Blood culture : from theoretical aspects to best practice guidelines

Brigitte Lamy, CHU Nice, Hôpital l'Archet2
Université de Nice Sofia Antipolis

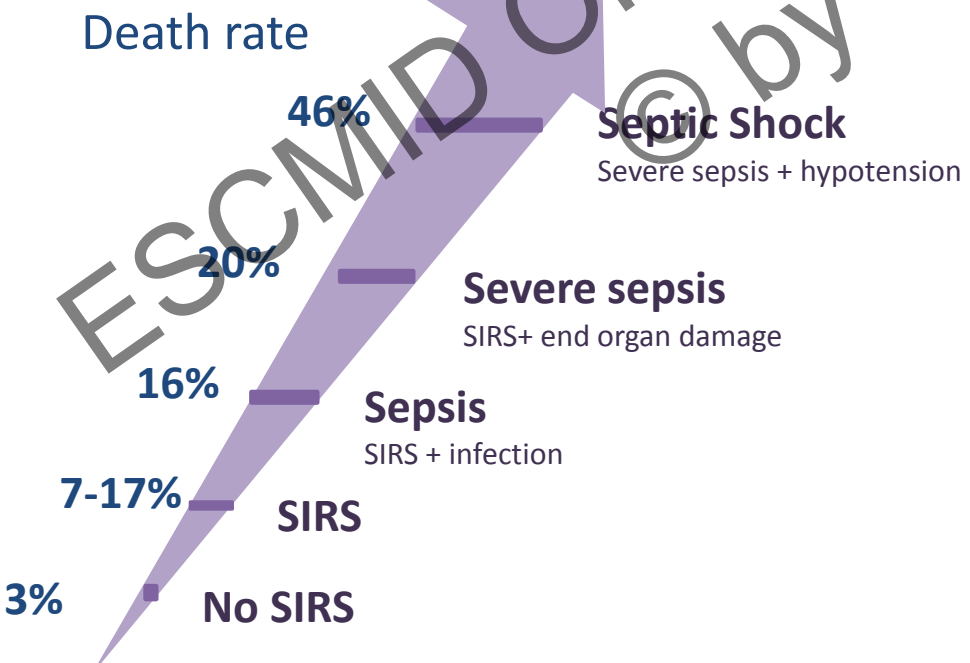
ESCMID Postgraduate education course

Improving the diagnosis of bloodstream infection – advancing technology and quality for better care

Nice, 28 Mars 2017

Blood culture for diagnosing BSI

- Intended to
  - Diagnose bloodstream infection (BSI)
  - Identify the causative agent
  - Guide antimicrobial therapy
- Lifethreatening infections



Stage	BC Positivity
Septic shock	69%
Severe Sepsis	25%
Sepsis	16%

Rangel-Frausto et al, JAMA, 1995

Bloodstream infections

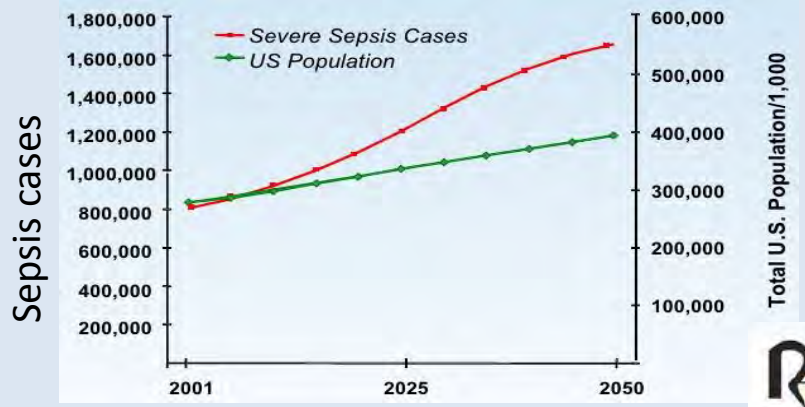
- Frequent and severe

Country/ region	Incidence rate of BSI per 100,000 person-year)	Short-term case fatality (%)	Mortality rate of BSI (per 100,000 person-year)	Estimated annual number of deaths following BSI
USA	174-204	13.5	23.5-27.5	72 349 – 84 823
Europe	166-189	13-20	24.6-37.8	157 750 - 276 318

From Goto et al., CMI, 2013

- Among the top 7 causes of death in Europe & USA
- Increasing…

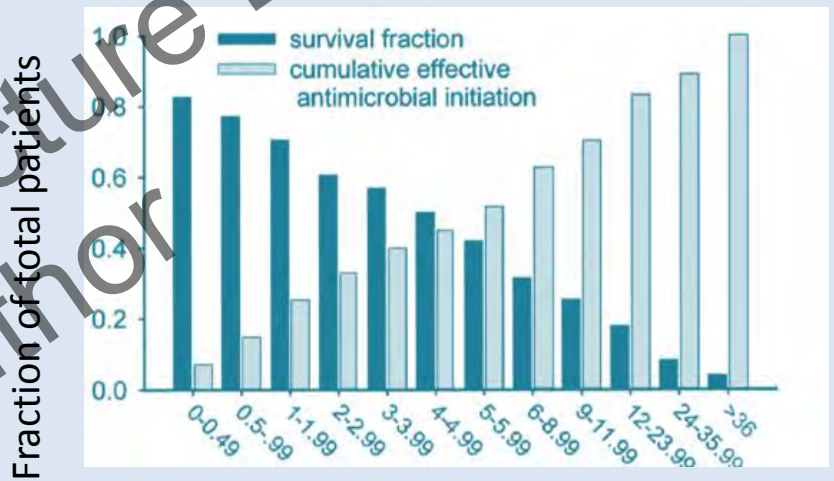
Projected incidence of severe sepsis, USA: 2001-2050



Better perform well and fast…

- BSI associated with:
  - Improved prognosis when causative microorganism(s) are recovered
  - Worse prognosis in case of delayed or absence of diagnosis

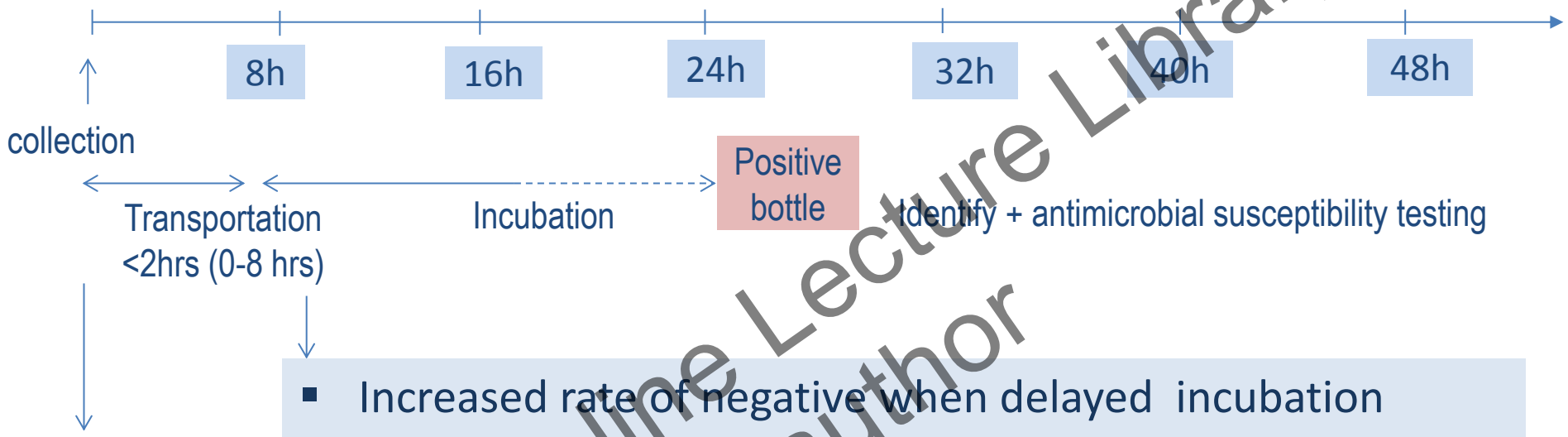
→ Efficient diagnosis must be sensitive, specific, and fast



Time from onset of septic shock to effective antimicrobial initiation (hrs)

