Diagnosis of vaccine preventable diseases

Mark Nicol

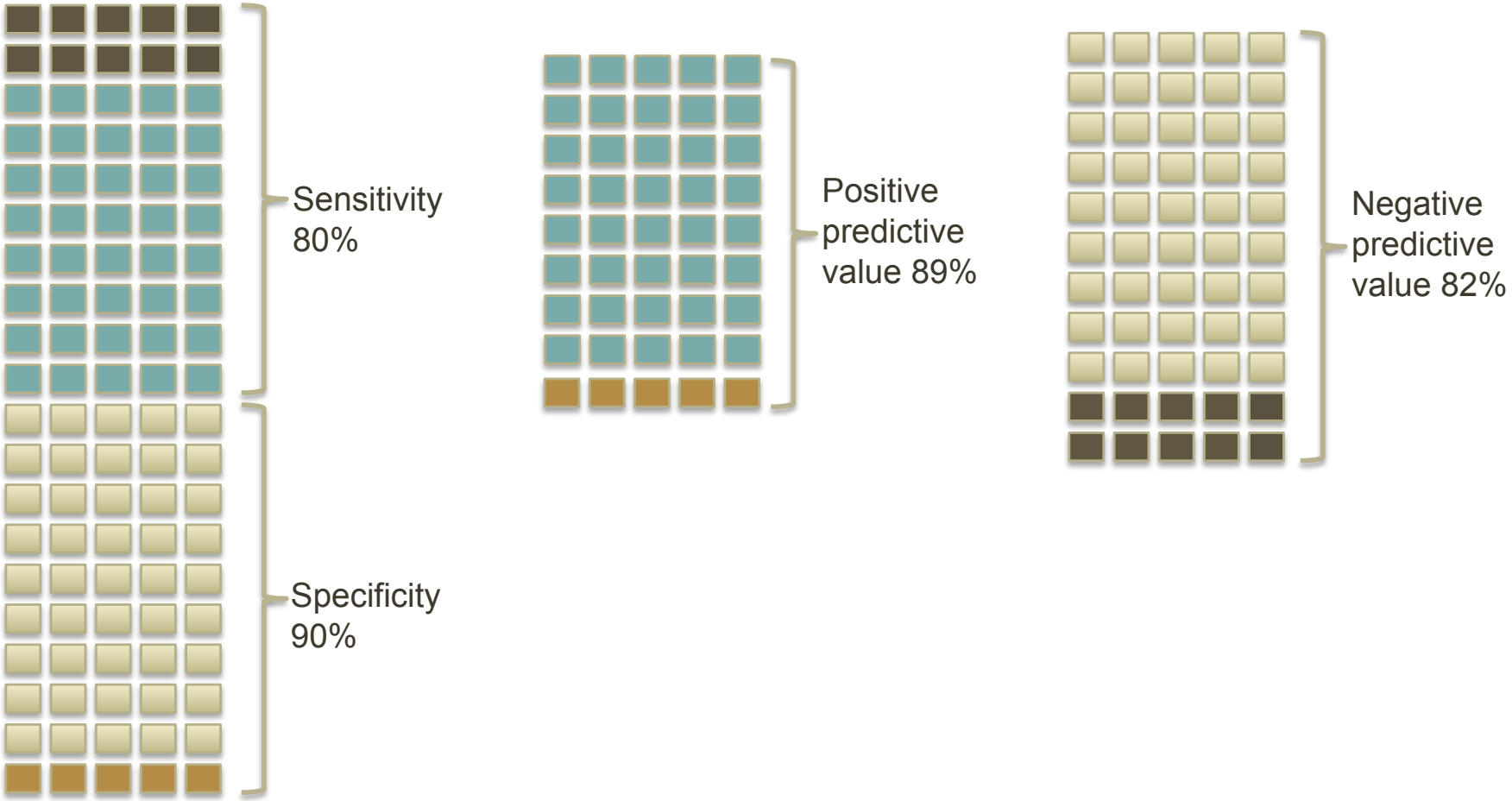
Division of Medical Microbiology and
Institute for Infectious Diseases and Molecular Medicine,
University of Cape Town and National Health Laboratory Service

Definitions…some reminders

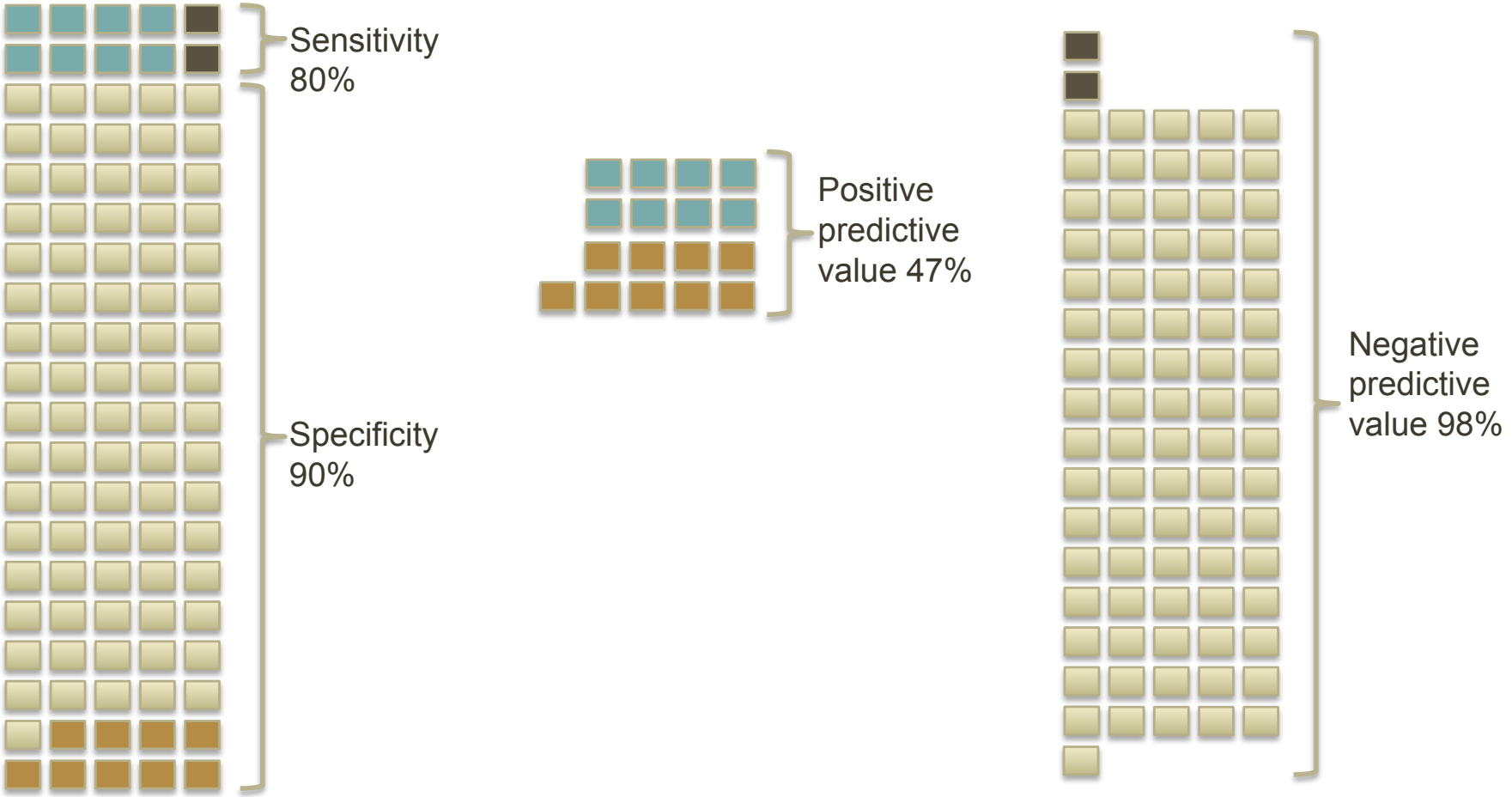
- Sensitivity
  - The proportion of those who have the disease who test positive
    - True test positives/Total with disease
- Specificity
  - The proportion of those without the disease who test negative
    - True test negatives/Total without disease
- Positive Predictive Value
  - The proportion of those with a positive test, who actually have the disease
    - True test positives/(True test positives + False test positives)
- Negative Predictive Value
  - The proportion of those with a negative test who actually don't have the disease
    - True test negatives/(True test negatives + False test negatives)

