Role of Microbiological Testing

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Sept 18-20th 2025

Congreso Internacional Epiclatino 2025

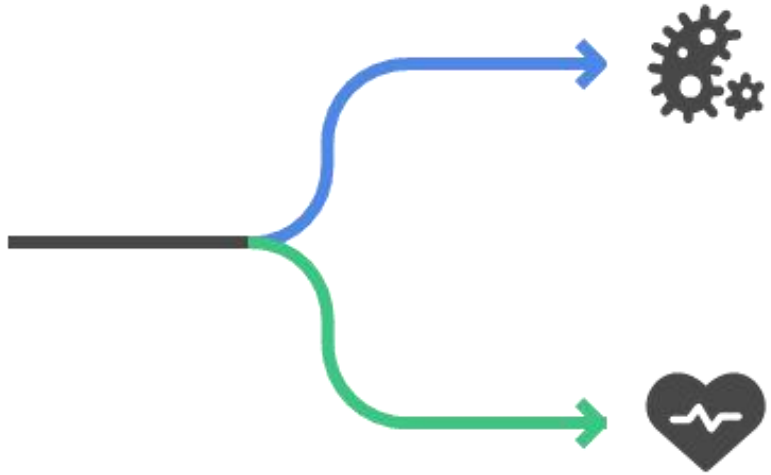
I have no financial relationships to disclose or Conflicts of Interest (COI) to resolve.

The data presented are derived from centers in United States and may not reflect practices across the world

Learning Objectives:

- **List culture-based and culture-independent microbiological tests available for sepsis diagnosis**
- **Identify strategies to optimize performance of culture-based microbiological tests**
- **Review the evidence supporting these strategies**

Tests used to diagnose neonatal infections



Microbiological Tests

Directly identify pathogens, crucial for targeted antibiotic use and sepsis management.

Host Response Biomarkers

Provide indirect markers of infection, useful when microbiological results are pending or suspicion is high.

Microbiological tests, particularly from clinical samples that should be sterile (blood and CSF) remain the 'gold standard' because they provide direct information for treatment