From Kumar et al., crit care med, 2006

Blood culture



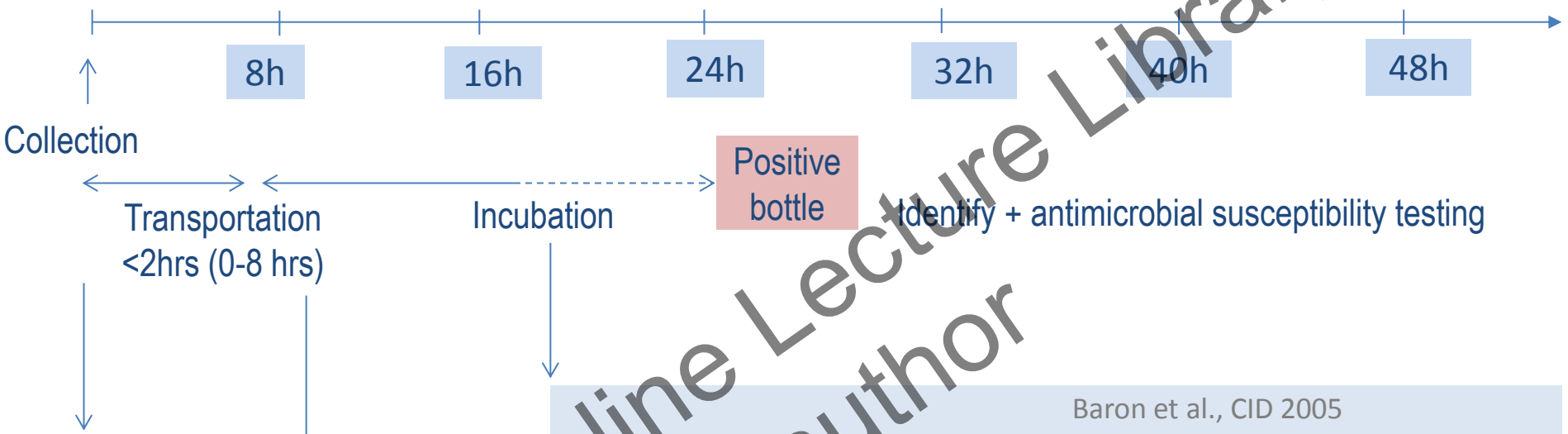
- Increased rate of negative when delayed incubation
  - Dead bacteria
  - Alive bacteria but flagged negative
  - → transport as quickly as possible
  - → if delayed, keep at room temperature
  - → Inspect bottle before loading (only 60%)

Lemming et al., 2004
Sautter et al., 2006

Media

- Current media highly fertile
- Highly varied microorganisms → aerobic + anaerobic
- Increased recovery with resins

Blood culture



Media

Baron et al., CID 2005
Petti et al., J Clin microbiol, 2006

- 5 to 7 days
- Very few positive between 5 and 7 days (<3%)
- Prolonged culture exceptionally useful even for fastidious bacteria (e.g., HACEK)

- Delay increases rate of False negative

- Perform well

Rationale for blood culture performance

- What volume of blood should be cultured ?
- How many BC sets (bottles/ samples) should be collected ?
- What timing between collections ?

→ Sensitivity (Se)

→ Specificity (Sp)

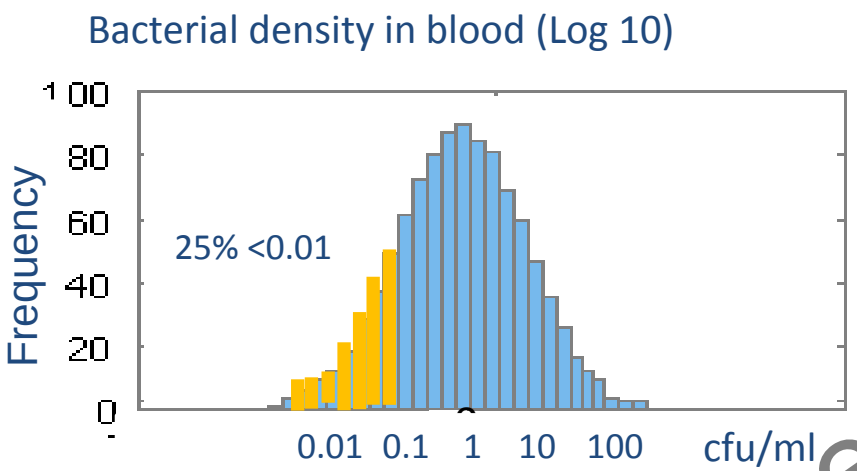
- ✓ Bacterial density (CFU.mL^{-1})
- ✓ Volume of blood per sample (mL)
- ✓ Number of punctures
- ✓ Risk to contaminate one sample

Usually 2 to 3 samples

Usually 2 bottles

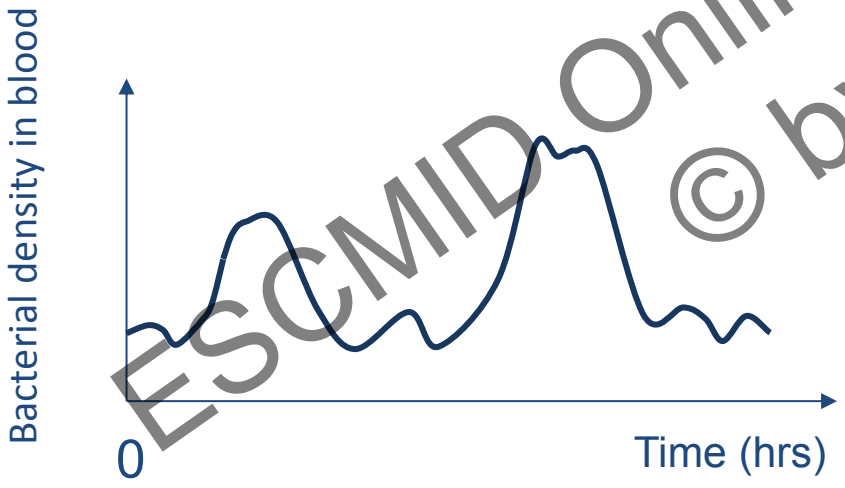
- Several samples of blood cultured [BC set] (n_s)
- Series is positive when at least one sample contains at least one bacterium
- Total Volume = No. Samples x No . bottles (volume per bottle)

Theoretical aspects of sensitivity



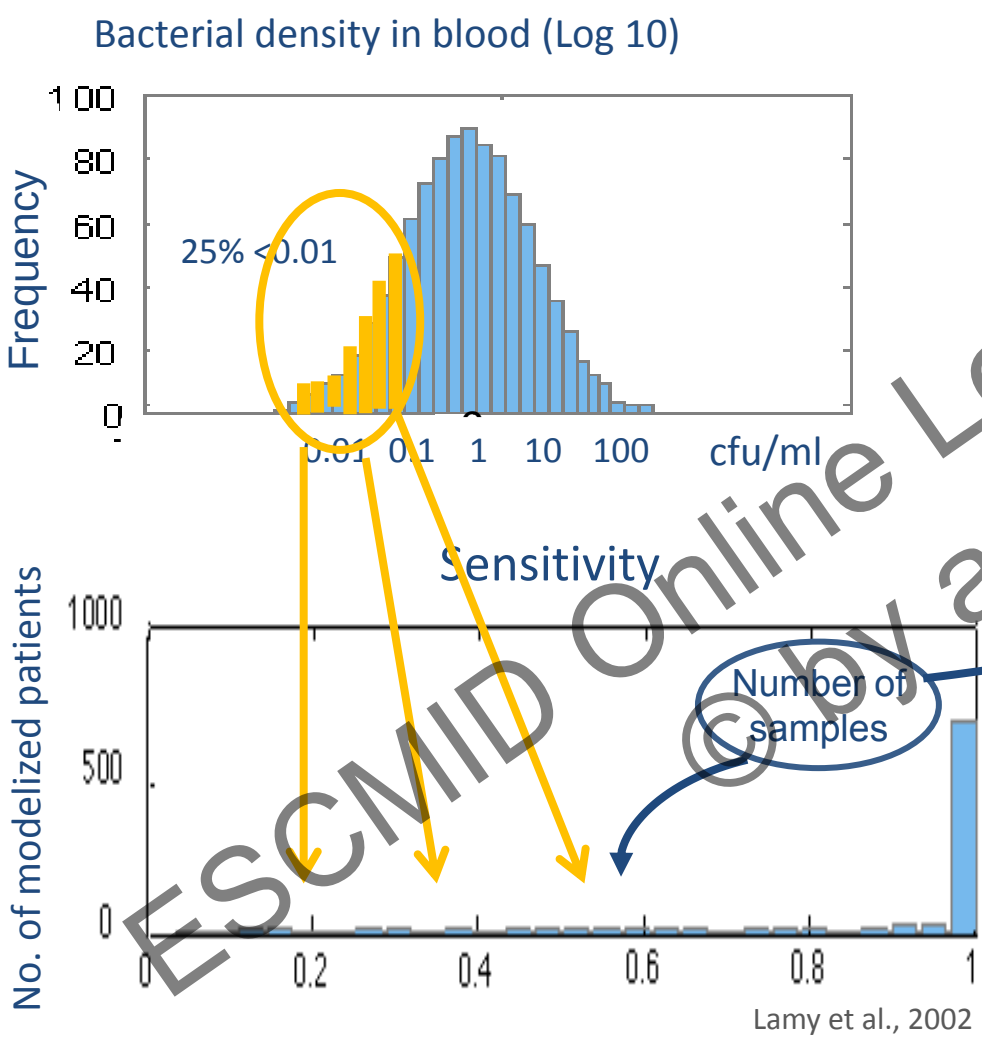
- Very low bacterial density in blood
  - Median : 1 cfu/ml
  - Does not depend on the type of bacteria