		Disease present		
		Yes	No	
Test results	Positive	TP	FP	PPV TP/TP +FP
	Negative	FN	TN	NPV TN/TN +FN
		Sensitivity TP/TP +FN	Specificity TN/TN +FP	

Sensitivity, specificity, PPV, NPV: prevalence 50%

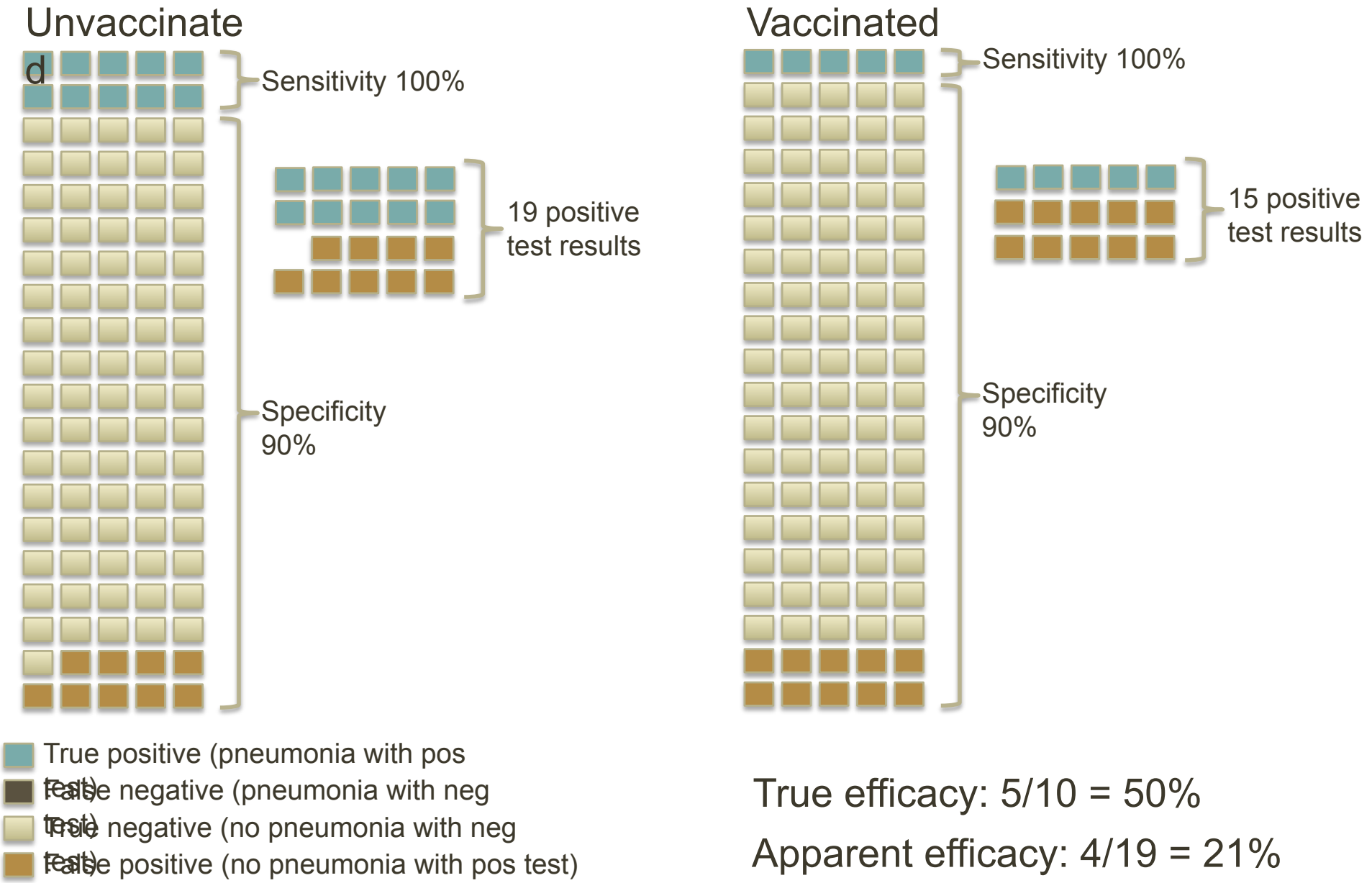


Sensitivity, specificity, PPV, NPV: prevalence 10%



So what is more important for vaccine studies: high sensitivity or high specificity?

