Understanding Test Performance

302-2110 Rev A May 2021

Agenda

Sensitivity and Specificity

Positive Predictive Value (PPV) and Negative Predictive Value (NPV)

Incidence and Prevalence

Screening and Diagnostic tests

Conclusion and Discussion

Exercises

Learning Objectives

The General Objective of this Module Is to Give You an Understanding of Test performance

At the end of the training, you will be able to:

- Discuss sensitivity, specificity, PPV, NPV, prevalence and incidence
- Apply your learnings to the Xpert® test Package Insert
- Discover the impacts of prevalence on the PPV and NPV
- Discover the purpose of PPV and NPV for diagnostic and screening tests
- Use the exercises to calculate the sensitivity, specificity, PPV and NPV of a test

Sensitivity & Specificity

Analytical & Clinical

Analytical Sensitivity

- Also called the Limit of Detection (LoD)
- Expressed in units/mL
- Units can be copies of nucleic acid (copies/mL), colony-forming units (CFU/ml), or inclusion-forming units (IFU/mL)
- Smallest amount of material that the given test can detect in a given volume
- Critical parameter for viral load tests (HIV, HCV, HBV) where the goal is to measure the lowest possible viral burden in patients on therapy.

Analytical Sensitivity

L.6 **ANALYTICAL SENSITIVITY**

Additional studies were performed to determine the 95% confidence interval for the analytical limit of detection (LoD) of this assay. The limit of detection is defined as the lowest number of colony forming units (CFU) per sample that can be reproducibly distinguished from negative samples with 95% confidence. The analytical LoD was determined by testing 20 replicates of different concentrations of *M. tuberculosis* cells spiked into negative clinical sputum samples. Under the conditions of the study, results indicate that the LoD point estimate for *M. tuberculosis* is 131 CFU/mL with a 95% confidence interval ranging from 106.2 CFU to 176.4 CFU. The estimate and confidence levels were determined using logistic regression with data (number of positives per number of tests at each level) taken at different concentrations.

The confidence intervals were determined using the maximum likelihood estimates on the logistic model parameters using the large sample variance-covariance matrix.

Please refer to the full Xpert MTB/RIF Package Insert for more information (301-0192 and 301-0191)

Clinical Sensitivity

- Expressed as a percentage (%)
- Represents the fraction of the truly infected patients who are POSITIVE with the specific test.
- Assessed during clinical studies
- Critical parameter for diagnostic tests where the goal is to identify everyone who has the disease

Analytical and Clinical Specificity

Analytical specificity

- Represents the degree that a test only reacts with its target
- NO CROSS REACTIVITY with other bacterial species or viruses

Clinical specificity

- Expressed as a percentage (%)
- Represents the fraction of the truly NOT infected patients who are NEGATIVE using the given test (i.e., how frequently a test is negative when a particular disease is NOT present)

Analytical Specificity

L.7 ANALYTICAL SPECIFICITY (EXCLUSIVITY)

Cultures of 18 nontuberculosis mycobacteria, NTM (formerly MOTT), strains were tested with the Xpert MTB/RIF assay. Two or more replicates of each isolate were spiked into negative sputum samples and tested at a concentration of 10^6 CFU/mL. See Table 7.

Table 7. NTM strains tested for specificity

NTM Strains Tested (10^6 CFU/mL)			
1	*M. avium, SmT Mc2, 2500*	10	*M. genevenses, #51233*
2	*M. avium, SmD Mc2, 2501*	11	*M. xenopi, #2278*
3	*M. intracellulare, #35790*	12	*M. szulgai, Cap E9-1997*
4	*M. intracellulare, #35771*	13	*M. celatum, #51131*
5	*M. kansasii, #12478*	14	*M. marinum, Cap E10*
6	*M. scrofulaceum, Cap E5-1985*	15	*M. simiae, #25275*
7	*M. malmoense, #29571*	16	*M. asiaticum, E1-1985*
8	*M. fortuitum, #35754*	17	*M. thermoresistable, e22-1985*
9	*M. chelonae, #35749*	18	*M. flavescens, PoH 193D*

Please refer to the full Xpert MTB/RIF Package Insert for more information (301-0192 and 301-0191)

Analytical Specificity

- Other culture collections can be used

Analytical Specificity

Cultures from American Type Culture Collection (ATCC) and Culture Collection, University of Göteborg (CCUG) representing organisms closely related to *C. difficile* as well as normal and pathogenic rectal flora were tested in a cross-reactivity study. Two strains of non-toxin producing *C. difficile* were tested using the Xpert *C. difficile* Assay. The organisms tested were represented by 24 aerobic, 14 anaerobic and two microaerophilic species. Three replicates of each isolate were tested at a concentration of at least 10^9 CFU per reaction. Under the conditions of the study, all isolates were reported toxinogenic *C. difficile* negative; none of the isolates were detected by the Xpert *C. difficile* Assay. Positive and Negative controls were included in the study. The Analytical specificity was 100%.

Please refer to the full Xpert C.*difficile* Package Insert for more information (300-9291)

Calculating Test Performance

Sensitivity and specificity

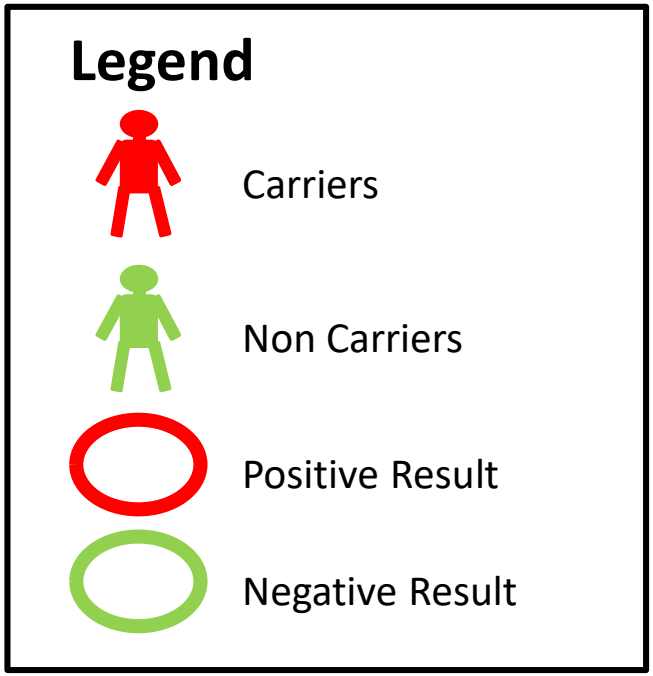
Performance characteristics – Sensitivity & Specificity

- **Sensitivity & Specificity** are the two basic measures of the inherent accuracy of a diagnostic test

	Real Positive	Real Negative	Total
Positive with your test	a (True Positives)	b (False Positives)	a+b
Negative with your Test	c (False Negatives)	d (True Negatives)	c+d
Total	a+c	b+d	-

- Sensitivity represents the "true positive rate" = a/a+c
- Specificity represents the "true negative rate," =d/b+d

100% Sensitivity and **100%** Specificity



Sensitivity: Patients with **Positive test results** in the positive patients population = **100%**

Specificity : Patients with **Negative test results** in the negative patients population = **100%**

Sensitivity and Specificity

- A Sensitivity of 100% : means that the test recognizes all people carrying the micro-organisms as **POSITIVE**
  - Sensitivity alone does not tell us how well the test predicts the Negative cases
- A Specificity of 100% : means that the test recognizes all people free of the micro-organisms as **NEGATIVE**
  - Specificity alone does not tell us how well the test recognizes the Positive cases
- A good test must have a good **Specificity** and **Sensitivity**!