Lee et al. JCM, 2007

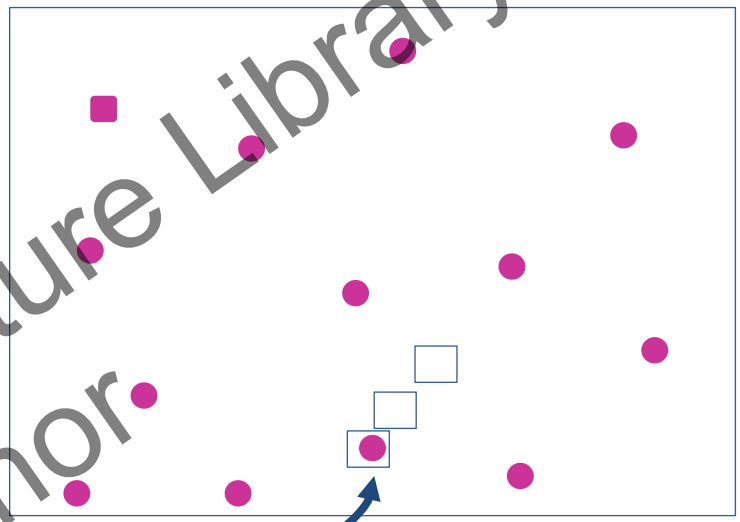


- Wide variation with time
  - 0.001-1000 CFU/ml

Theoretical aspects of sensitivity



Lamy et al., 2002



From Jonsson et al, 1993
Lamy et al., 2002

Likelihood to sample at least one bacterium = probability according to a Poisson distribution of the mean number of bacteria in a sample unit

- Very low bacterial burden in blood
  - → Detection increases with the amount of blood cultured
  - → Large volume of blood for ensuring a good sensitivity

Bacteremia and BC in adult

	Positivity, n (%)		OR	CI 95 %	P
	No	Yes			
Gender					
Female	118 (91.5)	11 (8.5)			
Male	172 (79.6)	44 (20.4)	2.74	1.36–5.53	*0.005*
Diagnosis on admission					
Sepsis/septic shock/other shock states	123 (78.8)	33 (21.2)			0.063
Respiratory failure	68 (94.4)	4 (5.6)	0.22	0.08–0.65	0.006
Neurologic disorders	46 (88.5)	6 (11.5)	0.49	0.19–1.24	0.130
Solid organ transplantation	18 (81.8)	4 (18.2)	0.83	0.26–2.62	0.748
Others	35 (81.8)	8 (18.6)	0.85	0.36–2.01	0.715
Median age (in years)[a]	68 (52–82)	68 (56–78)	1.00	0.98–1.01	0.884
Number of comorbidities[a]	3 (2–4)	3 (2–4)	1.00	0.82–1.22	0.972
Number of blood cultures pairs[a]	2 (1–3)	3 (2–7)	1.28	1.16–1.42	*<0.001*
Mean body temperature (°C) at time of collection[a]	36.7 (36.1–37.3)	37.0 (36.0–37.6)	1.20	0.90–1.62	0.220
Body temperature ≥37.8 °C at time of collection					
No	212 (86.5)	33 (13.5)			
Yes	78 (78.0)	22 (22.0)	1.81	1.00–3.30	0.052
Body temperature ≥39.0 °C at time of collection					
No	285 (84.8)	51 (15.2)			
Yes	5 (55.6)	4 (44.4)	4.47	1.16–17.21	*0.029*
Collected median volume (mL)[a]	35 (20–52)	56 (34–95)	1.02	1.01–1.03	*<0.001*
Collected median weight (g)[a]	35 (21–53)	54 (32–82)	1.02	1.01–1.02	*<0.001*

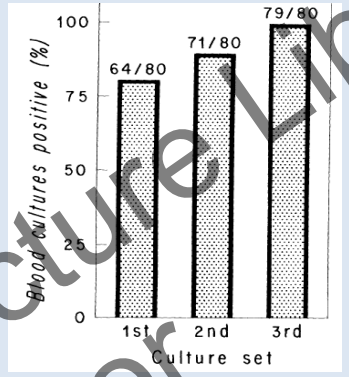
Neves et al., 2013 BMC Res notes

Bacteremia and BC in adult

1975

SUBJECT REVIEW
Blood Cultures
Principles and Techniques

JOHN A. WASHINGTON II, M.D.
DEPARTMENT OF MICROBIOLOGY



2004 -2007

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2007, p. 2765–2769
0095-1137/07/$08.00+0 doi:10.1128/JCM.00140-07
Copyright © 2007, American Society for Microbiology. All Rights Reserved.
Vol. 45, No. 9

Is the Volume of Blood Cultured Still a Significant Factor in the Diagnosis of Bloodstream Infections?

Emilio Bouza,* Dolores Sousa,[2] Marta Rodríguez-Créixems,[1] Juan García Lechuz,[1] and Patricia Muñoz[1]

MAJOR ARTICLE

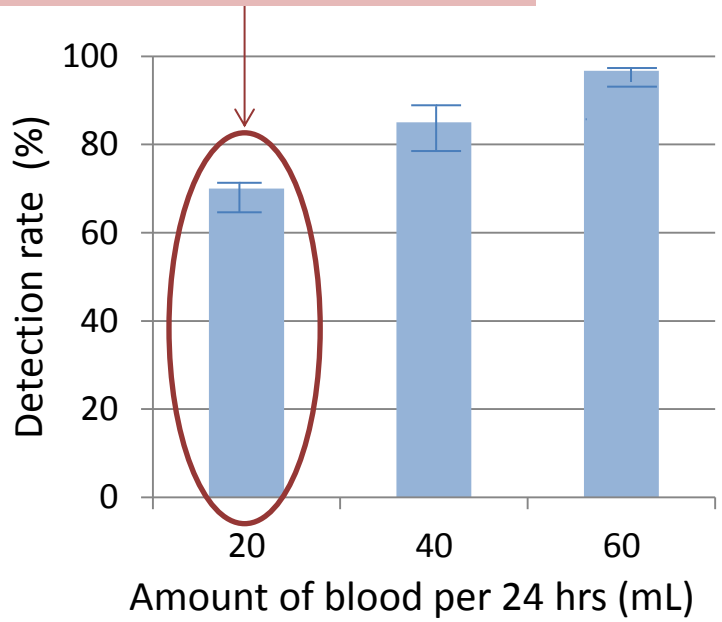
Optimal Testing Parameters for Blood Cultures

F. R. Cockerill III,[1,2] J. W. Wilson,[2] E. A. Vetter,[1] K. M. Goodman,[4] C. A. Torgerson,[1] W. S. Harmsen,[3] C. D. Schleck,[3] D. M. Ilstrup,[3] J. A. Washington II,[5] and W. R. Wilson[2]

[1]Department of Pathology, Division of Microbiology, [2]Department of Internal Medicine, Division of Infectious Diseases, [3]Section of Biostatistics, Department of Health Sciences Research, and [4]Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic and Foundation and Mayo Medical School, Rochester, Minnesota; and [5]Division of Microbiology, Cleveland Clinic Foundation, Cleveland, Ohio

