPV*)

-If the test is negative, what is the probability that the patient is disease-free? (*Negative predictive value of the test, NPV*)

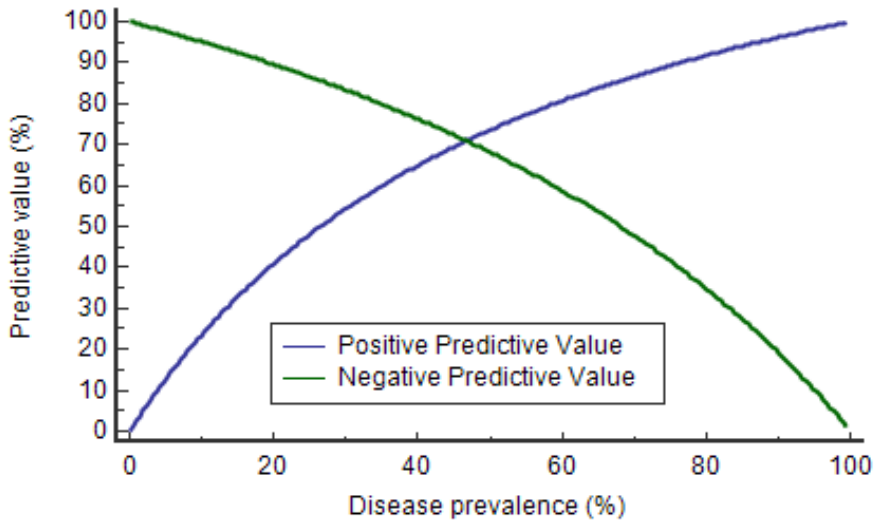
Results of Screening	POPULATION: Disease	POPULATION: No Disease	Totals
Positive	80	100	180
Negative	20	800	820
Totals	100	900	1,000

Positive predictive value $= \frac{80}{180} = 44\%$

Negative predictive value $= \frac{800}{820} = 98\%$

Predictive Values

- The PPV and NPV depend on:
  - Disease prevalence in population of interest
  - Sensitivity and specificity of the test itself



Relationship between disease prevalence and predictive value in a test with 99% sensitivity and 95% specificity

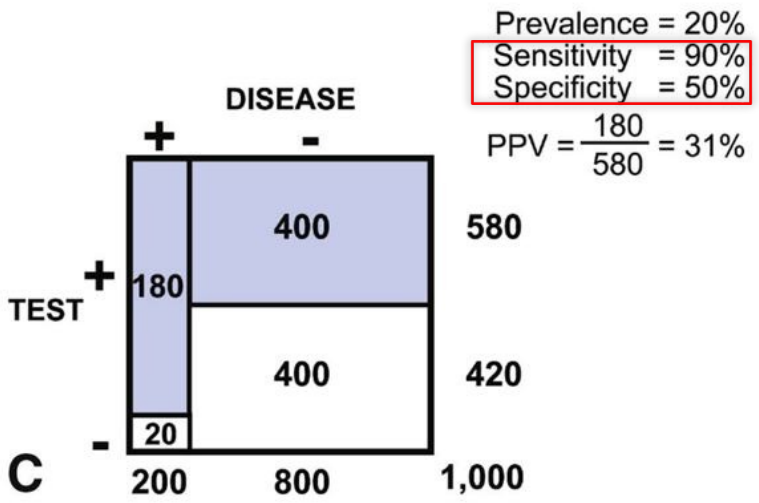
EXAMPLE: SENSITIVITY = 99%, SPECIFICITY = 95%					
Disease Prevalence	**Test Results**	**Sick**	**Not Sick**	**Totals**	**Positive Predictive Value**
1%	+	99	495	594	$\frac{99}{594} = 17\,\%$
	–	1	9,405	9,406	
	Totals	100	9,900	10,000	
5%	+	495	475	970	$\frac{495}{970} = 51\,\%$
	–	5	9,025	9,030	
	Totals	500	9,500	10,000	