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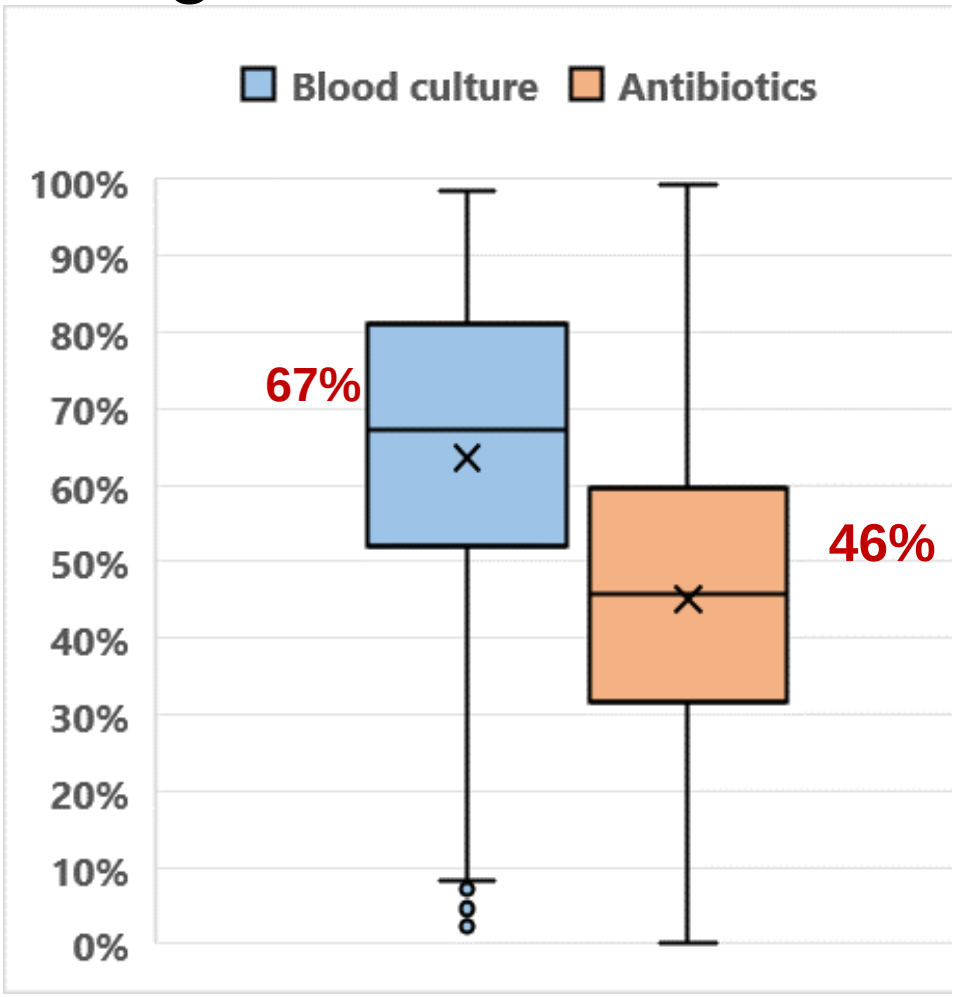
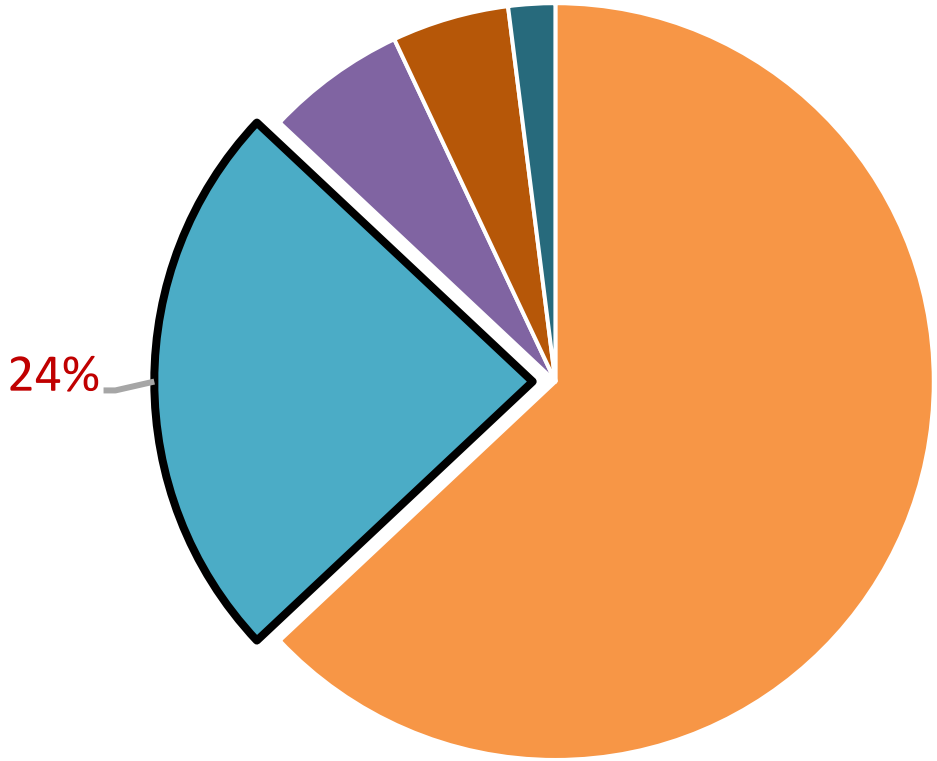
Trust (lack of) in cultures and antibiotic use