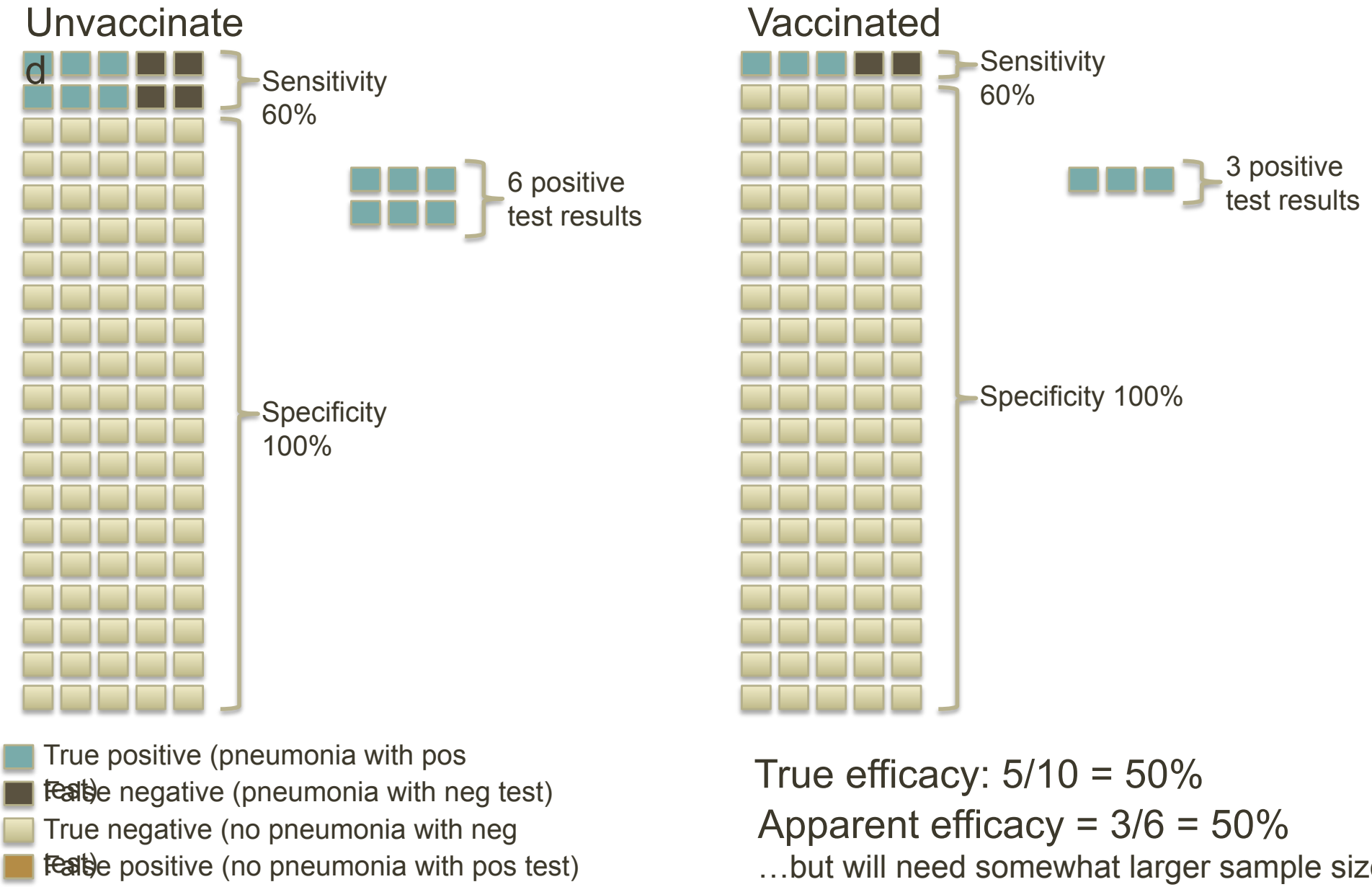
Specificity is important as prevalence is typically low for vaccine studies



True efficacy: 5/10 = 50%

Apparent efficacy: 4/19 = 21%

Sensitivity matters less…



True efficacy: 5/10 = 50%

Apparent efficacy = 3/6 = 50%

…but will need somewhat larger sample size

Microbiological diagnostics as vaccine trial endpoints

- Microbiological diagnosis as a reference standard
  - Advantages of microbiological endpoints
    - Improved specificity vs. clinical diagnosis
    - Ability to serotype/genotype strains
  - Disadvantages of microbiological endpoints
    - May be less sensitive than clinical diagnosis
    - May lack specificity
      - Colonization vs. infection
- Examples
  - tuberculosis
  - childhood pneumonia

Diagnostics for childhood tuberculosis: fumbling in the dark…

- Quandaries in diagnosing TB in children
  - Based on clinical diagnosis, we think that
    - culture is a poor reference standard (20-50%)
    - microscopy is infrequently helpful (<10%)
  - However, clinical diagnosis is probably even worse
    - chest radiography interpretation is variable
    - clinical scoring systems seldom concur
- Problem when evaluating novel diagnostics
- Problem for vaccine trials

Interpretation of CXR is highly inconsistent

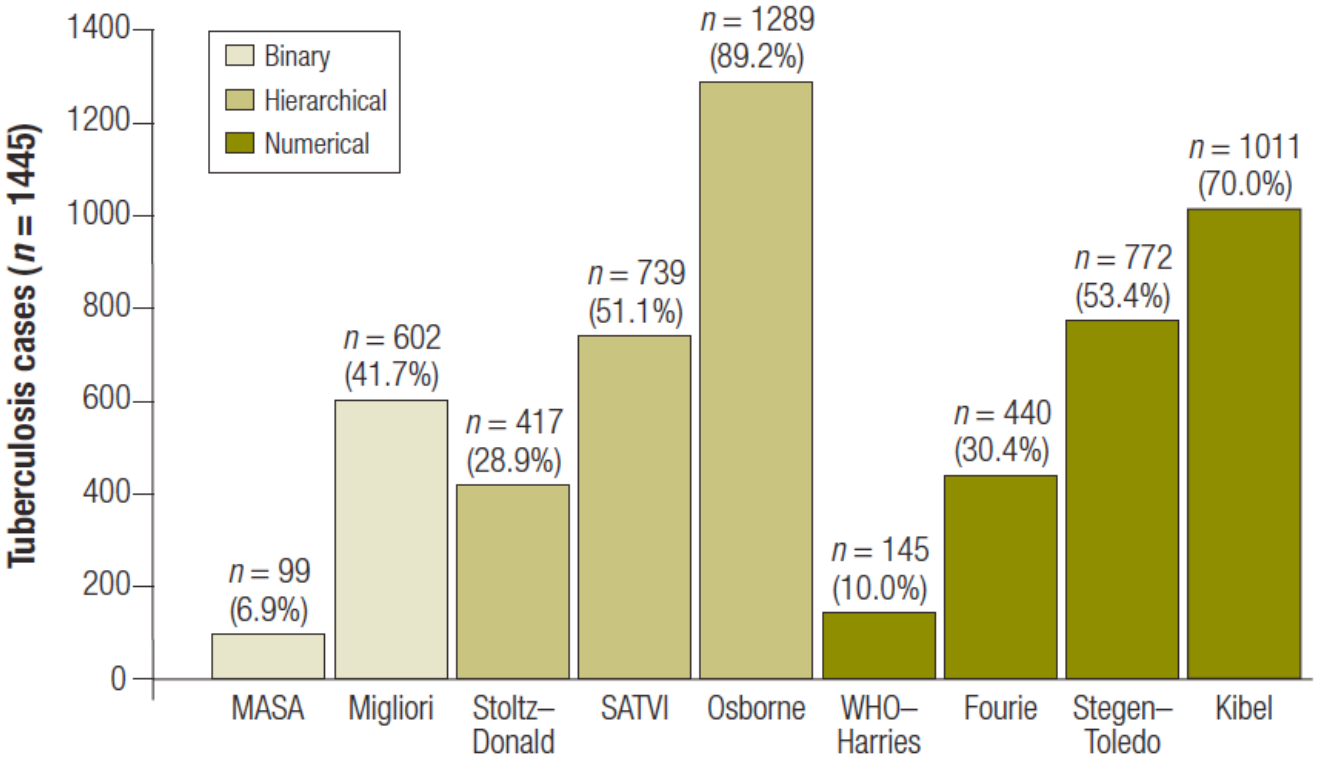
Table 1. **Results of chest radiograph assessment by three independent paediatric reviewers, grouped by certainty of tuberculosis diagnosis, South Africa, 2001–2006**