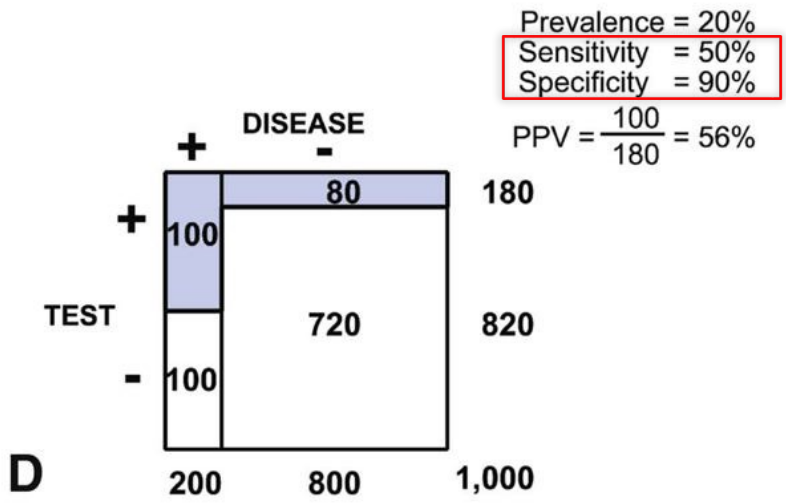
Implications

- Diagnostic tests with high PPV in clinical settings (high prevalence) may have low PPV in the largely healthy general population (low prevalence).
- **Screening tests are much more effective when disease prevalence is high**
- Screening for some diseases in the general population can be inefficient relative to the effort involved

Specificity and PPV

- Greater specificity improves PPV
  - Reduces the number of false positives
  - High specificity has a greater impact on PPV than high sensitivity





Why does specificity have a greater effect than sensitivity on predictive value?

- Because we are dealing with infrequent cases of disease diseases, the majority of the population is D-

- Any change to the D- group affects a greater number of people than would a comparable change to the D+ group.

EXAMPLE: PREVALENCE = 10%, SENSITIVITY = 100%					
Specificity	**Test Results**	**Sick**	**Not Sick**	**Totals**	**Predictive Value**
70%	+	1,000	2,700	3,700	$\frac{1,000}{3,700} = 27\%$
	–	0	6,300	6,300	
	Totals	1,000	9,000	10,000	
95%	+	1,000	450	1,450	$\frac{1,000}{1,450} = 69\%$
	–	0	8,550	8,550	
	Totals	1,000	9,000	10,000	

PPV values: general population vs. high risk group

Age (Years)	Women without a Family History of Breast Cancer	Women with a Family History of Breast Cancer
30–39	3%	4%
40–49	4%	13%
50–59	9%	22%
60–69	17%	14%
70	19%	24%

PPV increases with age and among women who have a family history of breast cancer

Reliability of Screening and Diagnostic Tests

Reliability

- Another important aspect of diagnostic testing is whether the results are reliable
  - Reliability=repeatability=reproducibility

Different types of variation:

- Intra-subject variation
- Intra-observer variation
- Inter-observer variation

Intra-subject variation

- Variation in results of a test conducted on the same individual
  - Over a short period of time
- Difference due to changes occurring within an individual

Blood Pressure (mmHg)	Female Aged 27 yrs	Female Aged 62 yrs	Male Aged 33 yrs
Basal	110/70	132/82	152/109
Lowest hour	86/47	102/61	123/ 78
Highest hour	126/79	172/94	153/107
Casual	108/64	155/93	157/109
From Richardson DW, Honour AJ, Fenton GW, etal: Variation in arterial pressure throughout the day and night. Clin Sci 26:445, 1964.			

Intra-observer variation
Inter-observer variation

Intra-observer: Variation in the result of a test due to the same observer examining the result at different times

- E.g., Dr. W, a radiologist, who looks at the same X-ray at two different times
- More subjective interpretation in test results, greater chance for intra-observer variability

Inter-observer: Variation in the result of a test due to multiple observers examining the test result

- Two observers may not give the same result
- Interested in the extent to which multiple examiners agree (or not)

Quantifying Agreement

		Observer 2	
		+	-
Observer 1	+	a	b
	-	c	d

- Concordant cells: a and d
- Discordant cells: b and c
  - Perfect agreement occurs when b=0 and c=0

- **Percent agreement** = [(a+d) / (a+b+c+d)] *100

Percent Agreement

The Inter-Observer Variation of Chest Radiograph Reading in Acute Lower Respiratory Tract Infection among Children

Gabriel Xavier-Souza,[1] Ana Luisa Vilas-Boas, MD,[1] Maria-Socorro Heitz Fontoura, MD, PhD,[1] César Augusto Araújo-Neto, MD,[2] Sandra C. S. Andrade, MD,[3] Maria-Regina Alves Cardoso, PhD,[4] Cristiana Maria Nascimento-Carvalho, MD, PhD[1]* and the PNEUMOPAC-Efficacy Study Group

TABLE 3—The Agreement Between the Radiologists on Each of the Radiological Findings Among Outpatient Children With Acute Lower Respiratory Tract Infection

		Radiologist 2		
Radiological finding	Radiologist 1	Yes	No	Agreement (%)
Alveolar infiltrate				83.2
	Yes	139	118	
	No	12	505	