All gestations at admission



Culture-negative Infection



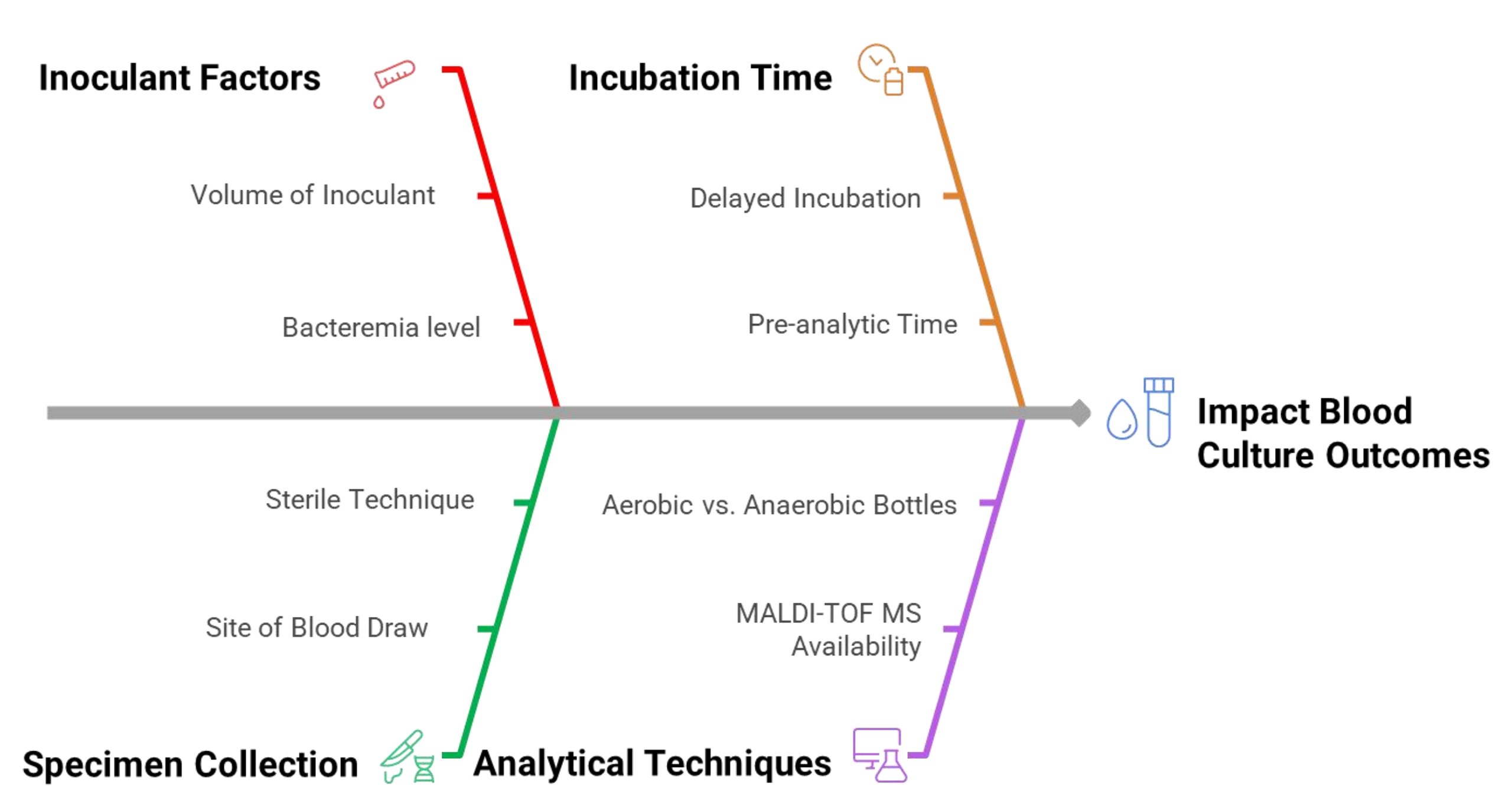
Barboza-Zevallos et al, Jpeds, 2024; Cantey JB et al, PIDJ, 2015

Perceived Low Sensitivity of Blood Cultures is False

CFU/mL	Inoculate volume			
	0.5 mL	1 mL	2 mL	4 mL
1	0.39	0.63	0.87	0.98
2	0.63	0.87	0.98	0.99
3	0.78	0.95	0.99	0.99
4	0.87	0.98	0.99	0.99
5	0.92	0.99	0.99	0.99
10	0.97	0.99	0.99	0.99
100	1.0	1.0	1.0	1.0

What about ultra-low bacteremia?