Diagnostic certainty[a]	Reviewer 1		Reviewer 2		Reviewer 3		Final classification	
	No.	%	No.	%	No.	%	No.	%
Highly likely to have tuberculosis	16	1.1	29	2.0	171	11.8		
Likely to have tuberculosis	20	1.4	38	2.6	323	22.4		
Suspected of having tuberculosis	124	8.6	145	10.0	242	16.7		
Positive	**160**	**11.1**	**212**	**14.6**	**736**	**50.9**	**271**	**18.8**
Inconclusive	45	3.1	35	2.4	82	5.7		
Abnormal but not tuberculosis	102	7.1	139	9.6	312	21.6		
Normal	1038	71.8	778	53.9	59	4.1		
Negative	**1185**	**82.0**	**952**	**65.9**	**453**	**31.4**	**1174**	**81.2**
Not read	100	6.9	281	19.5	256	17.7		
Total	**1445**	**100**	**1445**	**100**	**1445**	**100**	**1445**	**100**

Hatherill M et al. Bull World Health Organ 2010;88:312-320

Structured scoring systems?

Fig. 1. **Frequency of cases classified as tuberculosis with various scoring systems, with hierarchical and numerical outcomes condensed to a binary "tuberculosis/not tuberculosis" output , South Africa, 2001–2006**

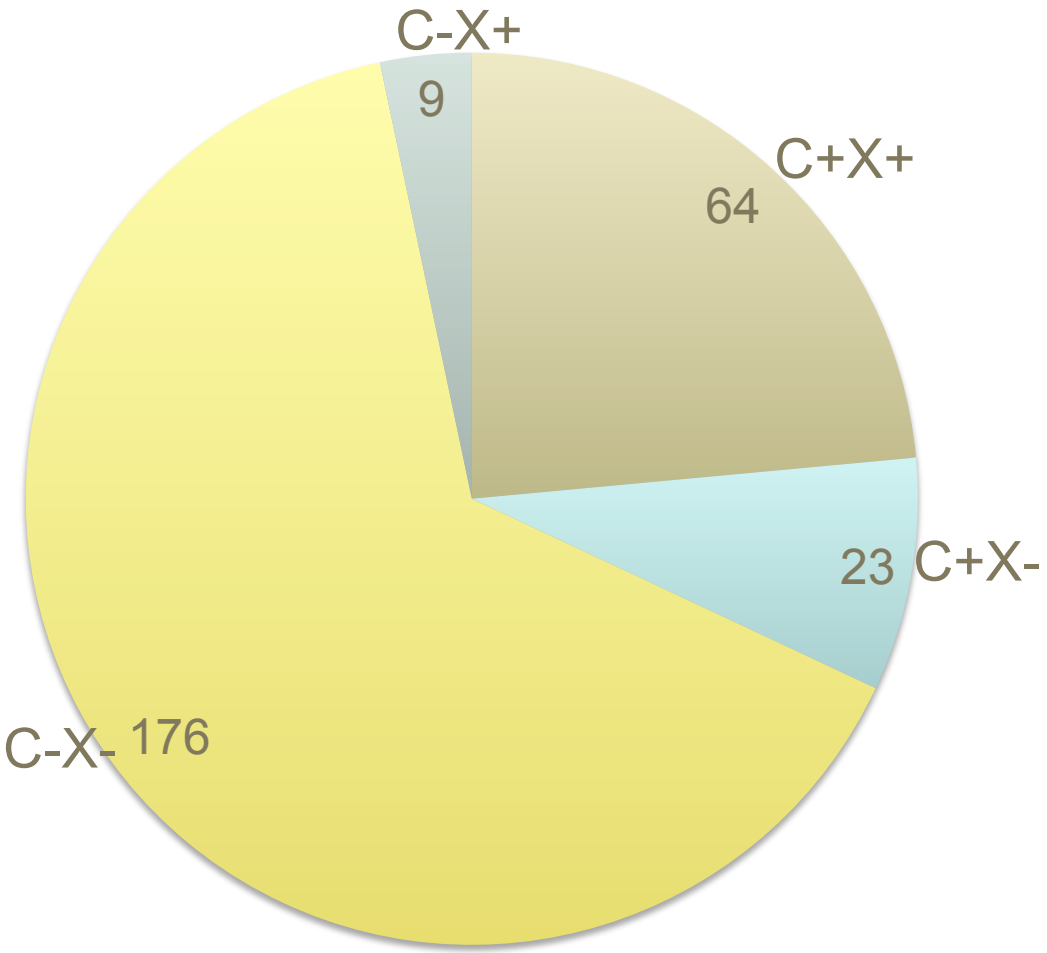


MASA, Medical Association of South Africa; SATVI, South African Tuberculosis Vaccine Initiative; WHO, World Health Organization.

Hatherill M et al. Bull World Health Organ 2010;88:312-320

Does microbiology solve the problem?

Culture and Xpert results in children started on TB treatment
n=272



27% Xpert positive

32% Culture positive

65% Both negative

Difference in yield of Xpert vs. culture = 14 cases (5%)