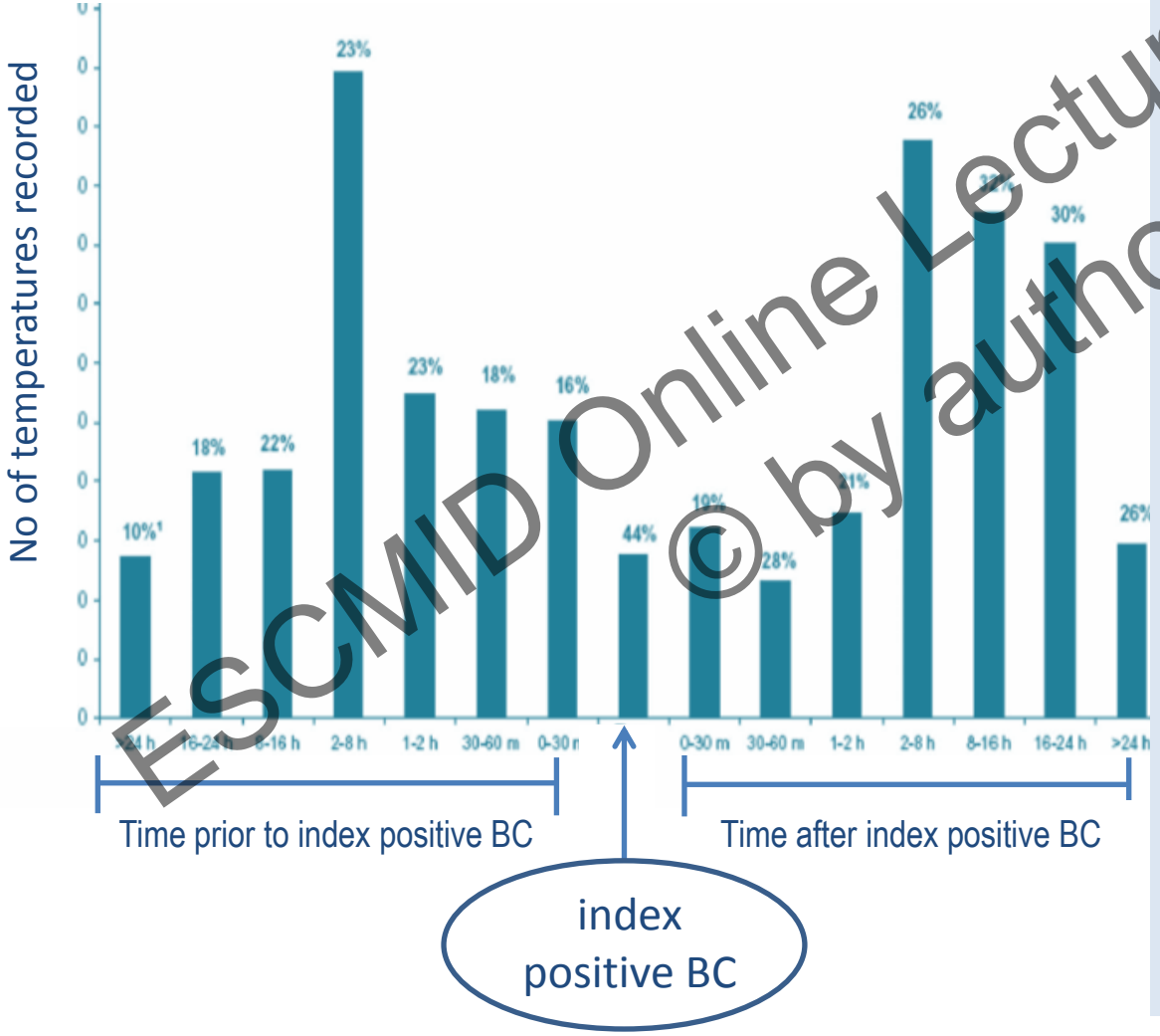
- 3 BC sets (total of 6 bottles)
- Corresponds up to 60 mL
- **minimum** 20-30 mL

Solitary BC
To be discouraged



Timing of collection: when should BC be drawn ? (1)

Distribution of temperature spike around index positive BC (1,436 adult patients)



- Obtaining BC at temperature spikes **does not improve** BSI detection
- More than the timing of BC the clinical intuition

Riedel et al, 2008, J Infect Chemother, 46: 1381

Timing of collection: when should BC be drawn ? (2)

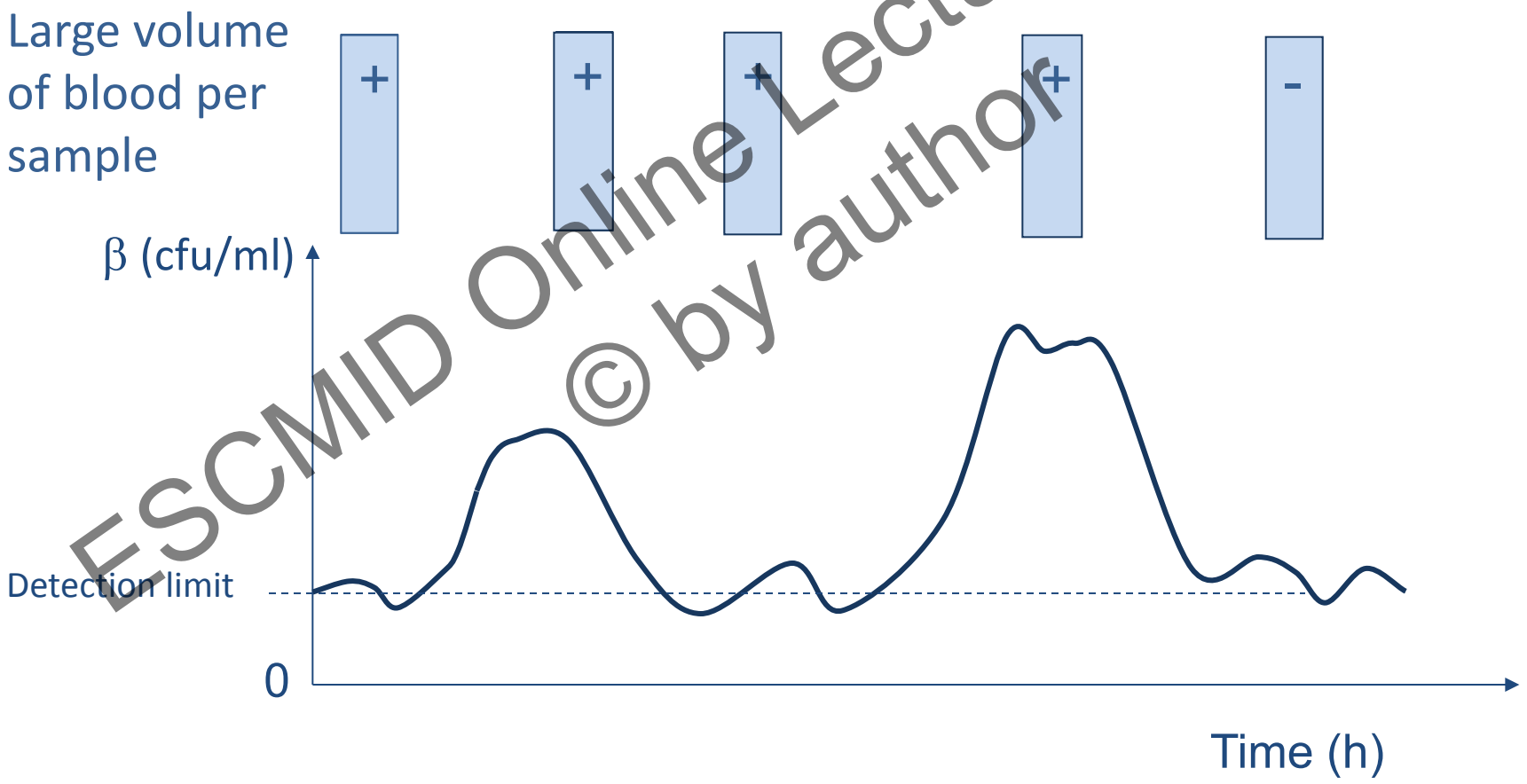
volume : 2 x 20 mL

Li et al, JCM, 1994

Interval between cultures	10 min-2h apart	2h-24h apart	Anytime within 24h	0 min
No. of bacteremic episodes	30	72	210	184
Yield added by extra vol (%)	17	17	20	19

The larger the volume, the higher the sensitivity

The larger the volume, the higher the sensitivity (whatever the number of draws, whenever the draws)

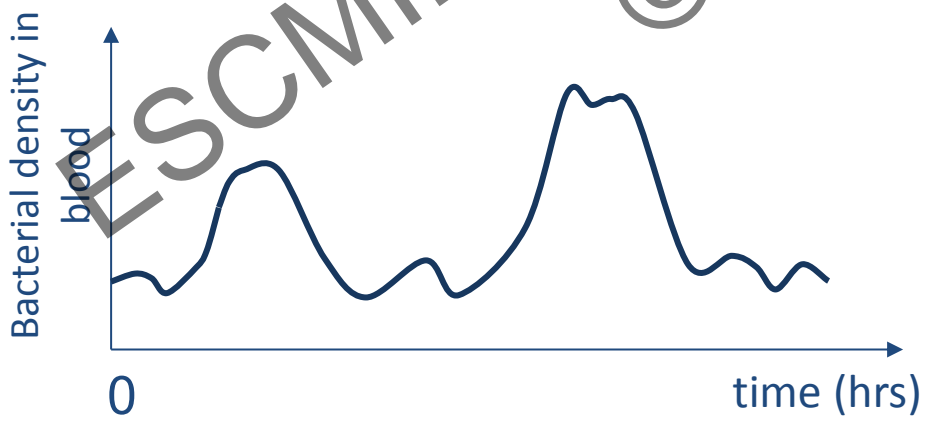


Timing of collection: when should BC be drawn ?