Schelonka *et al*, Journal of Pediatrics, 1996; Lancaster *et al*, JCM, 2015



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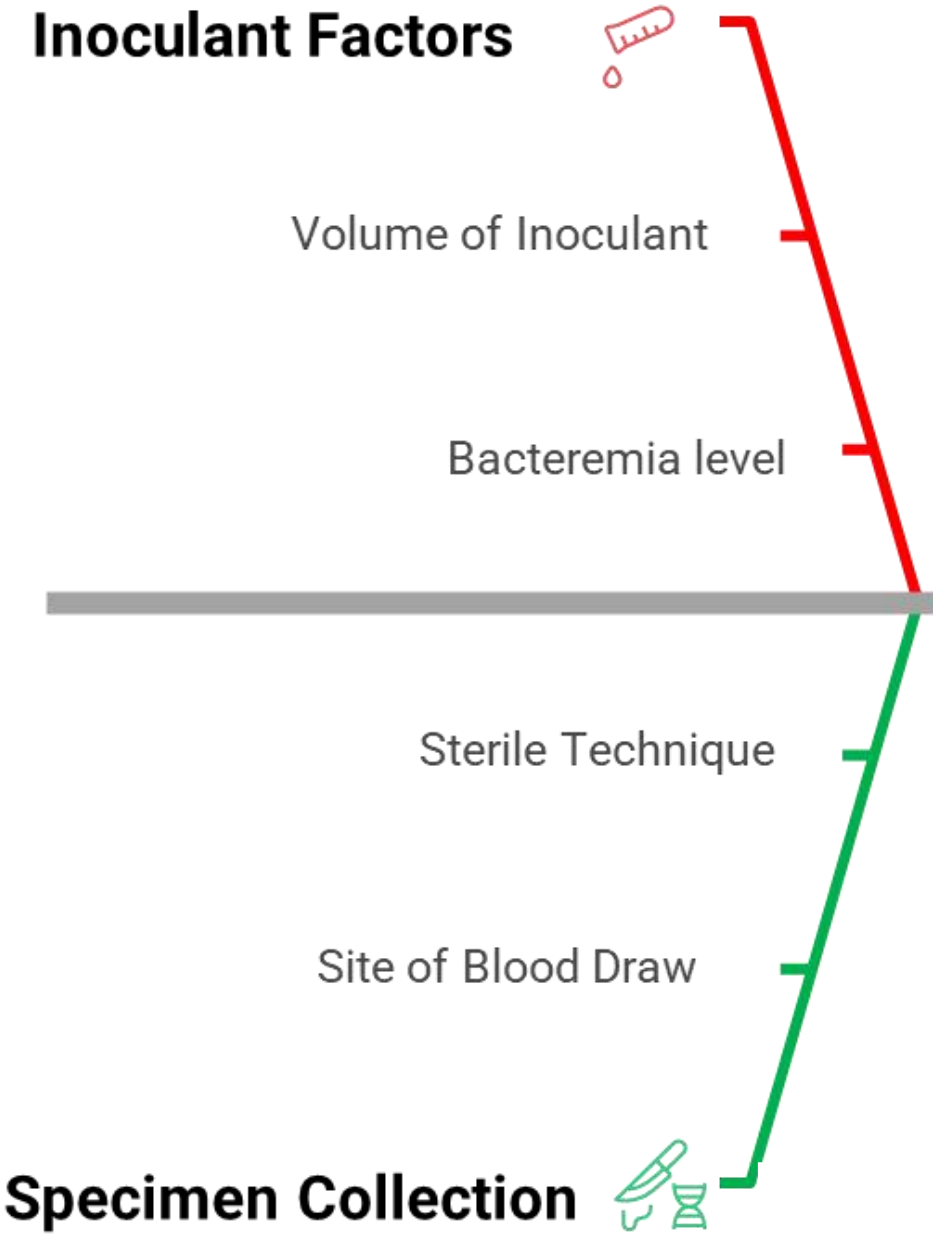

Impact Blood Culture Outcomes

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Blood culture performance depends on inoculant volume

CFU/mL	Inoculate volume			
	0.5 mL	1 mL	2 mL	4 mL
1	0.39	0.63	0.87	0.98
2	0.63	0.87	0.98	0.99
3	0.78	0.95	0.99	0.99
4	0.87	0.98	0.99	0.99
5	0.92	0.99	0.99	0.99
10	0.97	0.99	0.99	0.99
100	1.0	1.0	1.0	1.0

How sure are you of the inoculant volume?