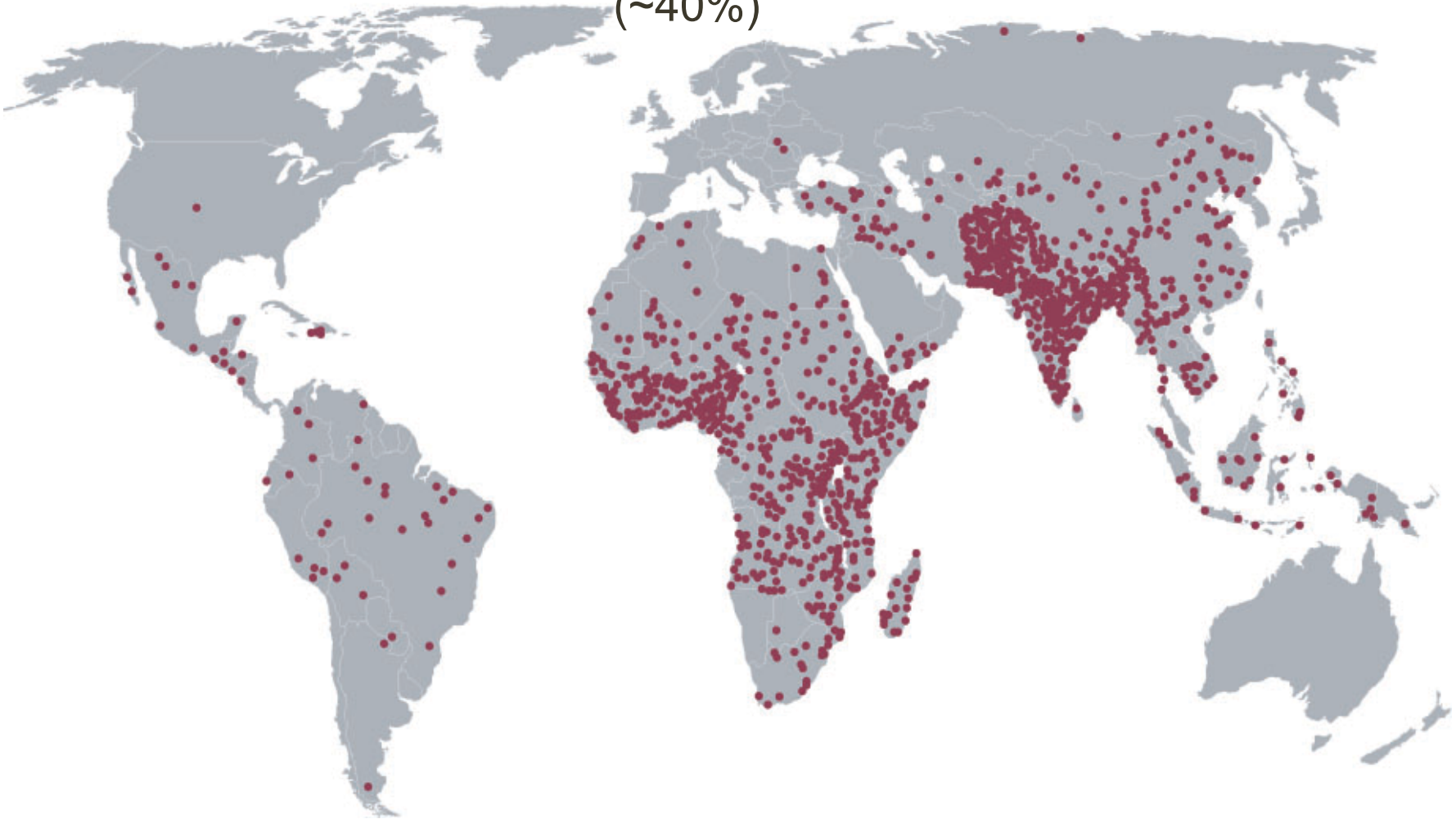
Zar, Nicol. Unpublished

Diagnostic uncertainty for childhood TB: implications for vaccine studies

- Lack of sensitive, specific case definition
- If use microbiology to define cases
  - High specificity, low sensitivity
  - Will require very large sample sizes: not feasible
- If use a combined case definition: clinical/ radiological plus microbiological
  - Higher sensitivity, low specificity
  - Will result in false low estimates of vaccine efficacy: potentially rejecting promising vaccines

Nearly 70% of child pneumonia deaths occur in Africa & South Asia

Pneumococcus is the leading cause of child pneumonia deaths (~40%)



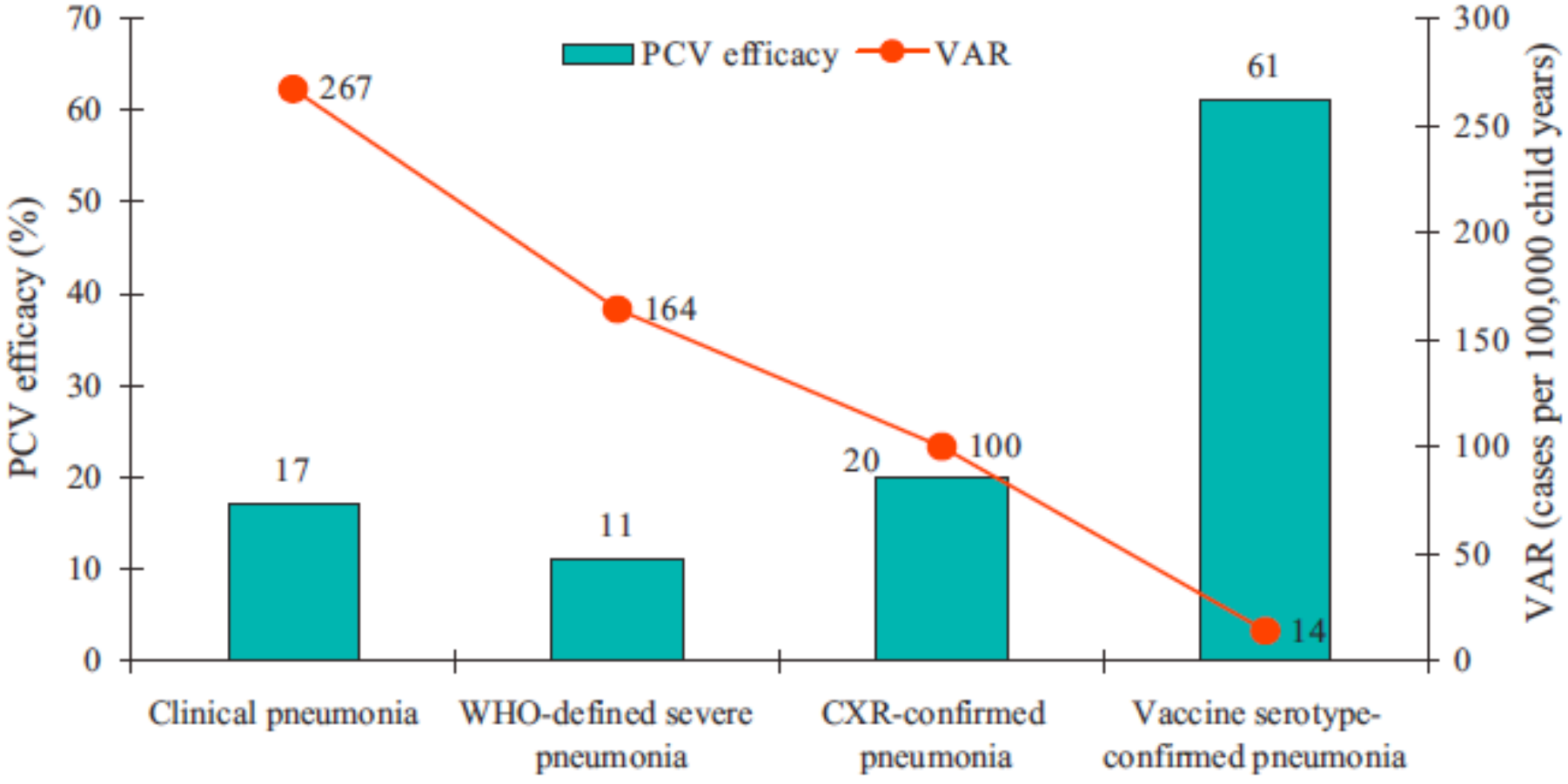
Each dot represents 1000 deaths (Williams BG et al Lancet 2002)

Slide courtesy Martin Antonio

Diagnostics for pneumococcal disease

- Estimates of vaccine efficacy depend on ability to detect:
  - Disease: invasive pneumococcal disease (bacteraemia, meningitis), pneumonia, (otitis media)
  - Colonization: nasopharyngeal (interrupt transmission)