volume : 2 x 20 mL

Li et al, JCM, 1994

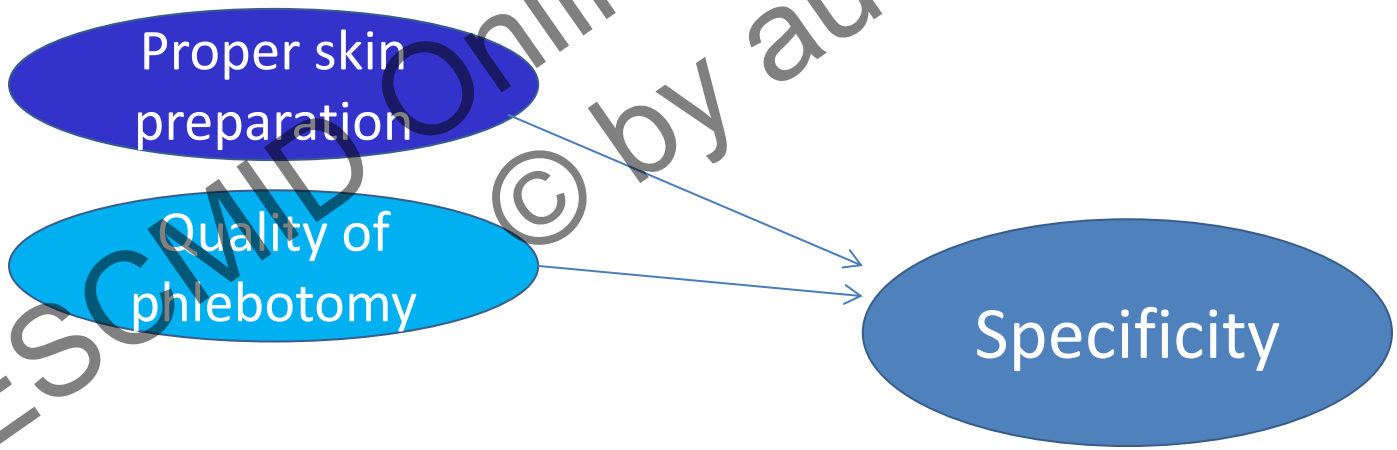
Interval between cultures	10 min-2h apart	2h-24h apart	Anytime within 24h	0 min
No. of bacteremic episodes	30	72	210	184
Yield added by extra vol (%)	17	17	20	19



- Obtaining BC at temperature spikes **does not improve** detection
- More than the timing of BC the clinical intuition
- More than the timing of BC or the Number of samples, the **total amount of blood** cultured
- **Intermittent bacteremia** over a short period (<24hrs) is **exceptional** (0.6%)

Factors determining BC specificity

- False-positive = contaminants
  - → Bacteria recovered from a bottle but not originating from the patient's blood
  - → Origin: bacterium from skin introduced during sample collection
  - → Concern: CNS involved in CRBSI

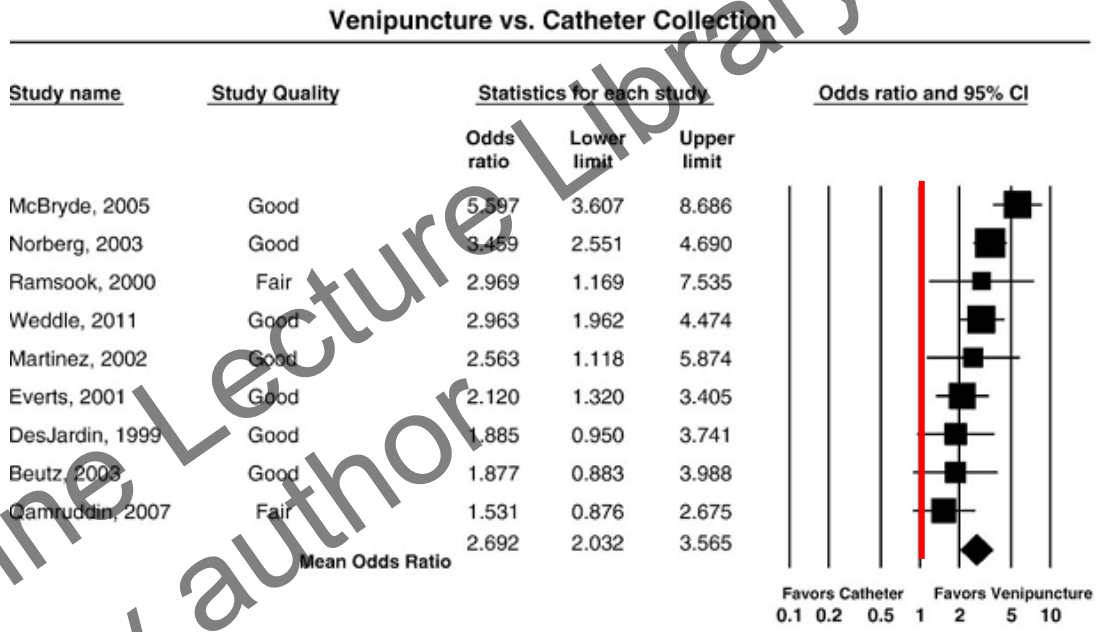


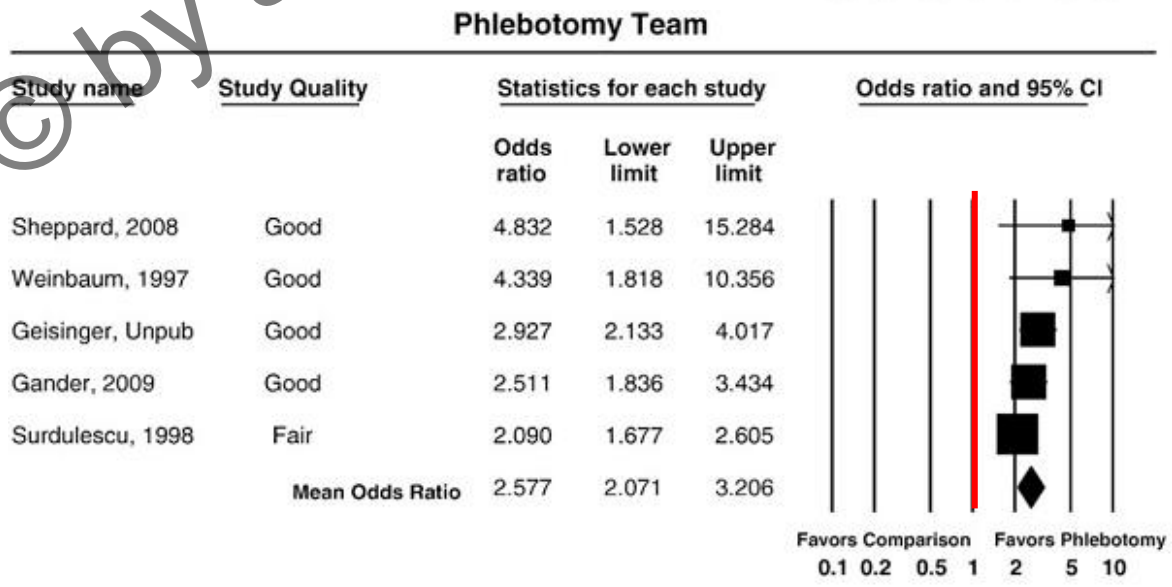
How to control contamination

- Venepuncture

- Quality of phlebotomy

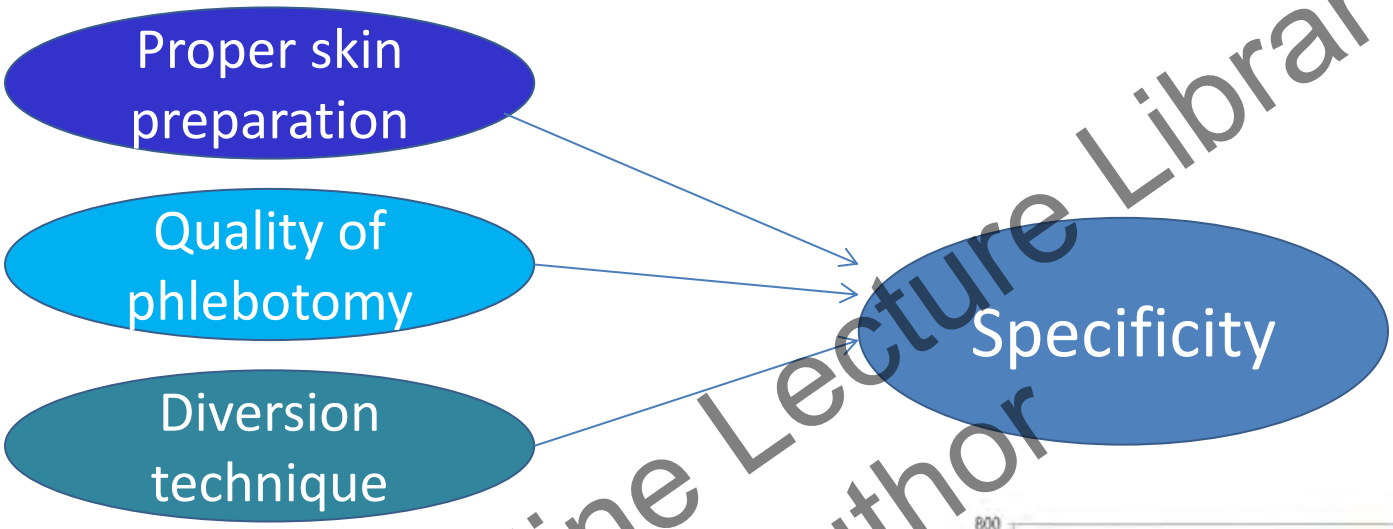
(Sylvie Dargère's talk)