Xpert® C.*difficile* performance characteristics

Table 1. Performance characteristics of the Xpert *C. difficile* Assay as compared to direct culture

		Toxinogenic Culture		
		C. difficile pos	027/NAP1/BI pos	Negative
Xpert *C. difficile*	Toxin B+	34	0	16
	027/NAP1/BI	0	0	1
	Negative	0	0	234

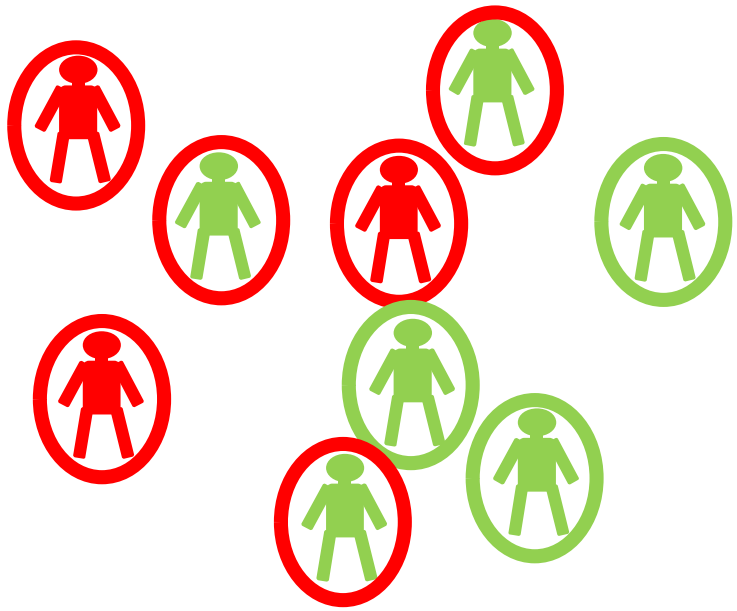
Sensitivity	100%
Specificity	93%

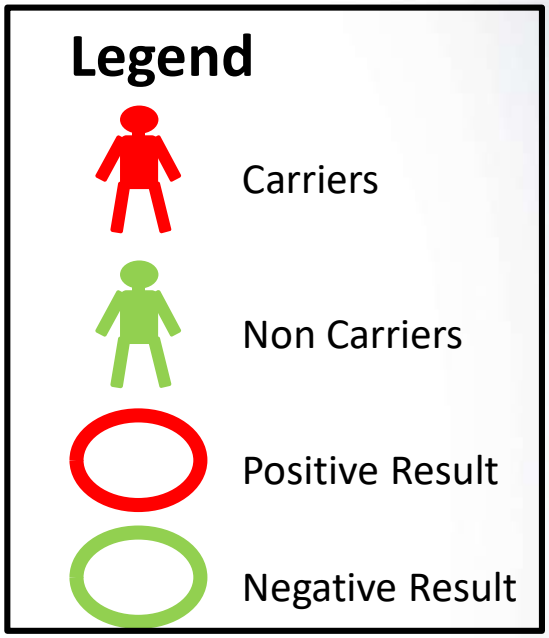
34/34 234/251

- Sensitivity = 34/34+0 x 100 = 100%
- Specificity = 234/251+0 x 100 = 93%

Please refer to the full Xpert C.*difficile* Package Insert for more information (300-9291)

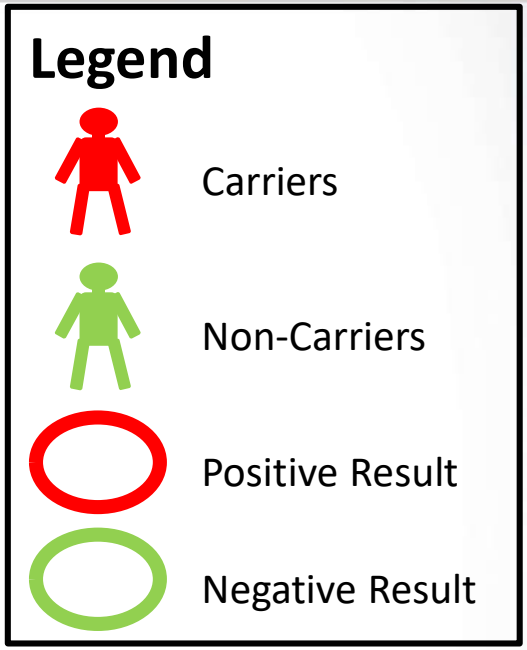
Example 1





- **Sensitivity:** Patients with Positive test results in the carrier population = 100%
- **Specificity:** Patients with Negative test results in the non-carrier population = 50%

Example 2



- **Sensitivity:** Patients with Positive test results in the carrier population = 66%
- **Specificity:** Patients with Negative test results in the non-carrier population = 100%

Example: Xpert® MRSA versus Culture

- Direct culture vs reference Culture Method
  - Positive Agreement = Sensitivity
  - Negative Agreement = Specificity

Table 3. Xpert MRSA Compared to Direct Culture Method

		Direct Culture			
		+	-		
Xpert MRSA	+	165	61	226	**Positive Agreement:** 94.3%
	-	10	838	848	**Negative Agreement:** 93.2%
		175	899	1074	PPV[a]: 73.0%
					NPV[b]: 98.8%

Table 2. Xpert MRSA Compared to Reference Culture Method

		Culture			
		+	-		
Xpert MRSA	+	182	44	226	Positive Agreement: 86.3%
	-	29	819	848	Negative Agreement: 94.9%
		211	863	1074[a]	PPV[b]: 80.5%
					NPV[c]: 96.6%

Please refer to the full Xpert C.*difficile* Package Insert for more information (300-9291)

Direct Culture versus Reference Culture

- Direct culture (16-18 hours) is less sensitive than reference culture (overnight enrichment in Broth)

Evaluation of *Brilliance* MRSA 2 Agar for Detection of Methicillin-Resistant *Staphylococcus aureus* in Clinical Samples

J. Veenemans, C. Verhulst, R. Punselie, P. H. J. van Keulen, J. A. J. W. Kluytmans

Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda, The Netherlands

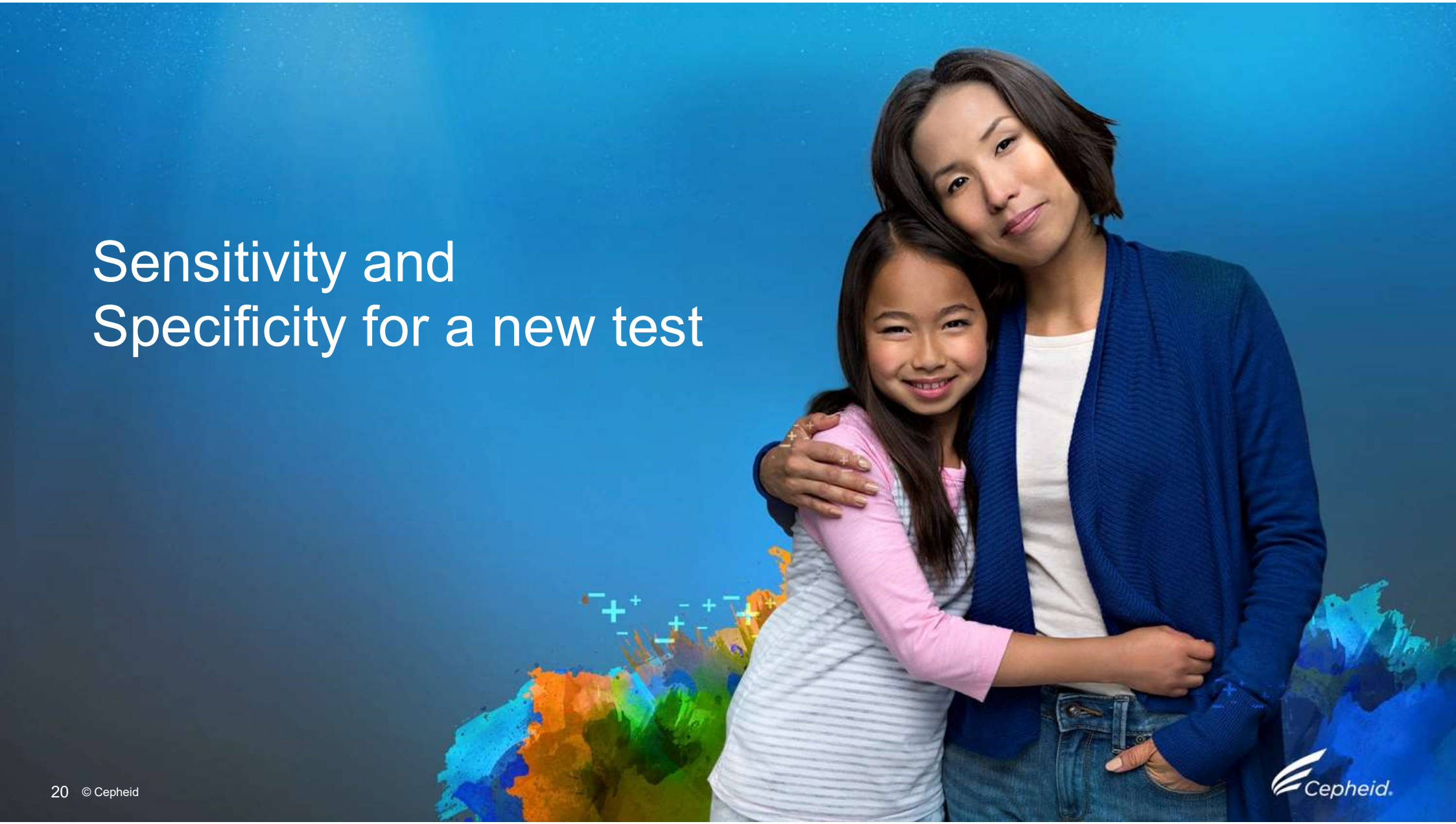
Journal of Clinical Microbiology p. 1026–1027
March 2013 Volume 51 Number 3