Pneumococcal vaccines: protection against pneumonia in children

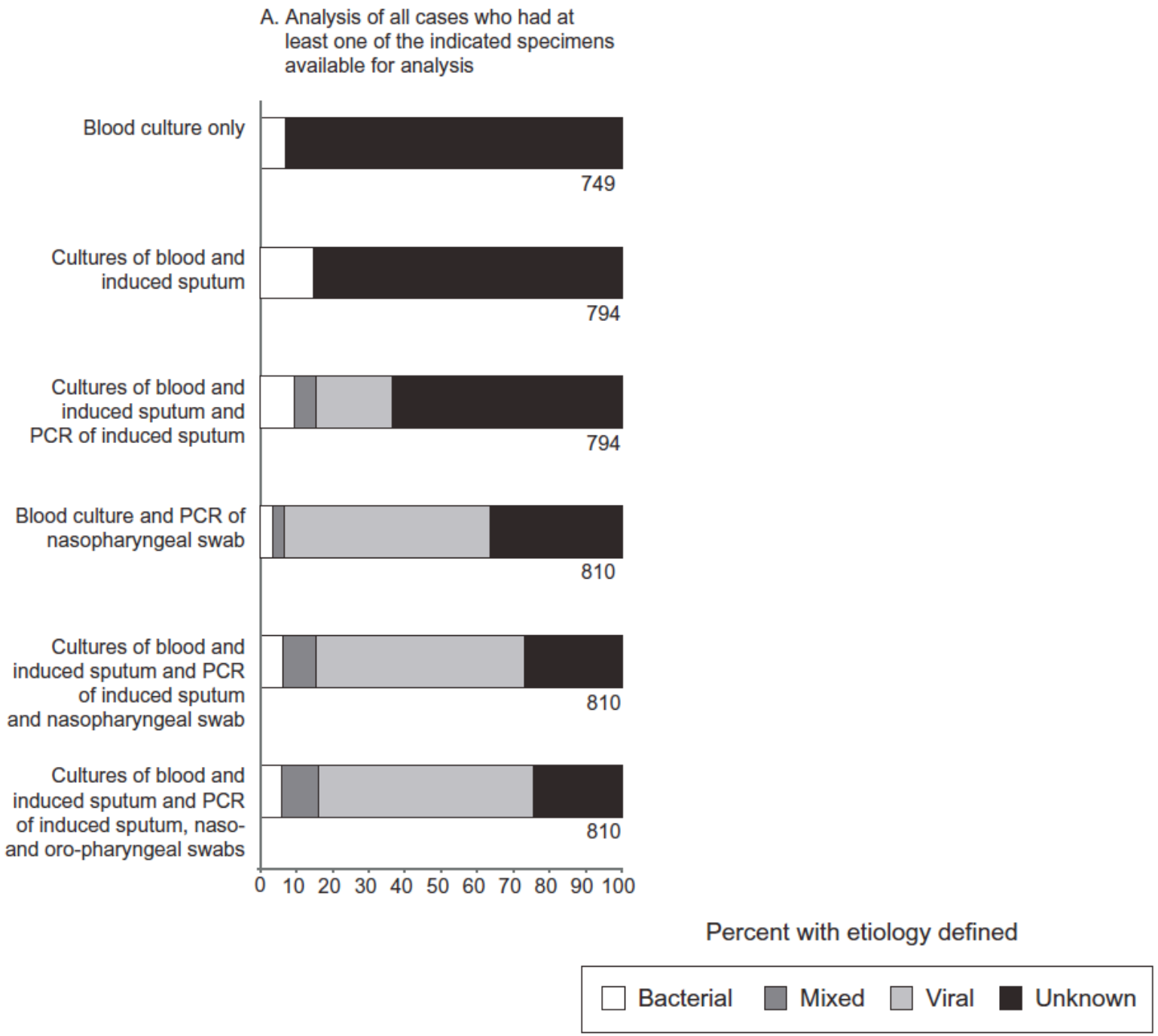


PCV=pneumococcal conjugate vaccine; WHO=World Health Organization; CXR=chest X-ray

Von Gottberg et al. Vaccine 2012

Methods for determining aetiology of pneumonia

- Microscopy and culture of respiratory tract secretions
  - Poor sensitivity of blood culture for pneumonia
    - Only small proportion of patients with pneumococcal pneumonia are bacteraemic (5%)
  - Poor specificity of sputum culture for pneumonia
    - Need to distinguish colonization vs. infection
- Antigen detection
  - C-polysaccharide in urine
  - Adults: sens 70%, spec >90%
  - Children: positive in 22-67% of carriers
- Serology
- Nucleic acid detection on respiratory samples
  - Singleplex (*lytA* for pneumococcus)
  - Multiplex panels
  - Same problem with specificity as culture



Hammitt LL et al. CID 2012:54 (Suppl 2)

Table 5. Detection of Respiratory Pathogens by Multiplex PCR of Nasopharyngeal Swab Specimens in Case Patients Aged 1–59 Months With Severe or Very Severe Pneumonia and in Controls

	Controls Without URTI (n = 142)		Controls With URTI (n = 227)		All Controls (n = 369)		Case Patients (n = 805)		aOR for Case Patients vs Controls Without URTI		aOR for Case Patients vs All Controls	
Pathogen	No.	%	No.	%	No.	%	No.	%	aOR	95% CI	aOR	95% CI
RSV A	2	1.4	14	6.2	16	4.3	136	16.9	**12.5**	3.1–51.5	**3.8**	2.2–6.6
RSV B	0	0.0	3	1.3	3	0.8	77	9.6	∞	<.001[a]	**11.9**	3.7–38.2
Adenovirus	15	10.6	13	5.7	28	7.6	39	4.8	0.5	.3–1.0	0.7	.4–1.2
Rhinovirus	32	22.5	50	22.0	82	22.2	184	22.9	1.0	.6–1.5	1.0	.7–1.3
Parainfluenza 1	1	0.7	4	1.8	5	1.4	9	1.1	1.4	.2–11.6	0.9	.3–2.7
Parainfluenza 2	1	0.7	7	3.1	8	2.2	5	0.6	0.8	.1–7.1	**0.3**	.1–.8
Parainfluenza 3	6	4.2	16	7.1	22	6.0	47	5.8	1.3	.6–3.2	0.9	.5–1.6
Parainfluenza 4	2	1.4	2	0.9	4	1.1	11	1.4	1.1	.2–5.0	1.4	.4–4.5
Influenza A	1	0.7	4	1.8	5	1.4	7	0.9	1.4	.2–11.4	0.7	.2–2.2
Influenza B	0	0.0	0	0.0	0	0.0	2	0.3	∞	1.0[a]	∞	1.0[a]
Influenza C	0	0.0	2	0.9	2	0.5	3	0.4	∞	1.0[a]	0.8	.1–4.8
Coronavirus 229E	5	3.5	10	4.4	15	4.1	17	2.1	0.7	.3–1.9	0.6	.3–1.1
Coronavirus OC43	2	1.4	9	4.0	11	3.0	22	2.7	2.0	.5–8.8	1.0	.5–2.1
Coronavirus NL63	0	0.0	2	0.9	2	0.5	4	0.5	∞	1.0[a]	1.0	.2–5.4
HMPV	1	0.7	3	1.3	4	1.1	25	3.1	4.6	.6–34.4	2.8	.9–8.1
Mycoplasma pneumoniae	2	1.4	2	0.9	4	1.1	3	0.4	0.3	.1–1.9	0.5	.1–2.1
Any pathogen	59	41.6	116	51.1	175	47.4	489	60.8	…	…	…	…
Any virus	57	40.1	115	50.7	172	46.6	486	60.4	…	…	…	…

Values in bold indicate significant differences at the $P < .05$ level; ∞ indicates OR equal to infinity.