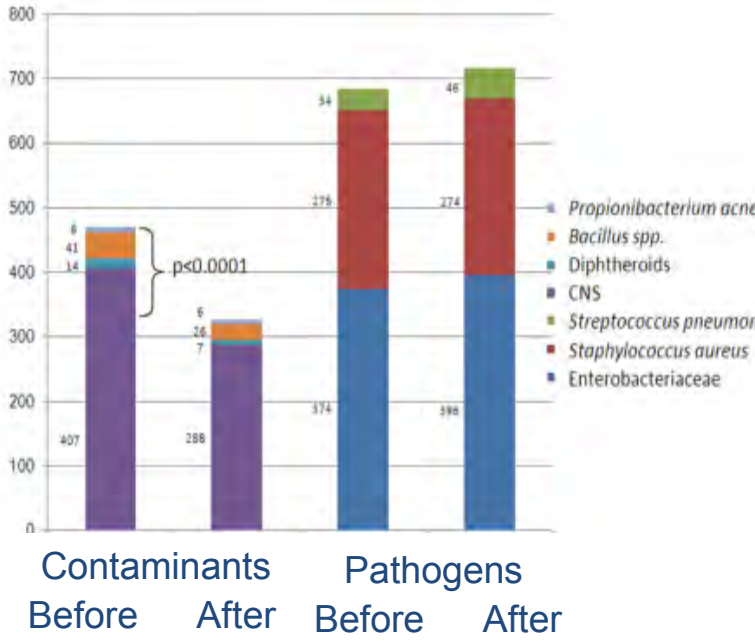


Snyder et al. Clin Biochem. 2012, 45:999-1011 (metaanalyse)

Factors determining BC specificity (2)

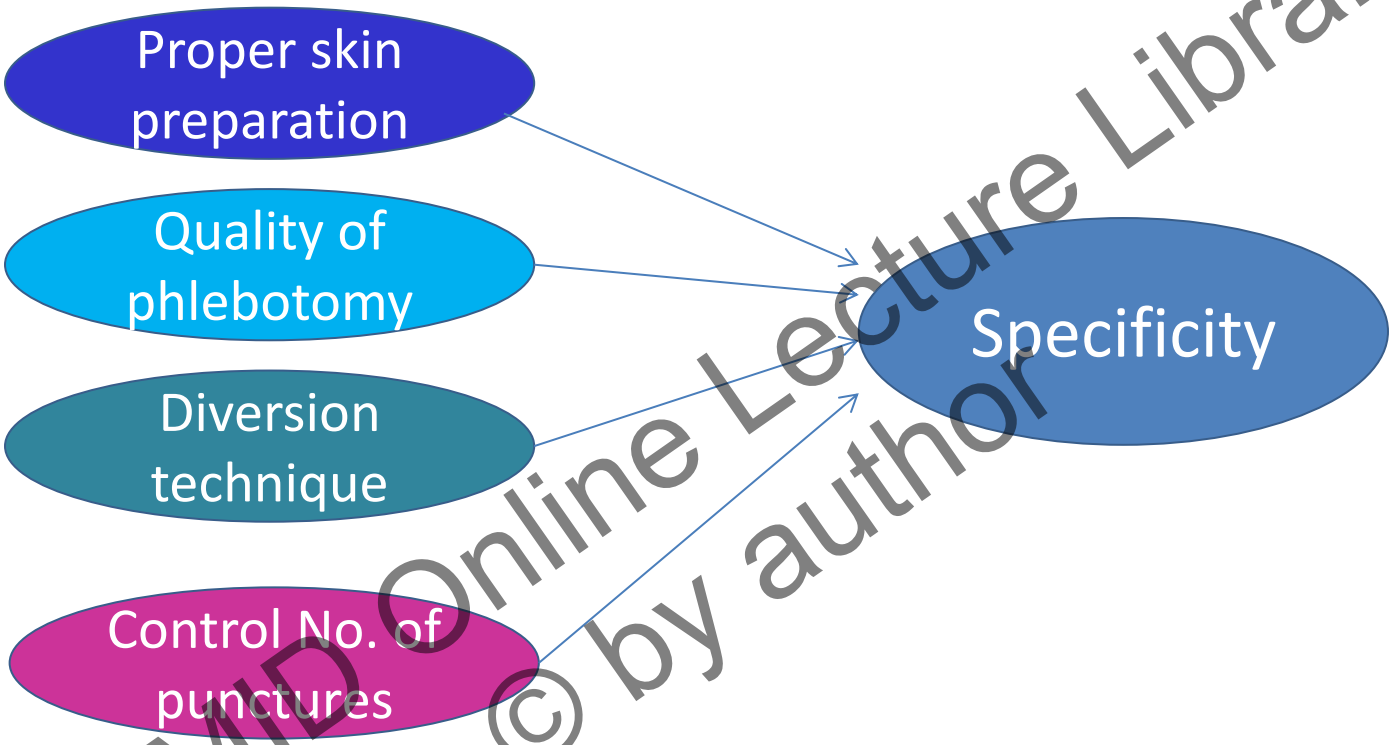


- The first mls of blood are discarded
  - Contamination more likely in the first ml(s)
  - Results in reduced contamination rate
- Limitations
- Unsolved questions :
  - How much is it reduced?
  - how many ml to discard?



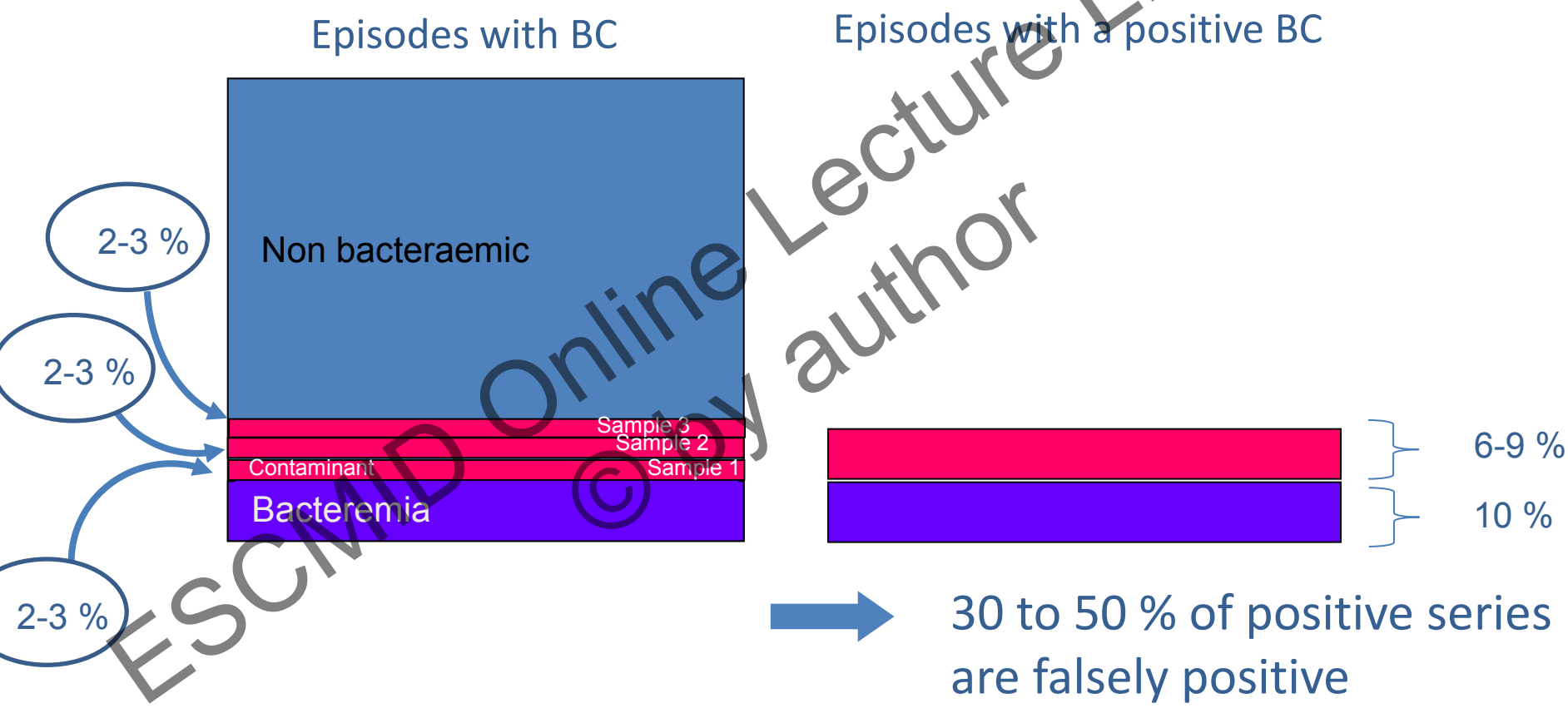
Patton & Schmitt, J. Clin Microbiol, 2010
Binkhamis & Forward, 2014

Factors determining BC specificity (3)



Impact of the number of draws on contaminants (1)