Schelonka *et al*, Journal of Pediatrics, 1996; Lancaster *et al*, JCM, 2015

Reports of Inadequate Blood Culture Inoculant Volume

Citation	Volume goal	% less than goal
Neal et al, 1986	0.5 mL	55% aerobic 58% anaerobic
Jawaheer et al, 1997	0.5 mL	40% all cultures
Connell et al, 2007	0.5 mL	65% pre-education 82% post-education
Singh et al, 2020	0.8 mL	4% pre-education 75% post-education

Optimize collection

Sterilization

Use chlorhexidine and allow to dry for infection control.

Written Policy

Site

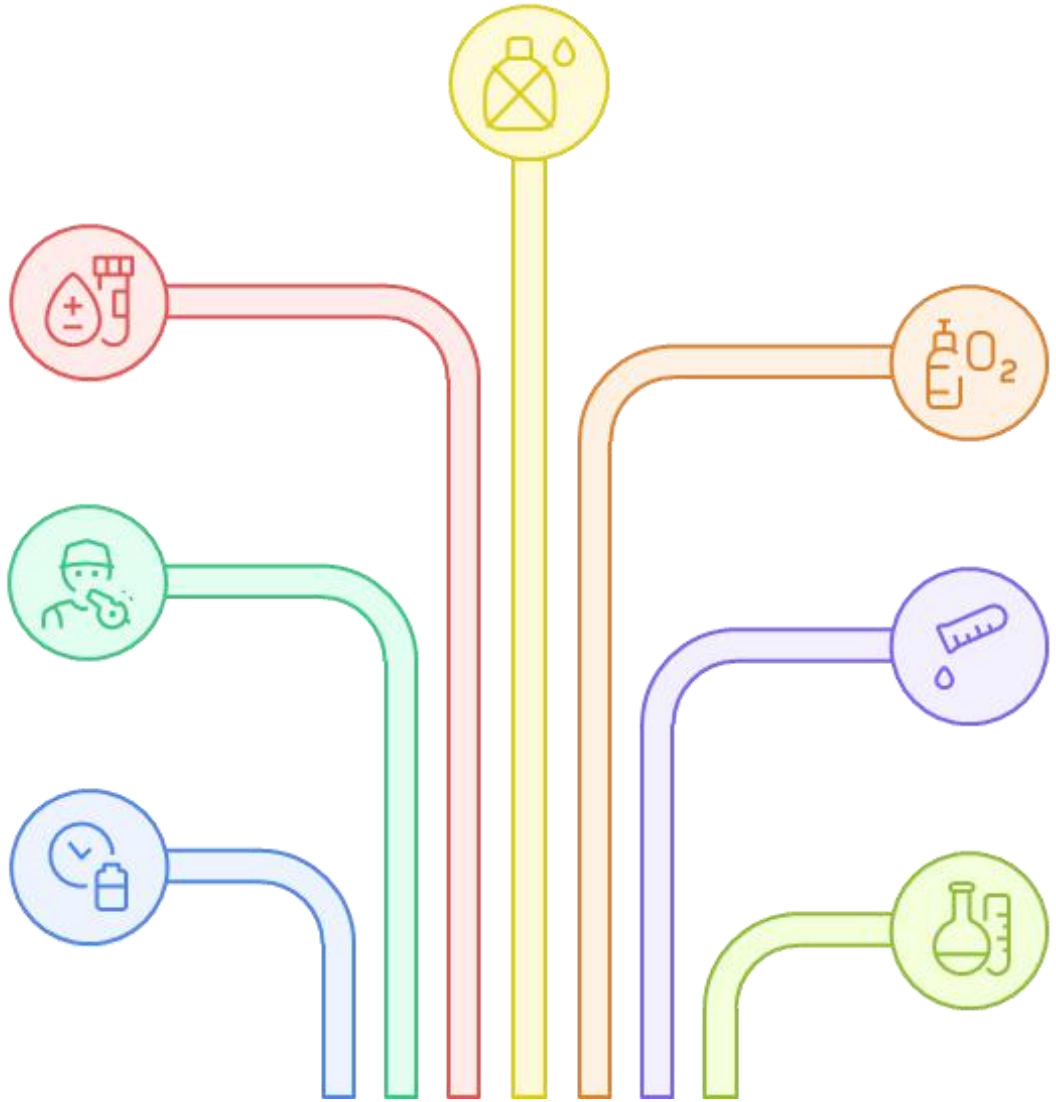
Choose phlebotomy or a fresh central catheter for optimal results.