Abbreviations: aOR, adjusted odds ratio (OR) (adjusted for age and season); CI, confidence interval; HMPV, human metapneumovirus; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; URTI, upper respiratory tract infection.

[a] *P* value for case patients vs controls (Fisher's exact *t* test).

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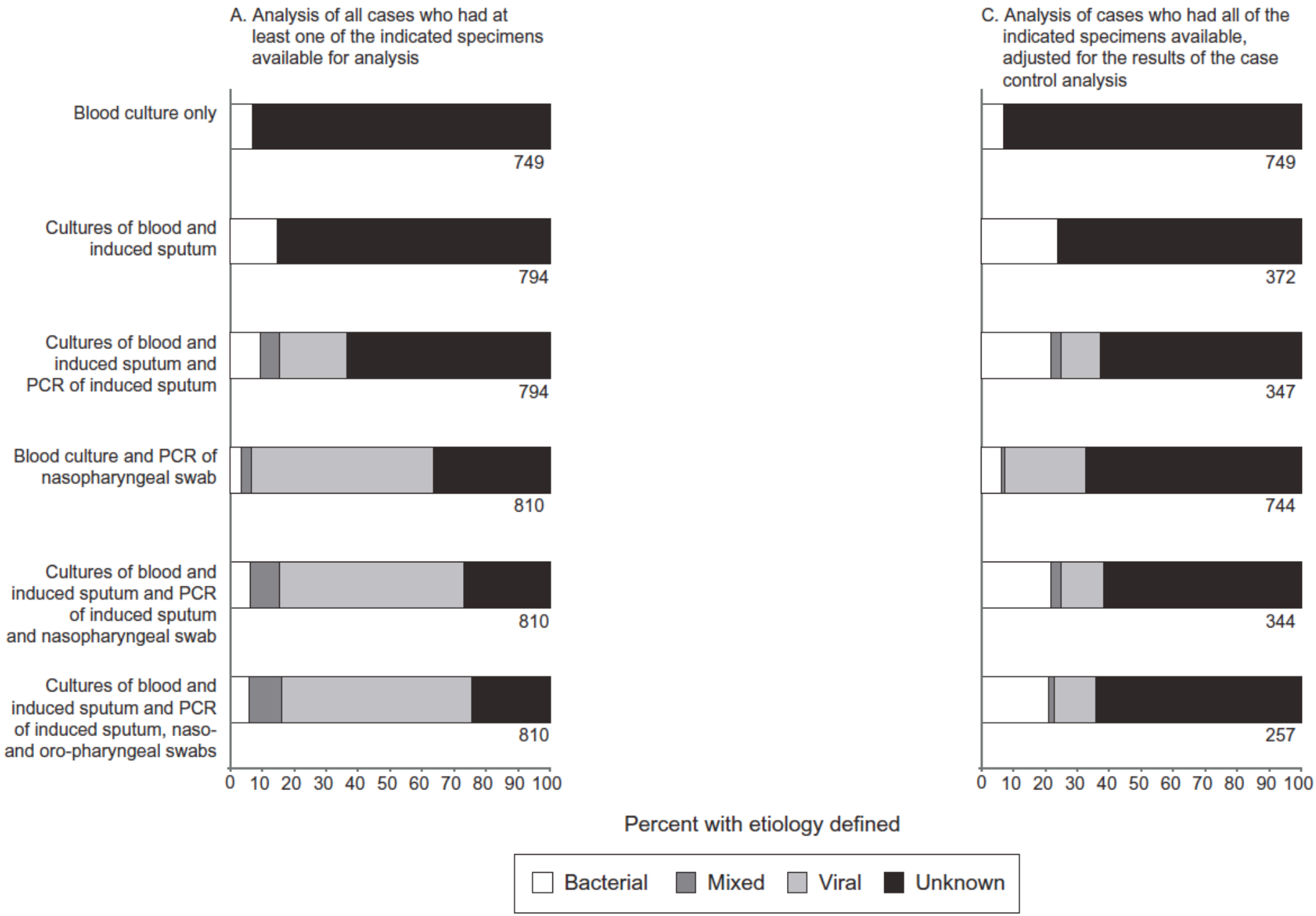

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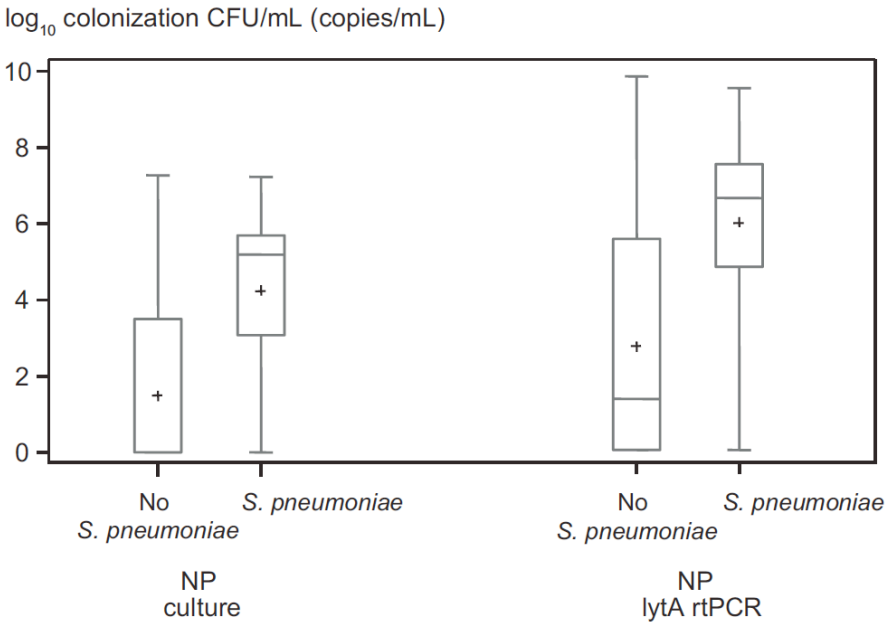
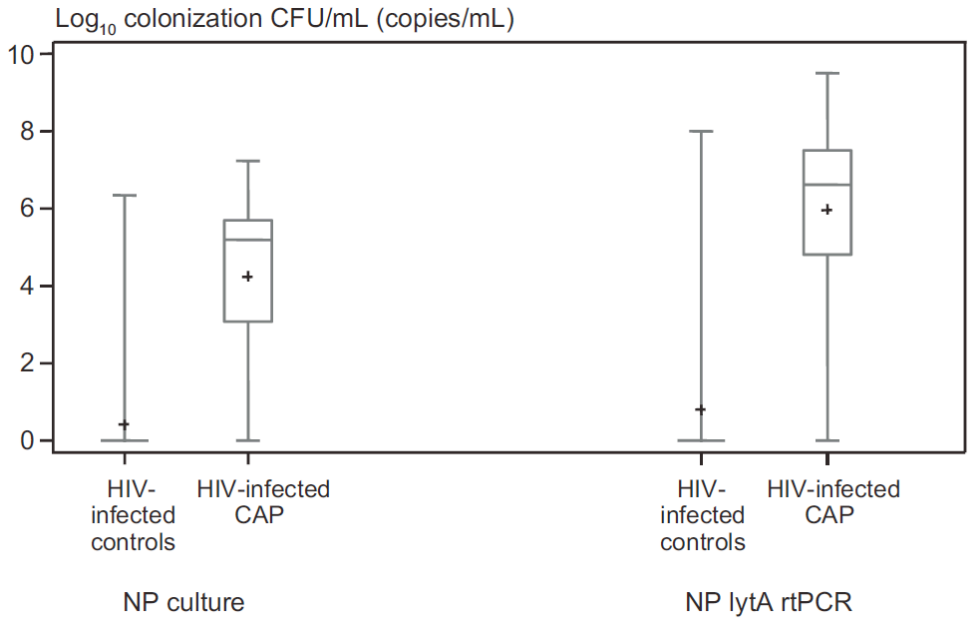
Summary