One positive sample within the series → inflation effect

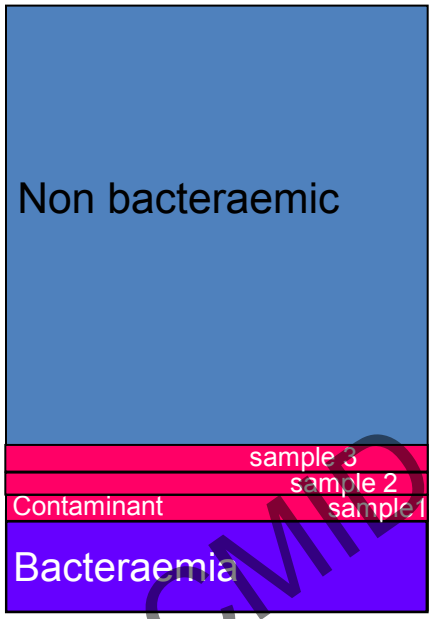


30 to 50 % of positive series are falsely positive

The higher the number of samples, the higher the false positive rate

The higher the contamination rate, the higher the false positive rate

Impact of the number of draws on contaminants (2)



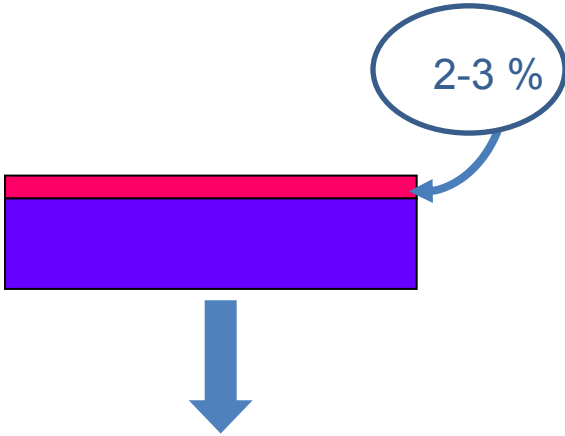
Episodes with a positive BC

3 samples of 2 bottles

30-50% contaminants

1 sample of 6 bottles

15-20 % of contaminants



Improve positive predictive value

Vicious circle of contamination

Guidelines

Sampling strategy (adult patients)

1 set = 2 bottles

1 set =4-6 bottles (not 2)

40-60 ml of blood cultured

2-3 paired bottles
2-3 samples

2-3 paired bottles
1 single sampling

1 single paired bottle
1 single sample

Multi-sampling strategy

Single sampling strategy

Solitary BC
Non compliant volume

Indicator of lack of quality

- Adequate number of bottles
- adequately filled (8-10 mL per bottle)
- Quickly managed

<20 mL, 90% of the cases

EMCM (2012); Baron et al, Clin Infect Dis, 2013; REMIC V5 (2015)

EMCM (2012); REMIC V5 (2015)

* ASM : 2 to 4 paired bottles

Sample strategy

2-3* paired bottles
2-3 samples

2-3 paired bottles
1 single sampling

Multi-sampling strategy

Single sampling strategy

- Adequate number of bottles
- adequately filled (8-10 mL per bottle)
- Quickly managed

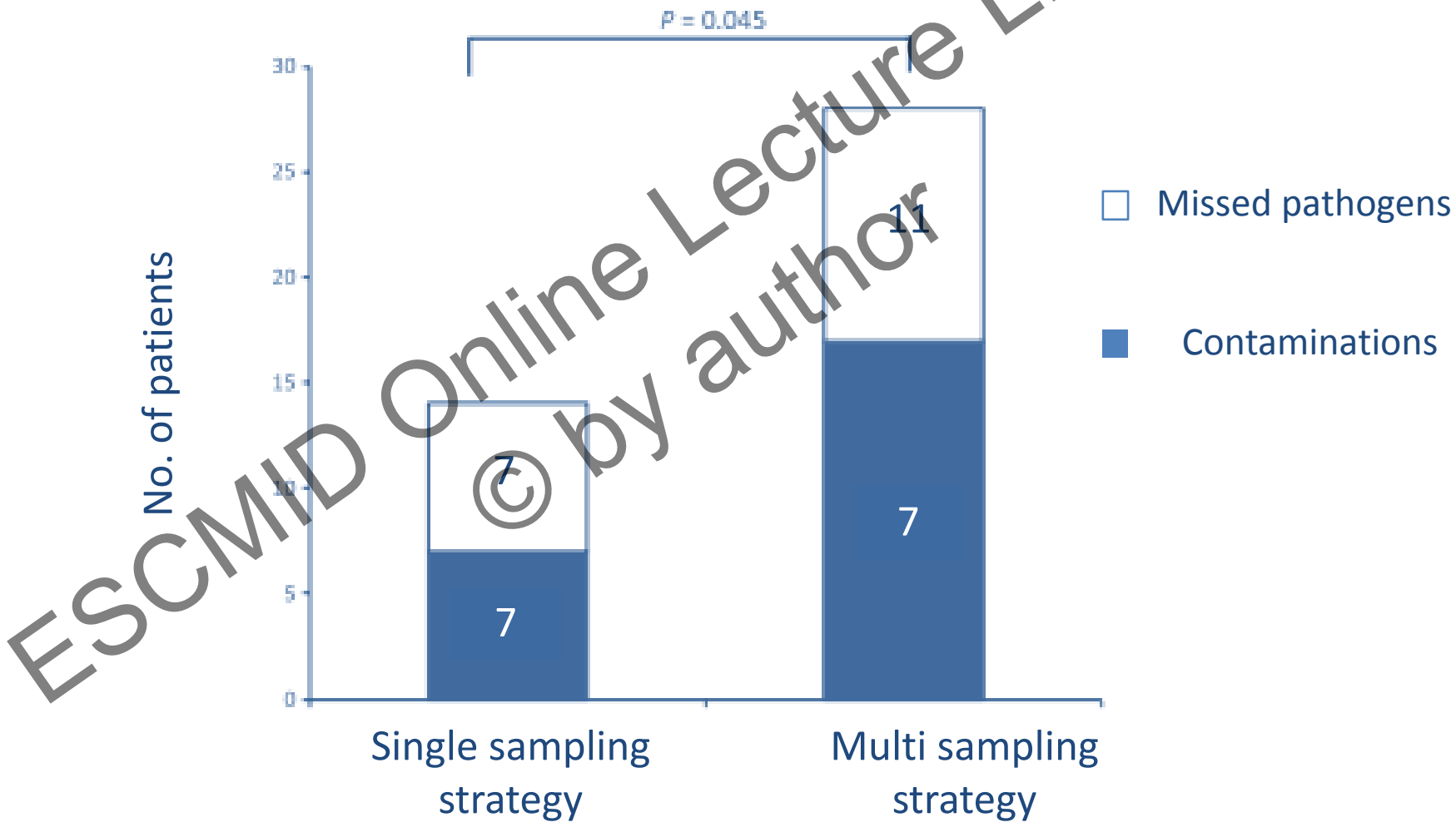
EMCM (2012); Baron et al, Clin Infect Dis, 2013; REMIC V5 (2015)
EMCM (2012); REMIC V5 (2015)

* ASM : 2 to 4 paired bottles

- Same volume, same sensitivity
- No Solitary BC with single sampling strategy
- 1 vs 3 collections
  → Reduced risk of contamination
  → Improved confort for patient
  → Earlier treatment
  → Decreased nurse workload
- Limitations
  - Number of studies
  - Infective endocarditis (Dukes criteria)

Performance of the Single sampling strategy

300 patients with positive BC



Dargere et al., Clin Microbiol Infection, 2014

Two or Three BC ?

- Guidelines not always very clear
- 3 BC → close to 60 ml = optimal diagnostic conditions
- 2 BC → close to 40 ml = very good diagnostic conditions

  …. If all bottles are adequately filled

French Nationwide evaluation

Total volume actually cultured	4 bottles all bottles >8ml	4 bottles all bottles <8 ml	6 bottles All bottles <8 ml
>30 ml	95%	0.15%	71.4%

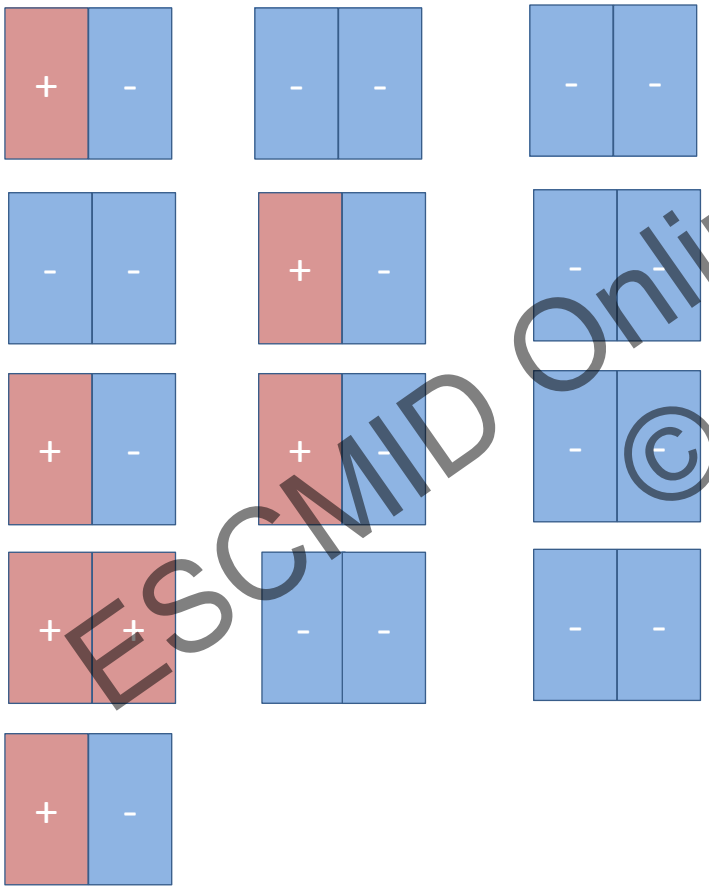
- Prefer 3 BC (6 bottles) if bottles are underfilled

  Note : Under-filling is (very) costly…

How to interpret results ?