Personnel

Select skilled personnel for accurate procedure execution.

Timing

Collect before antibiotic administration to avoid interference.



Bottles

Fill aerobic bottle first, then anaerobic for proper culture.

Volume

Collect 2 ml of blood, 1 ml per bottle for accuracy.

Transfer

Immediate lab transfer for timely inoculation.

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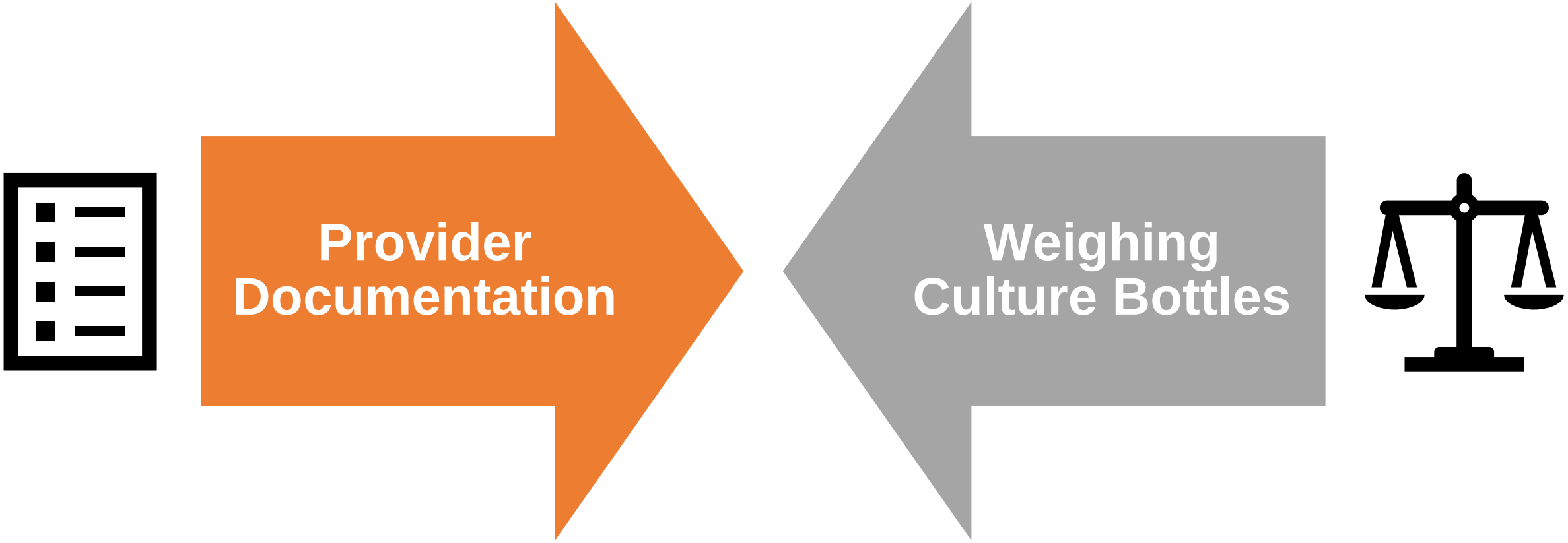
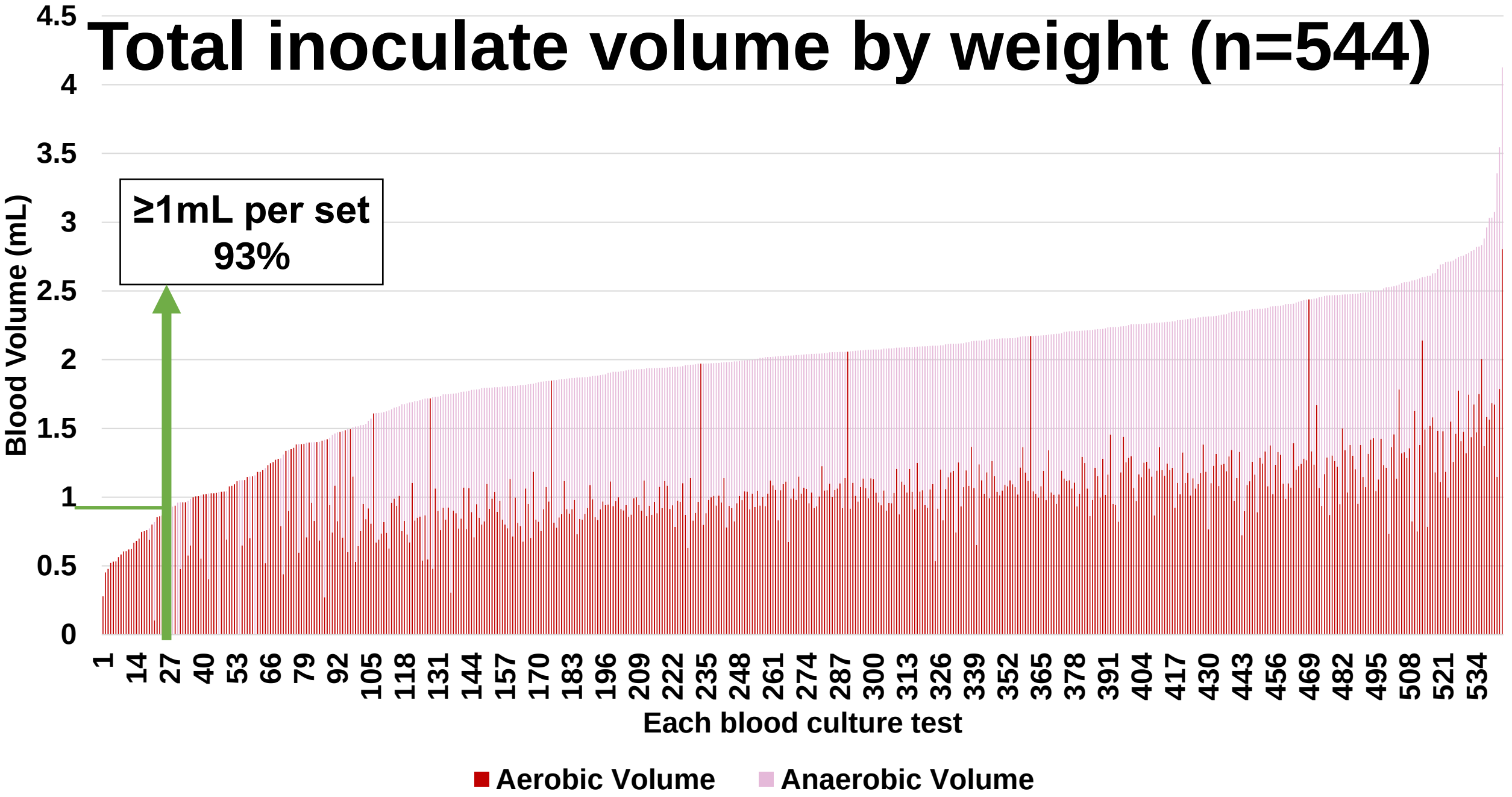
Tracking Technique