		Oxoid Brilliance MRSA 2 agar	bioMérieux MRSA-ID
Without Overnight Enrichment	Sensibility	65.70%	52%
	Specificity	99.8%	99%
With Overnight Enrichment	Sensibility	100%	98%
	Specificity	99.10%	98.8%

Veenemans, J., Verhulst, C., Punselie, R., Van Keulen, P.H.J. and Kluytmans, J.A.J.W., 2013. Evaluation of brilliance MRSA 2 agar for detection of methicillin-resistant Staphylococcus aureus in clinical samples. Journal of Clinical Microbiology, 51(3), pp.1026-1027.

Cepheid

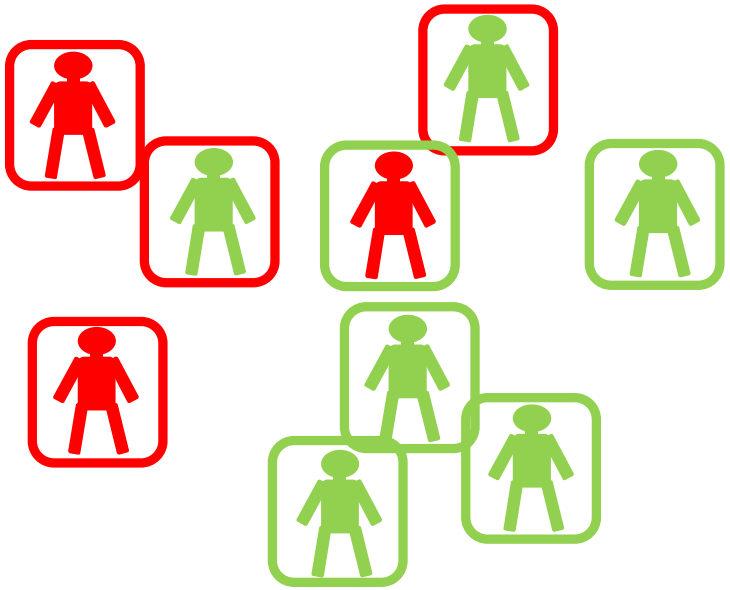
Sensitivity and Specificity for a new test



Reference Method or Gold Standard

- Sensitivity and specificity calculated compared to a reference method or gold standard (approved method currently used by laboratories for diagnosis).
- The diagnostic gold standard for active tuberculosis (TB) is the detection of Mycobacterium tuberculosis (MTB) by culture or molecular methods
- What is the impact of comparing a reference method (culture) to a more sensitive method (molecular)?
- How is sensitivity and specificity determined for the reference method?

Reference method vs actual disease status



Legend

Carriers

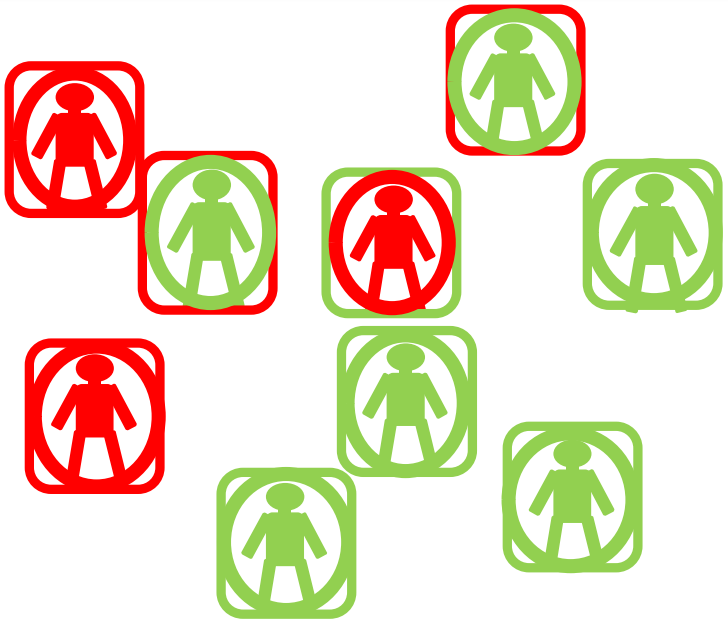
Non-Carriers

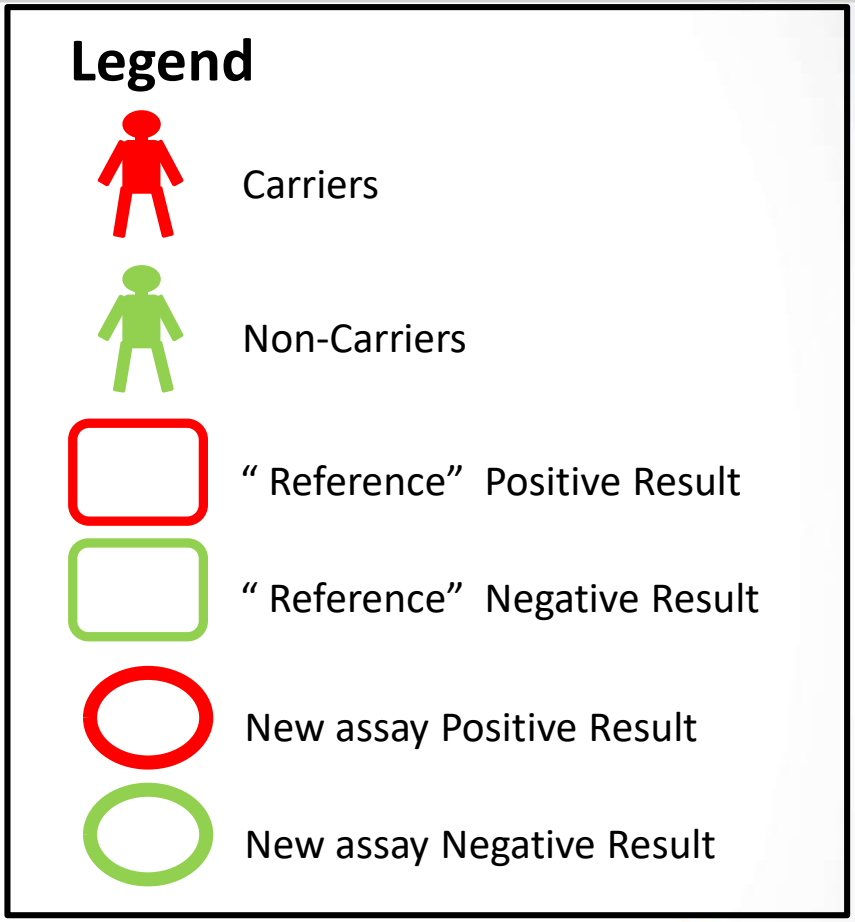
" Reference" Positive Result

" Reference" Negative Result

- Sensitivity = 2/3 = 67%
- Specificity = 4/6 = 67%

New method vs Reference method





- Positive Agreement Positive Agreement (clinical sensitivity) with reference = 50%
- Negative Agreement Negative Agreement (clinical specificity) with reference = 80%

Reference method vs actual disease status

- Test performance of the reference method - Patient status is unknown!
  - At this time it is impossible to establish if a positive test result is a real true positive or if a negative test result is a real true negative
  - Sensitivity and specificity of a new test are calculated against a reference method known as the gold standard (approved method currently used by laboratories for diagnosis).

- Implications
  - Bias : if the new test has better performances than the reference test.
  - Performances of the new test may appear artificially reduced.

Positive and Negative Predictive Values

PPV and NPV

PPV & NPV

- Sensitivity and Specificity represent technical performances of a diagnostic test
- Clinicians need answers to the following questions.

  – “What is the likelihood that this patient carries the micro-organisms when the test result is Positive?”
  – “What is the likelihood that this patient does not carry the micro-organisms when the test result is Negative?”