- To recognise that an isolate is a contaminant (CNS)

Multi-sampling strategy



Likelihood to contaminate
1 sample: 0.03 x 0.97 x 0.97 =0.028

Contaminant

Likelihood to contaminate
1 sample: 0.97 x 0.03 x 0.97 =0.028

Contaminant

Likelihood to contaminate
2 samples: 0.03 x 0.03 x 0.97 =0.0009

BSI

???

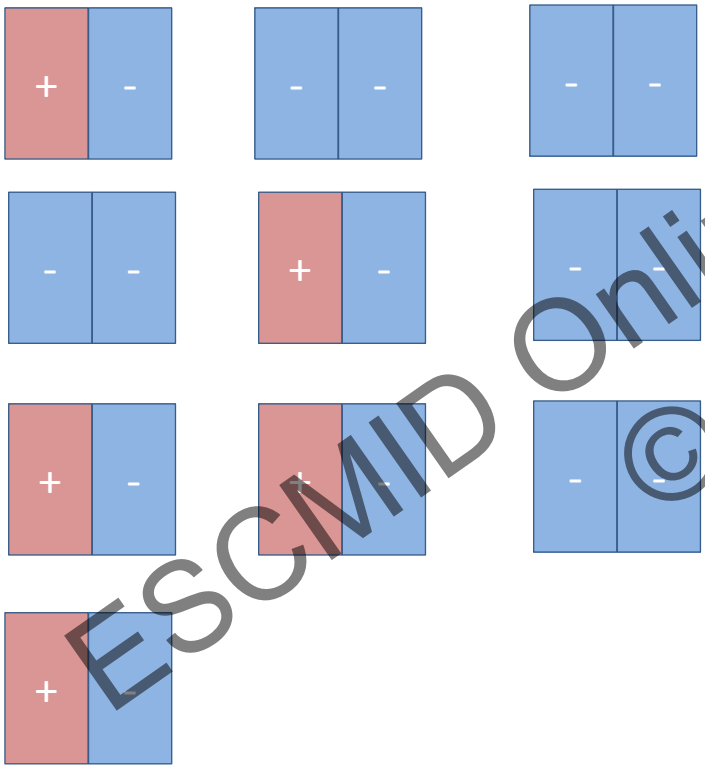
???

Rule of thumb = **No. of positive samples** ….and clinical data !

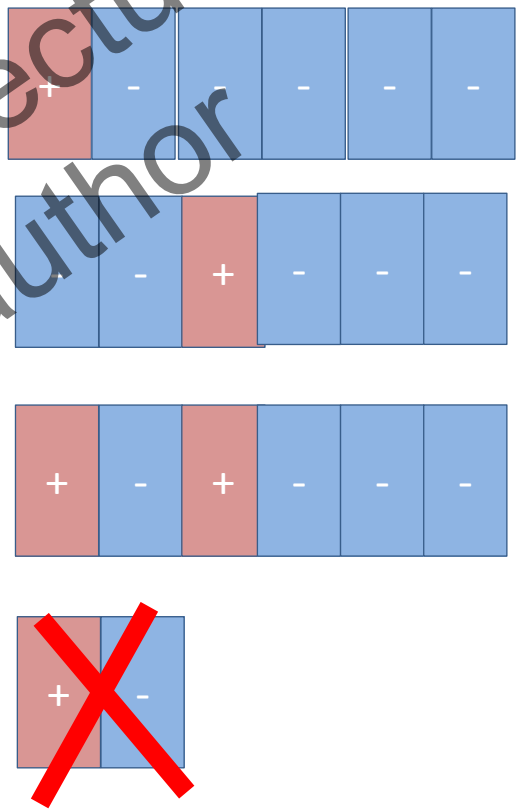
How to interpret results ?

- To recognise that an isolate is indeed a contaminant (CNS)

Multi-sampling strategy

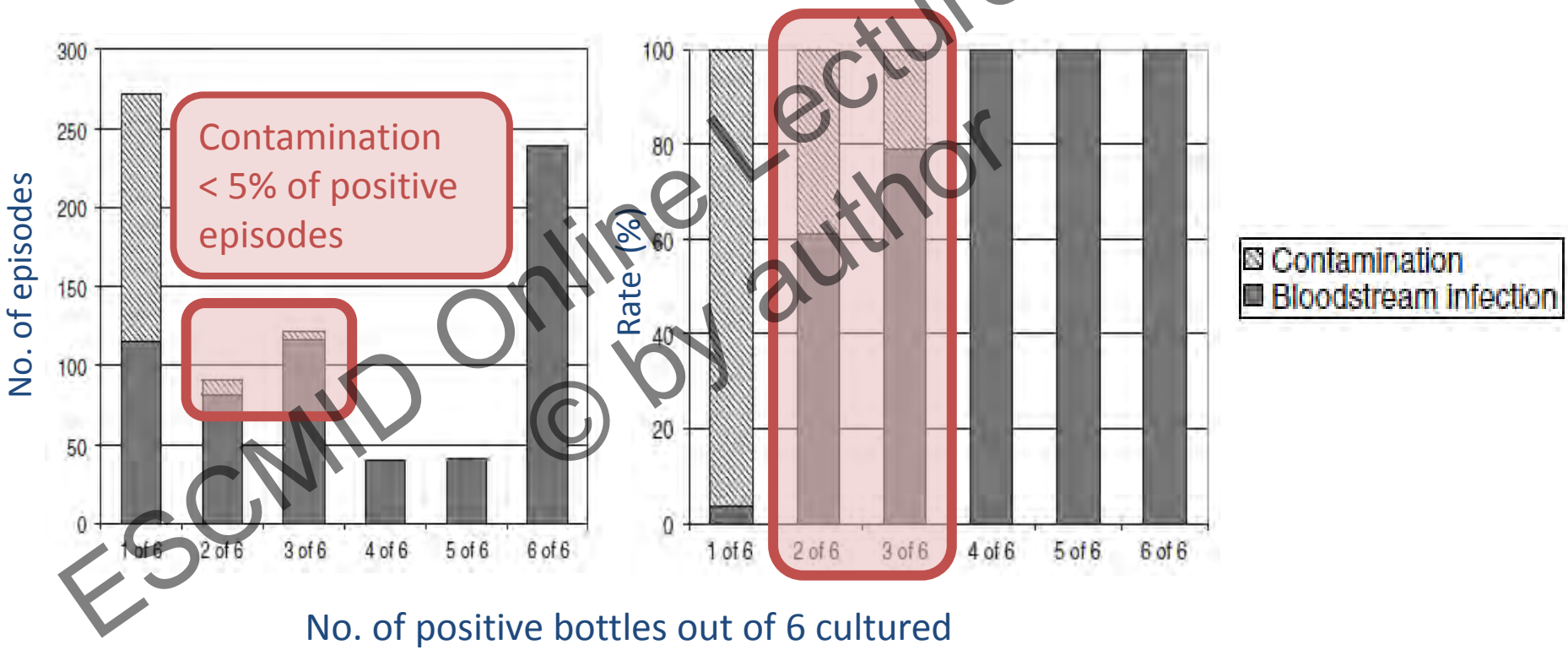


single-sampling strategy



~~Rule of thumb =~~ ~~**No. of positive samples**~~

Single sampling strategy: how to interpret results



➲ Clinical data and **No. of positive bottles**

Leyssene et al, Eur J Clin Microbiol Infect Dis, 2011; 30:5660.

Take home message

- Critical for sensitivity:
  - Total volume cultured (always think about the volume)
    - Total number of bottles
    - Actual volume of blood per bottle
    - Never culture only 2 bottles in adult
- Not critical for sensitivity
  - Number of samples (when the right total volume of blood being cultured)
  - Timing of collection
- Critical for specificity
  - Number of samples (the lowest)
  - Skin preparation
  - Type or phlebotomy
  - The first ml more likely to be contaminated

Thanks
for your
attention

Raoul Dufy
Le casino de la jetée
de Nice, 1927,