Percent agreement = (139 + 505) / (139 + 12 + 118 + 505)

Percent agreement for multiple categories

	Reading No. 1						
Reading No. 2	***Abnormal***		***Suspect***		***Doubtful***		***Normal***
Abnormal	[A]	+	B		C		D
Suspect	E		[F]	+	G		H
Doubtful	I		J		[K]	+	L
Normal	M		N		O		[P]

$$\text{Percent agreement} = \frac{A + F + K + P}{\text{Total readings}} \times 100$$

Kappa Statistic

Extent to which the observed agreement that the observers achieved exceeds that which would be expected by chance alone

Answers two questions:

1.How much better is the agreement in observers' readings than we we would expect by chance alone?

2. What is the most that two observers could have improved their agreement over the agreement that would be expected by chance alone

$$\text{Kappa} = \frac{\left(\begin{array}{c}\text{Percent agreement}\\ \text{observed}\end{array}\right) - \left(\begin{array}{c}\text{Percent agreement}\\ \text{expected by chance alone}\end{array}\right)}{100\% - \left(\begin{array}{c}\text{Percent agreement}\\ \text{expected by chance alone}\end{array}\right)}$$

Screening in the News

- Screening is a very complicated issue and is oftentimes difficult to explain to the general public
- Not always intuitive why more screening is sometimes a bad thing
  - Pap smears
  - Mammography
  - Prostate cancer screening
- The usual result? Confusion.

Screening mammography doesn't cut breast-cancer deaths, Canadian study says

HELEN BRANSWELL
TORONTO — The Canadian Press
Published Tuesday, Feb. 11, 2014 8:37PM EST
Last updated Tuesday, Feb. 11, 2014 8:39PM EST

More breast tumors detected in the mammography group (compared with annual physical examination by MD)

Number of deaths almost identical in the two study groups

Mammography found both benign and malignant tumors

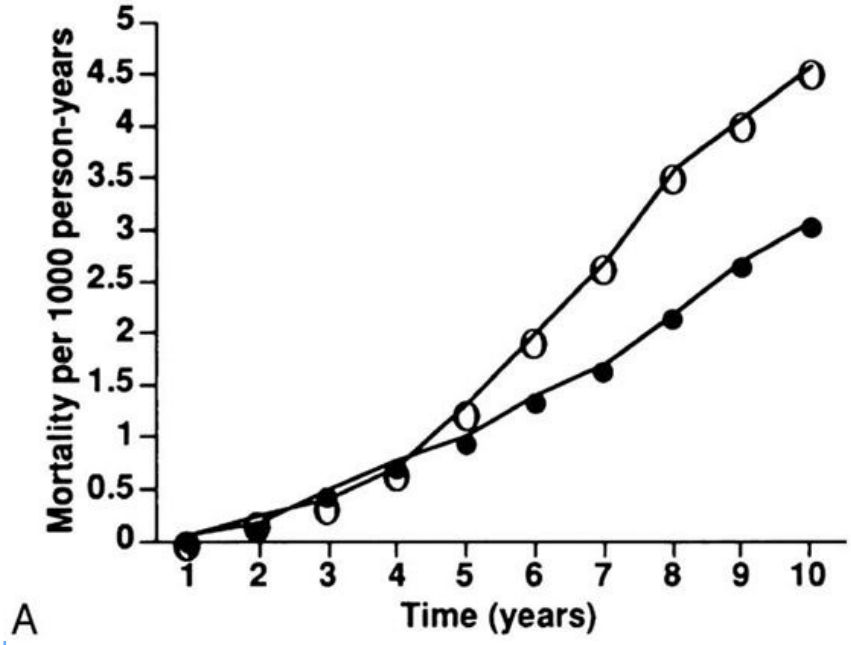
American Cancer Society urges later start for mammograms

CARLY WEEKS
The Globe and Mail
Published Tuesday, Oct. 20, 2015 11:00AM EDT
Last updated Wednesday, Oct. 21, 2015 10:10AM EDT