- The answers to these questions are known as the Positive and Negative Predictive Values (PPV and NPV)

Theory

	Real Positive	Real Negative	Total
Positive with your test	a (True Positives)	b (False Positives)	a+b
Negative with your Test	c (False Negatives)	d (True Negatives)	c+d
Total	a+c	b+d	-

- PPV is the Positive Predictive Value = a/a+b
- NPV is the Negative Predictive Value = d/c+d

Clinical Value of PPV and NPV

- PPV & NPV integrate the prevalence of the carriers in the population
- PPV is the probability that positive result is a true positive and gives indications on number of patients that will receive unnecessary treatment or quarantine.
- NPV is the probability that a negative result is a true negative and gives indications on number of patients who will not receive the appropriate treatment

Example: Xpert® MRSA screening

PPV	**Sensitivity**
Tells us the probability that the Patient is an MRSA carrier when Xpert MRSA is Positive	Tells us the probability that Xpert MRSA is positive with a Patient who is MRSA carrier
NPV	**Specificity**
Tells us the probability that the Patient is not an MRSA carrier when Xpert MRSA is Negative	Tells us the probability that Xpert MRSA is Negative with a Patient who is not an MRSA carrier
PPV & NPV	**Sensitivity & Specificity**
help the clinician decide how to manage the patient after they receive the result from the lab	help the Microbiologist to decide which test to use or if additional tests are required

Incidence and Prevalence

Incidence and Prevalence

- Incidence is the number of new cases in a given time period.
- The number of individuals who get a disease divided by the total number in the population per unit of time

- Prevalence measures the proportion of affected people in a population (new and old) with a particular disease at a given time.
- Similar to a snapshot of a particular disease

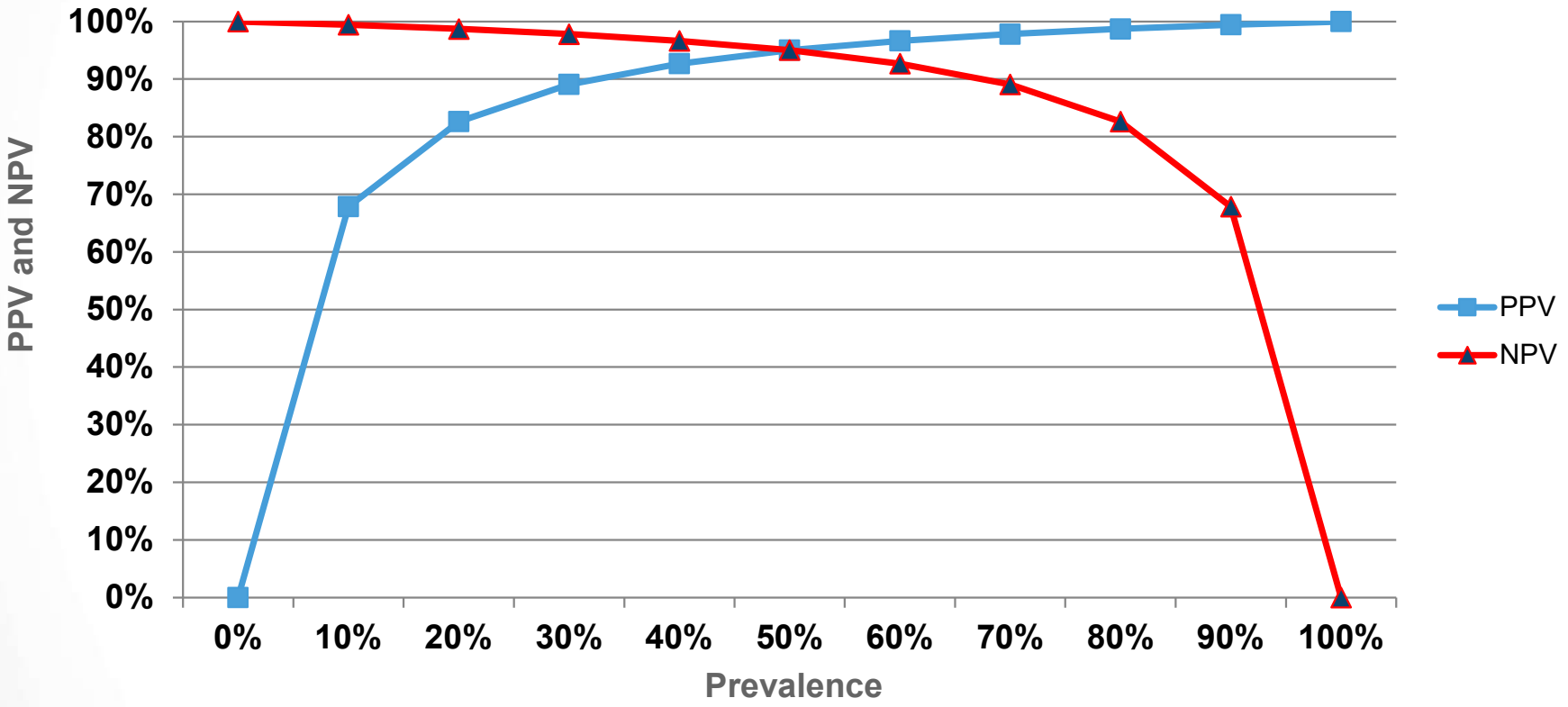
PPV & Prevalence

- Prevalence directly impacts PPV and NPV
- The Lower the prevalence for a disease , the lower the PPV for the test and the higher the NPV

- For the Exercise: Use established clinical performances within different prevalence scenarios and calculate the PPV and NPV