- Need to be aware of implications of the diagnostic endpoint chosen
  - Balance between increasing case detection and compromising specificity
  - There is seldom a true ‘gold standard’ available
  - Understand the limitations of both microbiological and clinical case definitions
  - Understand the performance characteristics of any novel test very thoroughly

Bacterial density matters





- Using lytA PCR >8000 copies/mL
  - sensitivity of 82% and specificity of 92% for pneumococcal CAP vs. asymptomatic colonization
  - proportion of CAP cases attributable to pneumococcus increased from 27% to 53%

Albrich WC et al. CID 2012; 54:601-9

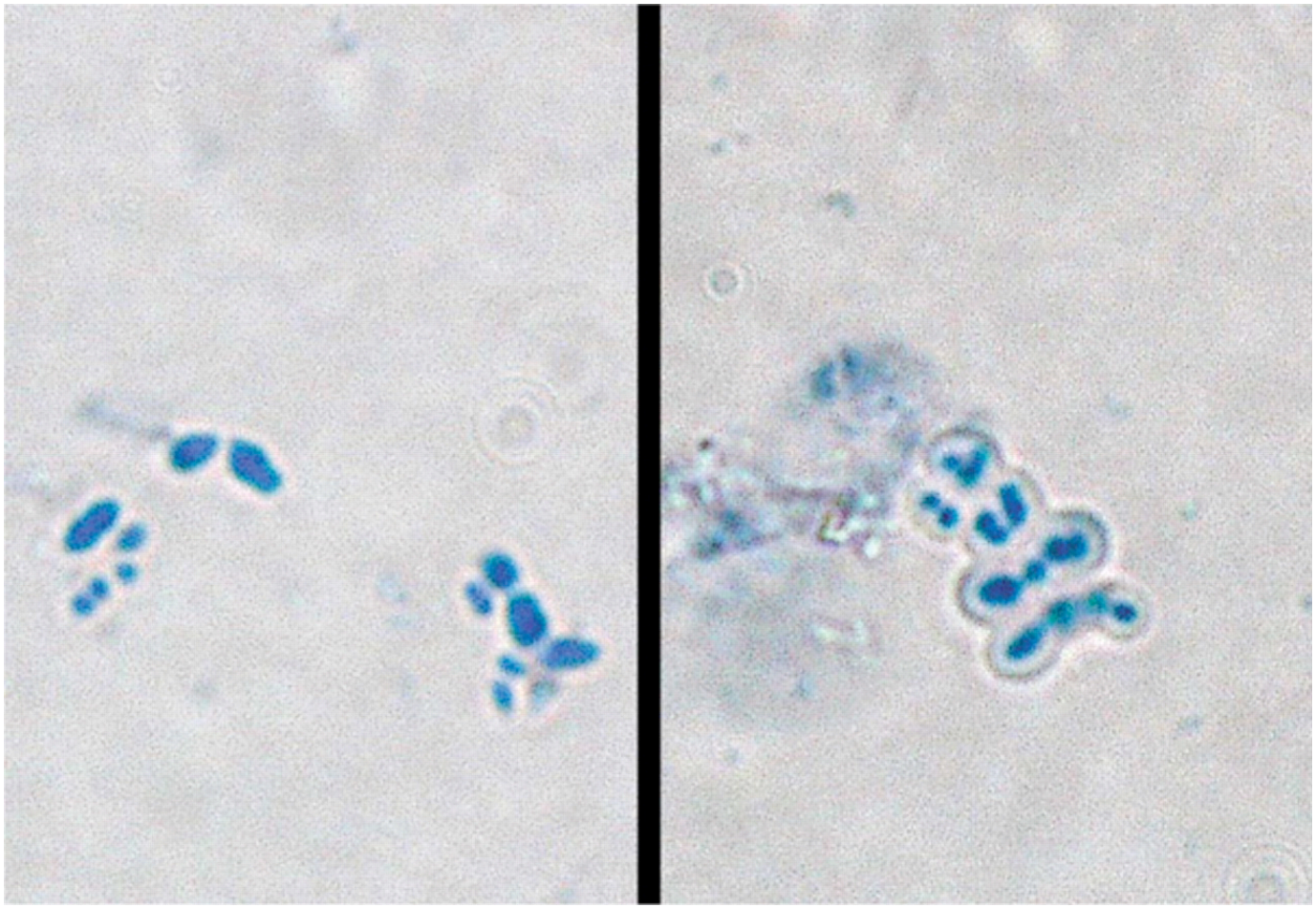
New methods for serotyping pneumococci

- Accurate assessment of serotype is important for
  - Vaccine formulation
  - Vaccine evaluation (impact on carriage)
  - Understanding pathogenesis

- Traditional methods for serotyping pneumococci are problematic
  - Need to detect colonization/infection with multiple serotypes
    - Culture may detect only predominant serotype
  - Quellung is labour-intensive and subjective

- Molecular identification of serotypes
  - Based on differences in capsular biosynthetic gene cluster
  - Real-time PCR, multiplex PCR, microarray, sequencing

Quellung reaction



Oxford Textbook of Medicine 5th edition

Validation of pneumococcal serotyping techniques

	Quellung Reaction	CDC Protocol	Sequetyping
Method	Agglutination	7 triplex real-time PCR reactions	Singleplex PCR + Sequencing
Assay spec	Gold standard technique	Need 21 sets of primers	Only one set of primers
Serotypes	All	21	84
Expertise required	High, error prone	moderate	moderate
Labour requirements	Time consuming	Time consuming	Quick
Costs	High	High relative to sequetyping	Cheap