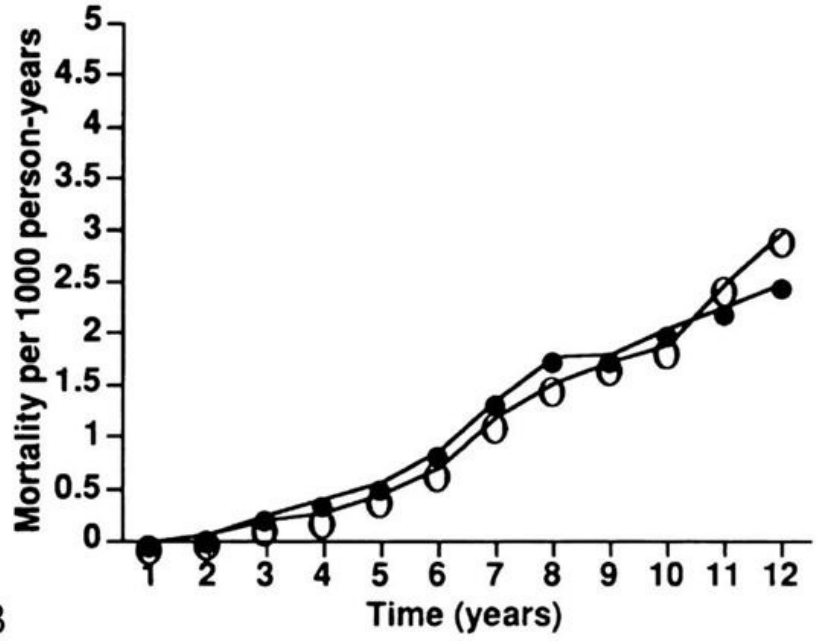
In the general population, regular mammograms before age 45 are likely to do more harm than good

Cumulative breast cancer mortality rates in screened and unscreened women **(A) ages 50 to 69 years and (B) ages 40 to 49 years.**

• = screened; ○ = unscreened.



A



B

Is a screening program effective?

Screening can be evaluated from two different perspectives

1. Process

E.g., Number of people screened, total costs per case found, proportion of positive tests that resulted in correct diagnosis and treatment

2. Outcome

E.g., reduction in mortality, morbidity, improved quality of life

Properties of a valid screening program

1. Disease detectable in an asymptomatic ("pre-clinical") period
   - Important to have a long pre-clinical phase

2. Early treatment (following early detection) provides benefit (survival, morbidity) over conventional treatment (standard diagnosis)

3. Benefits outweigh costs (financial and otherwise) of screening

Cost-Benefit Analysis

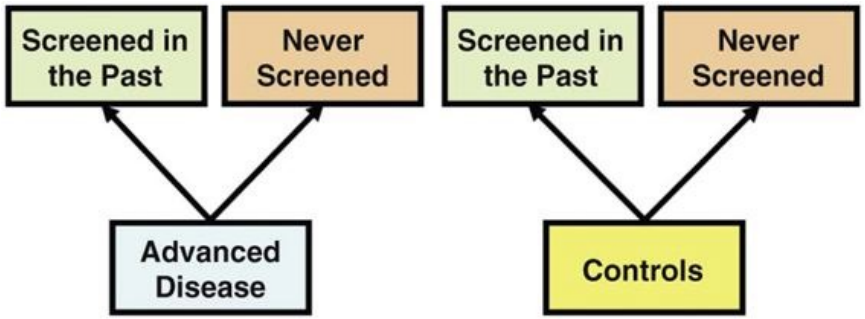
- Even if a screening test is inexpensive, should we be doing it in the general population?
  - What about the cost of the confirmatory tests required?

- Must consider non-financial costs
  - Anxiety/emotional distress
  - Inconvenience
  - Physically invasive
  - Side effects

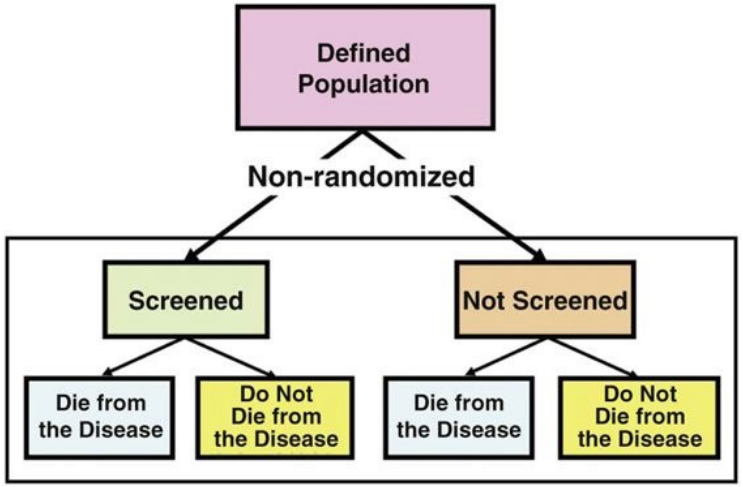
Evaluating Screening Programs: Non-Randomized Studies

Can use cohort or case-control studies to evaluate screening programs

Case-control study



Cohort study



Evaluating Screening Programs: Randomized Studies