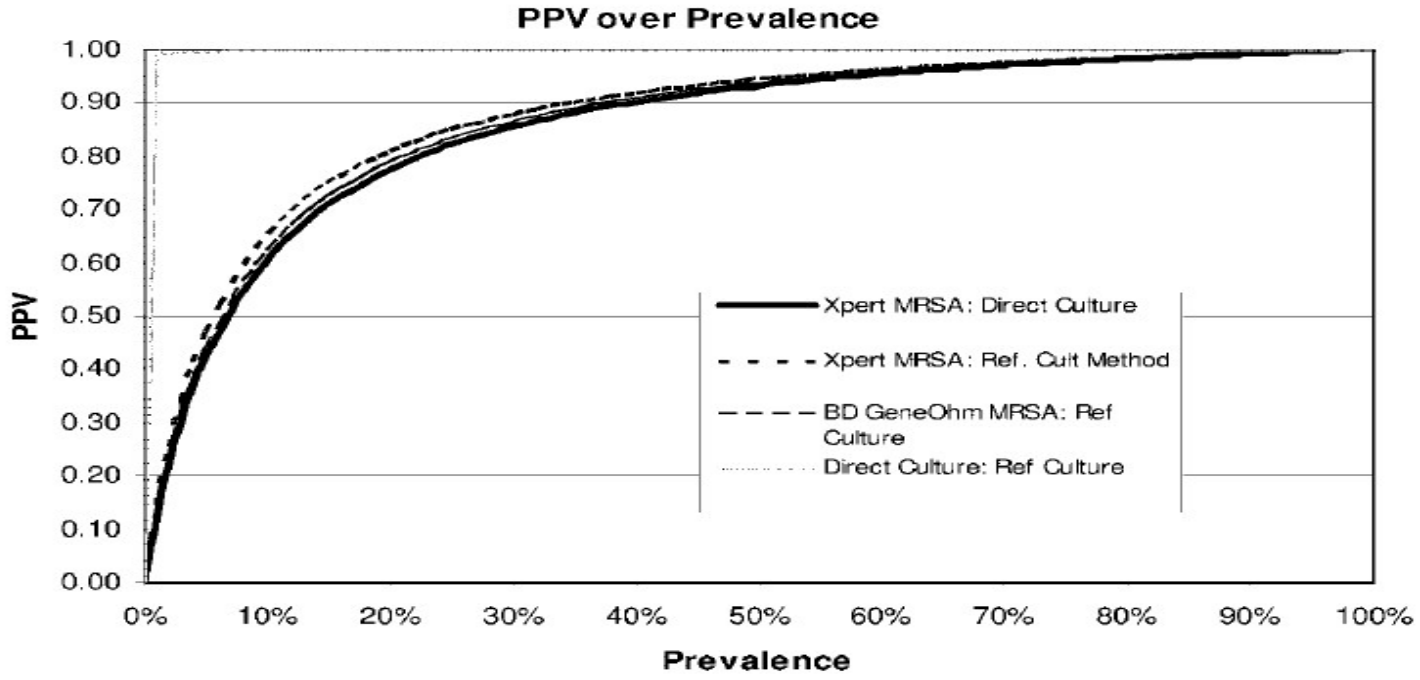
Impact of Prevalence on PPV and NPV

PPV and NPV over the Prevalence



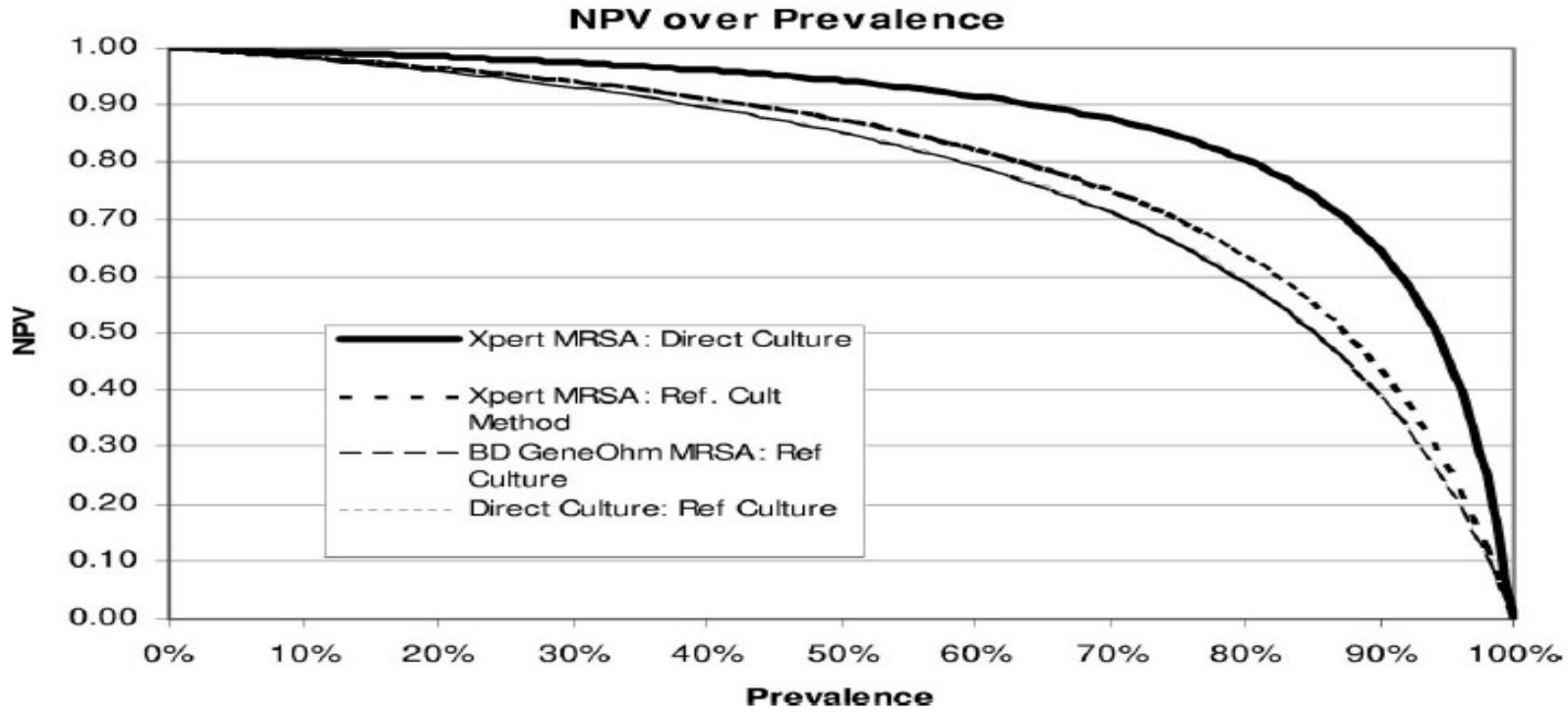
Goarant, C., Bourhy, P., d'Ortenzio, E., Dartevelle, S., Mauron, C., Soupé-Gilbert, M.E., Bruyère-Ostells, L., Gourinat, A.C., Picardeau, M., Nato, F. and Chanteau, S., 2013. Sensitivity and specificity of a new vertical flow rapid diagnostic test for the serodiagnosis of human leptospirosis. PLoS Negl Trop Dis, 7(6), p.e2289.

PPV over the Prevalence



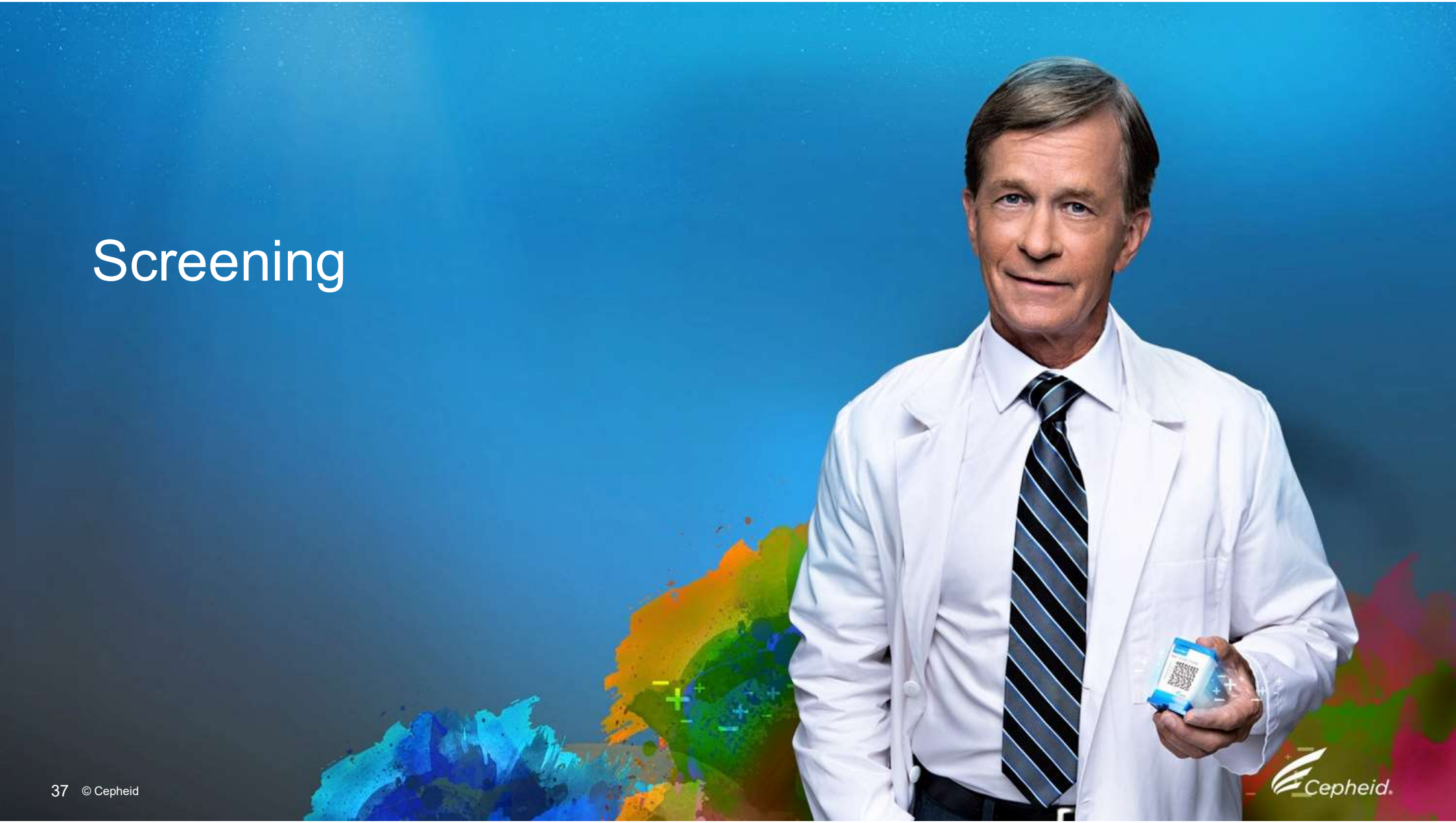
Multicenter Evaluation of the Cepheid Xpert Methicillin-Resistant Staphylococcus aureus (MRSA) Test as a Rapid Screening Method for Detection of MRSA in Nares
D. M. Wolk, E. Picton,1 D. Johnson, T. Davis, P. Pancholi, C. C. Ginocchio, S. Finegold, D. F. Welch, M. de Boer, D. Fuller, M. C. Solomon, B. Rogers, M. S. Mehta, and L. R. Peterson JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2009, p. 758–764

NPV over the Prevalence



Multicenter Evaluation of the Cepheid Xpert Methicillin-Resistant Staphylococcus aureus (MRSA) Test as a Rapid Screening Method for Detection of MRSA in Nares
D. M. Wolk, E. Picton,1 D. Johnson, T. Davis, P. Pancholi, C. C. Ginocchio, S. Finegold, D. F. Welch, M. de Boer, D. Fuller, M. C. Solomon, B. Rogers, M. S. Mehta, and L. R. Peterson JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2009, p. 758–764

Screening



Screening

- Screening: is the process by which a patient without symptoms is identified
  - Screening for colonization is the most effective strategy in reducing prevalence and preventing infection. e.g., MRSA, VRE, CRE

- Positive Result impact:
  - Decolonization: to reduce their risk of infection occurring in themselves
  - Isolation: to prevent the spread of the bacteria to other vulnerable patients

- Negative Result impact:
  - No action taken

Screening test: example with VRE

- What is the impact of inaccurate results?

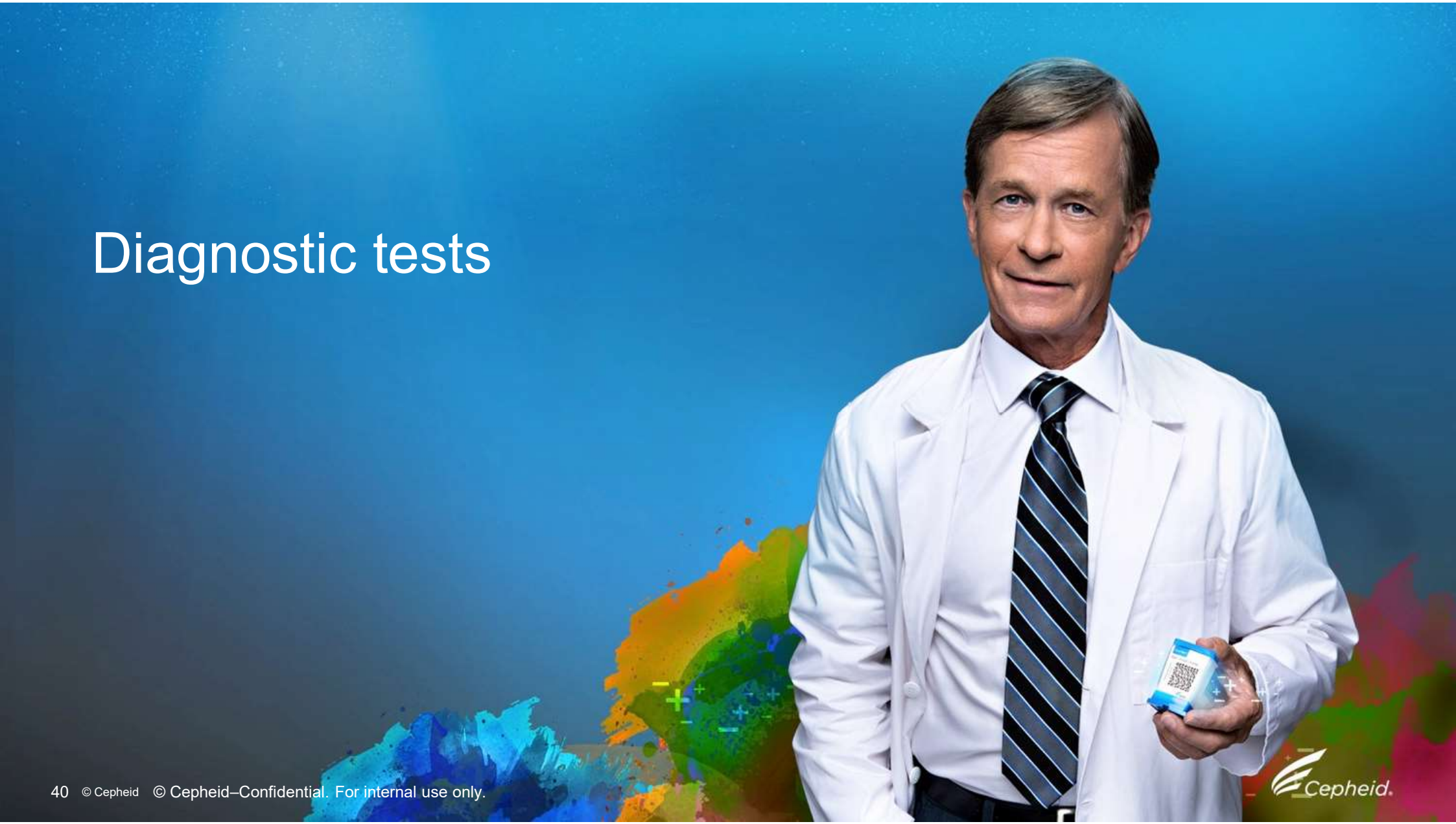
  – False Positive Result: Unnecessary decolonization or isolation from the ward

  – False Negative Result: Risk of self-infection or spread of the bacteria to other vulnerable patients

- NPV is the most important for physicians:
  – Confidence in the negative result
  – Patient with a negative test result is truly Negative

Cepheid.

Diagnostic tests



Diagnostic test

- Diagnostic testing: is the process by which patient with symptoms who are infected are identified e.g., bacterial or viral infections
- Detection and identification in order to initiate appropriate effective treatment
- Positive Result Impact: appropriate antibiotic is initiated, or empirical therapy is stopped
- Negative Result Impact: empirical therapy is not stopped, and investigations continue to identify the cause of infection

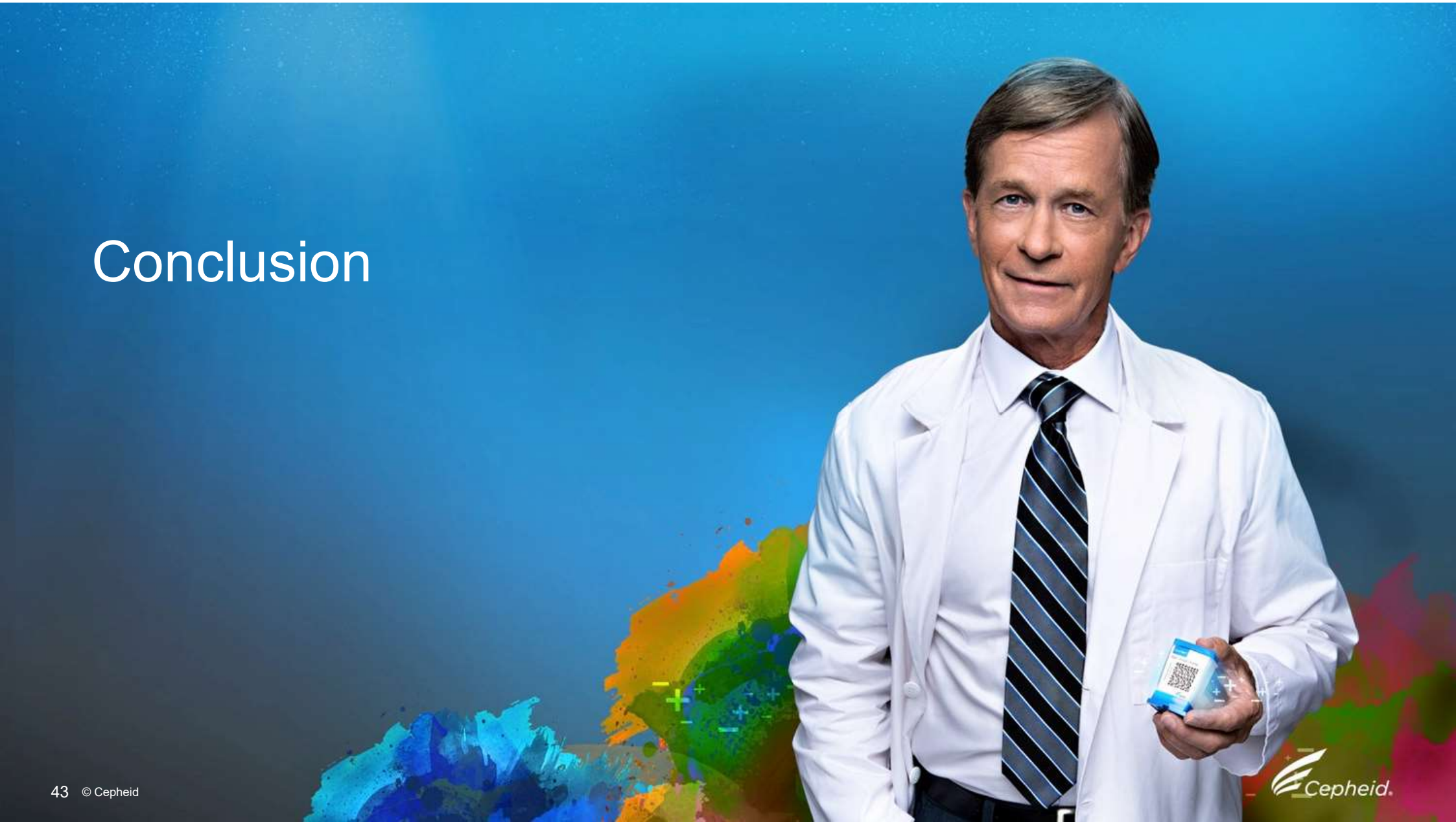
Diagnostic test: example with Tuberculosis

- What is the impact of inaccurate results?
- False Positive Result: inappropriate antibiotic is initiated which can have severe side effects on the patient, antibiotic stewardship and antimicrobial resistance.

- False Negative Result: targeted therapy is not initiated, and patient outcome does not improve, patient goes back in the community and can potentially infect others.

- PPV is the most important for physicians:
  - Confidence in the positive result
  - Patient with a positive test result is truly Positive

Conclusion



Summary

- Sensitivity represents the proportion of patients with a positive test result in the positive patient's population
- Specificity represents the proportion of patients with a negative test result in the negative patient's population
- Performance of a new test is evaluated against a reference method
- The newly evaluated test performance may be affected by the reference method performance

Conclusion

- PPV is the proportion of patients with a Positive test result who are truly **Positive**
- NPV is the proportion of patients with a Negative test result who are truly **Negative**
- PPV depends on the prevalence
- Microbiologists in the laboratory - Analytical Sensitivity and Specificity.
- Physicians - PPV and NPV.

Conclusion

- For PPV and NPV always consider the prevalence

- NPV is the most important for screening test
  - Xpert MRSA NxG : 98.9% (Prevalence 11%)
  - Xpert VanA/VanB : 98.3% (Prevalence 14%)

- PPV is the most important for diagnostic tests
  - Xpert MRSA/SA BC : 99.6% (SA) and 98.1% (MRSA) (Prevalence 30% and 13% respectively)
  - Xpert MTB/RIF : 99.1% (Prevalence 47%)

Exercise 1

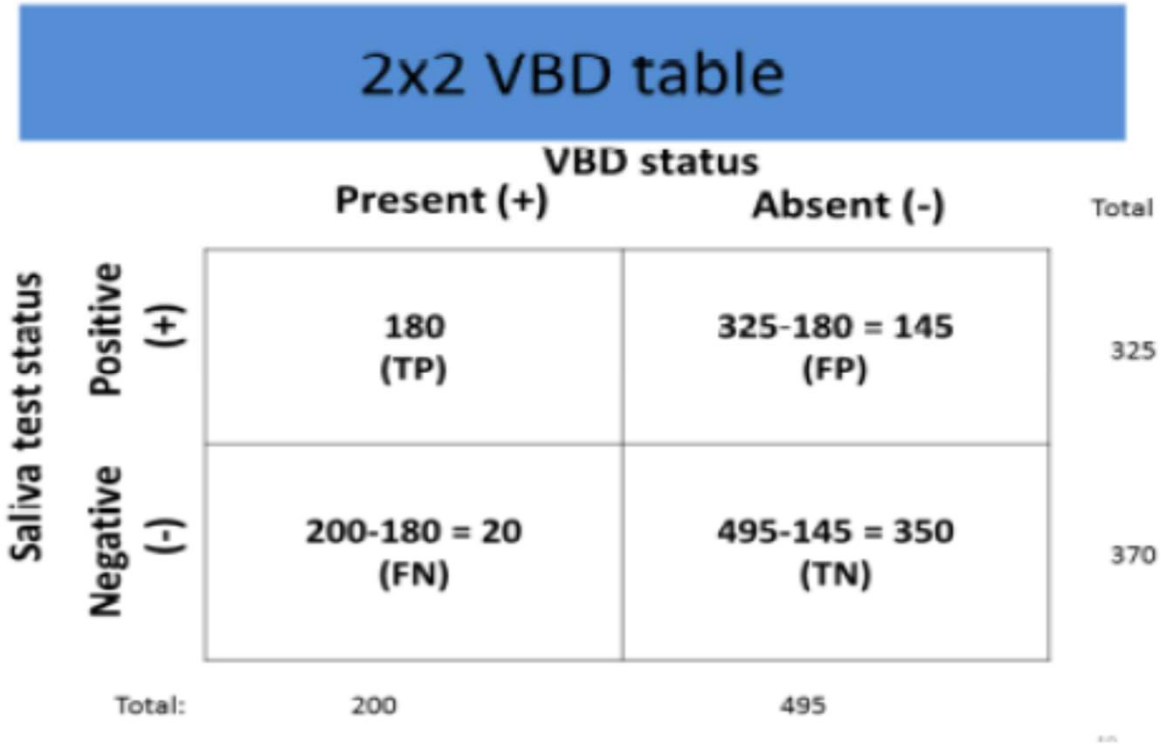
Calculating the sensitivity and specificity

Exercise

- A new saliva test has been developed to identify cases of VBD (very bad disease).
- The saliva test was given to 200 people with VBD and 495 people without VBD (according to the gold standard) .
- The tests were read and identified a total of 325 were positive. Of those, 180 were from people with VBD.

1. Calculate the sensitivity of the VBD saliva test.
2. Calculate the specificity of the VBD saliva test.

Answer

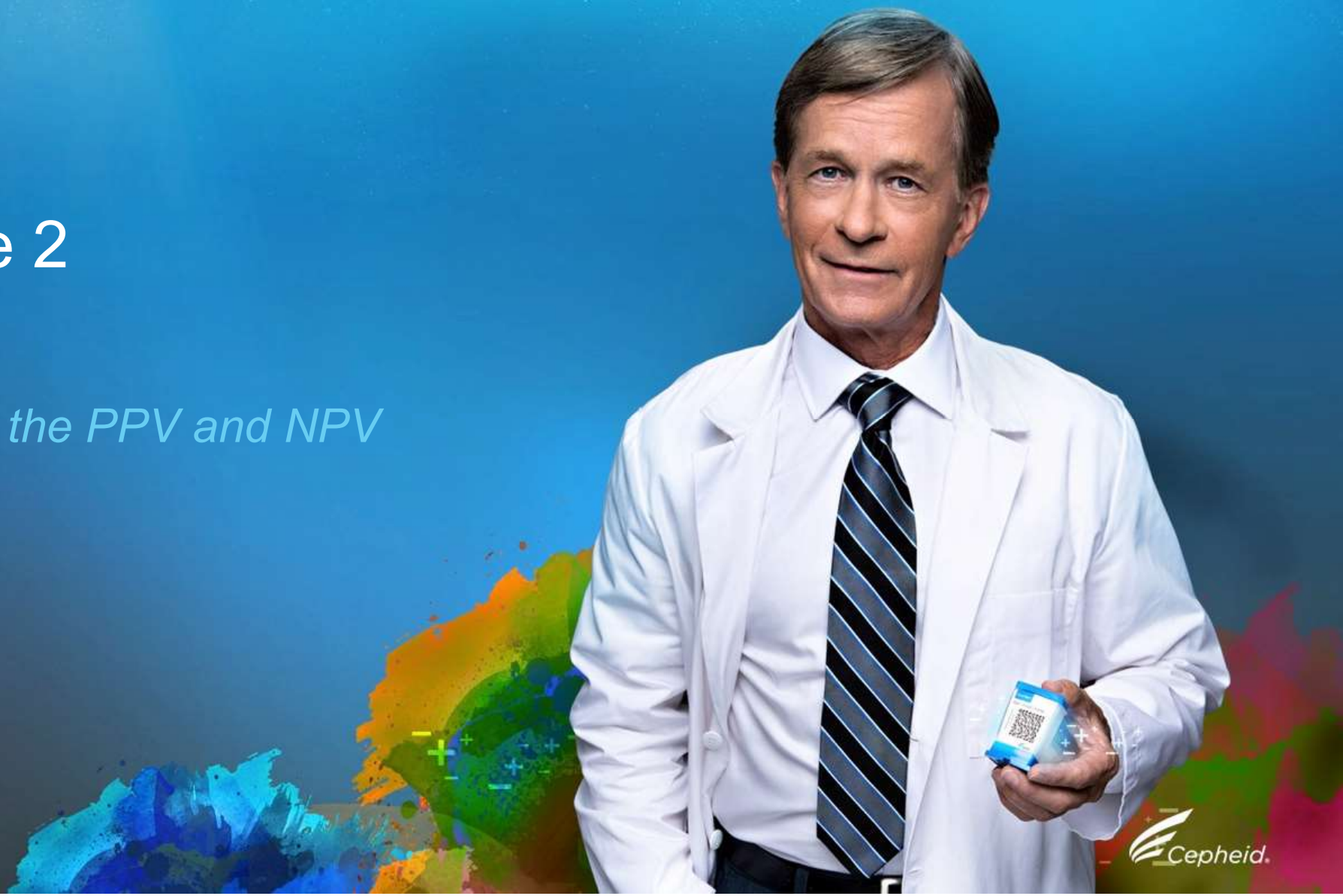


Sensitivity = TP / (TP+FN) = 180 / (180 +20) = 0.90 or 90%

Specificity = TN / (TN + FP) = 350/ (350 + 145) = 0.71 or 71%

Exercise 2

Calculating the PPV and NPV



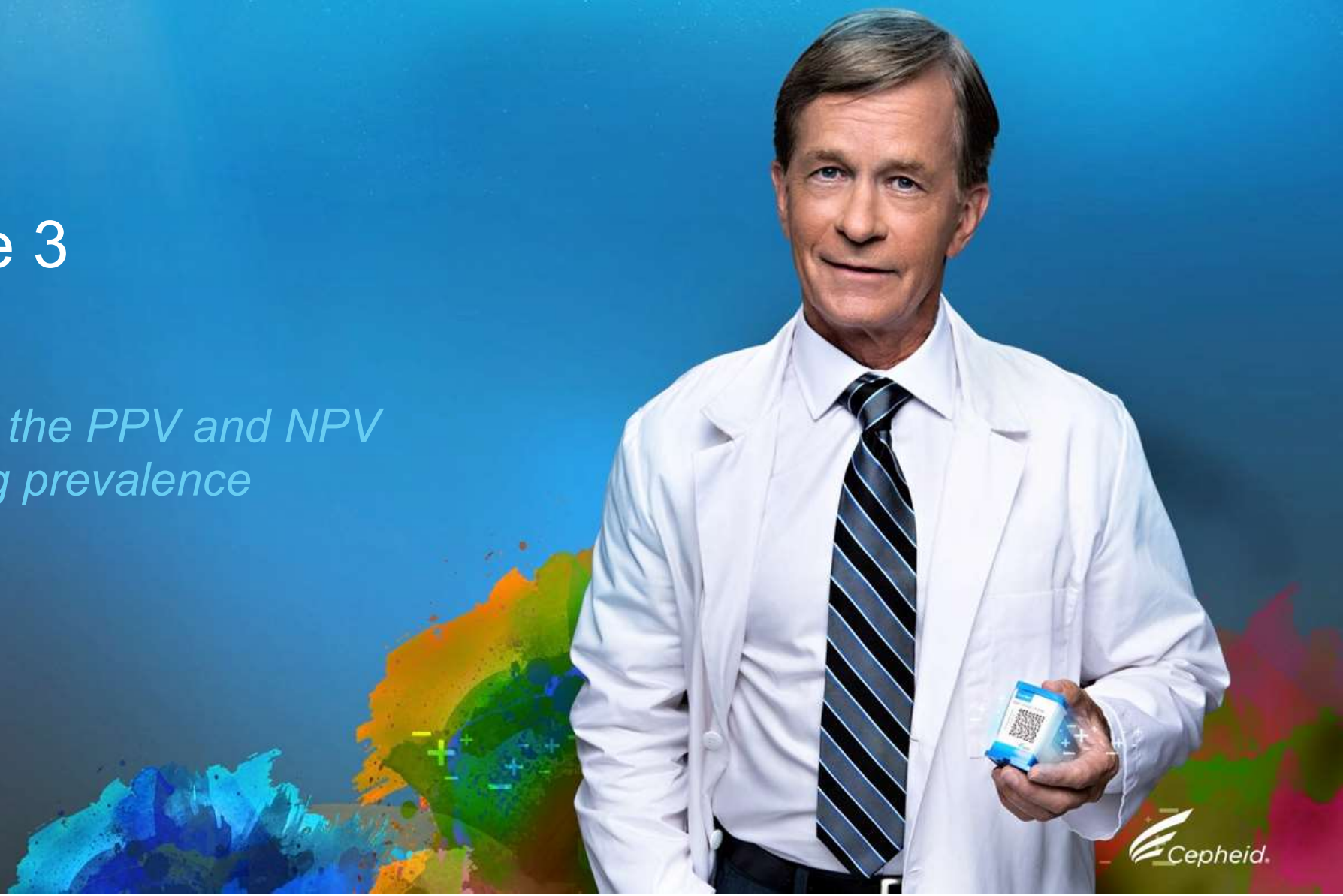
Exercise

- A new saliva test has been developed to identify cases of VBD (very bad disease).
- The saliva test was given to 200 people with VBD (according to the gold standard) and 495 people without VBD.
- The tests were read and identified a total of 325 were positive. Of those, 180 were from people with VBD.

1. Calculate the PPV of the VBD saliva test.
2. Calculate the NPV of the VBD saliva test.

Exercise 3

Calculating the PPV and NPV with varying prevalence



Exercise

- A New Test with established clinical performances for VBD is about to be adopted : Sensitivity : 96% and Specificity : 96%
- Prevalence of VBD in this population is 1% and 1000 patients will be tested with this new test (Tip: prevalence is 10/1000)

1. Determine the 2x2 table
2. Calculate the PPV test.
3. Calculate the NPV test.

Answer

- Prevalence is 10/1000 :
  1. 10 patients have the disease and 990 don't have the disease
  2. Number of patients that Test Positive = True Positive x Sensitivity (10x0.96 = 9.6 round up to 10)
  3. Number of patients that Test Negative = True Negative x Specificity (990x0.96 = 950.4 round up to 950)

		Disease Status		
		Pos	**Neg**	**Total**
New Test	**Pos**	10	40	50
	Neg	0	950	950
	Total	10	990	1000

PPV = 10/50 = 20%
NPV = 950/950 = 100%

Exercise

- The same test with established clinical performances for VBD is about to be adopted in another setting : Sensitivity : 96% and Specificity : 96%
- Prevalence of VBD in this population is 15% and 1000 patients will be tested with this new test (Tip: prevalence is 150/1000)

1. Determine the 2x2 table
2. Calculate the PPV test.
3. Calculate the NPV test.

Answer

- Prevalence is 150/1000 :
  1. 150 patients has the disease and 850 don't have the disease
  2. Number of patients that Test Pos= True Positive x Sensitivity (150x0.96 = 144)
  3. Number of patients that Test Neg= True Negative x Specificity (850x0.96 = 816)

		Disease Status		
		Pos	**Neg**	**Total**
New Test	**Pos**	144	34	178
	Neg	6	816	822
	Total	150	850	1000

PPV = 144/178 = 81%
NPV = 816/822 = 99.3%

Exercise

- The same test with established clinical performances for VBD is about to be adopted in another setting : Sensitivity : 98% and Specificity : 96%
- Prevalence of VBD in this population is 15% and 1000 patients will be tested with this new test (Tip: prevalence is 150/1000)

1. Determine the 2x2 table
2. Calculate the PPV test.
3. Calculate the NPV test.

Answer

- Prevalence is 150/1000 :
  1. 150 patients has the disease and 850 don't have the disease
  2. Number of patients that Test Pos = True Positive x Sensitivity (150x0.96 = 144)
  3. Number of patients that Test Neg = True Negative x Specificity (850x0.96 = 816)

		Disease Status		
		Pos	**Neg**	**Total**
New Test	**Pos**	148	34	182
	Neg	2	816	818
	Total	150	850	1000

PPV = 148/182 = 81%
NPV = 816/818 = 99.7%


Thank You.
www.Cepheid.com
GeneXpert
Xpert
Cepheid
Cepheid