One year, single center

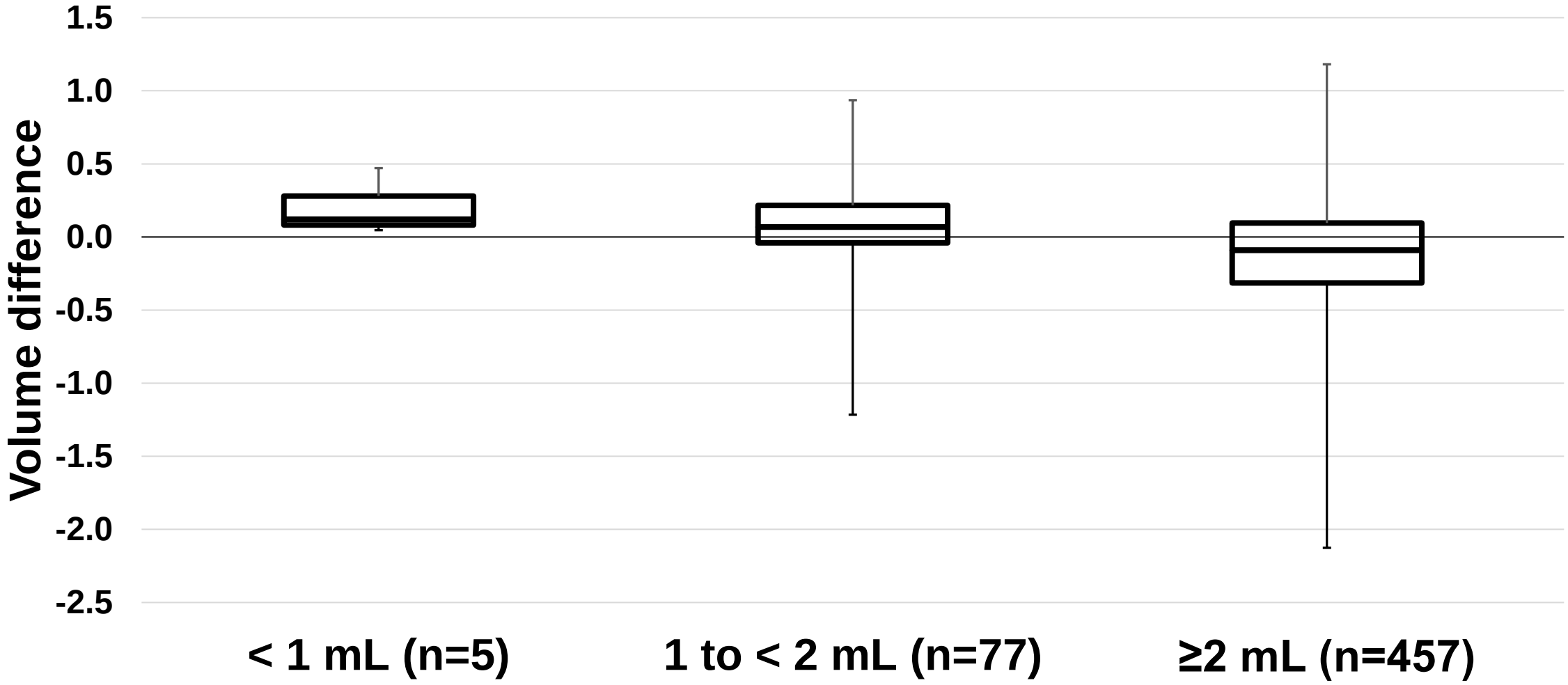
Woodford E …Mukhopadhyay S, *PedRes*, 2021


Total inoculate volume by weight (n=544)
≥1mL per set
93%
Blood Volume (mL)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1
14
27
40
53
66
79
92
105
118
131
144
157
170
183
196
209
222
235
248
261
274
287
300
313
326
339
352
365
378
391
404
417
430
443
456
469
482
495
508
521
534
Each blood culture test
Aerobic Volume
Anaerobic Volume

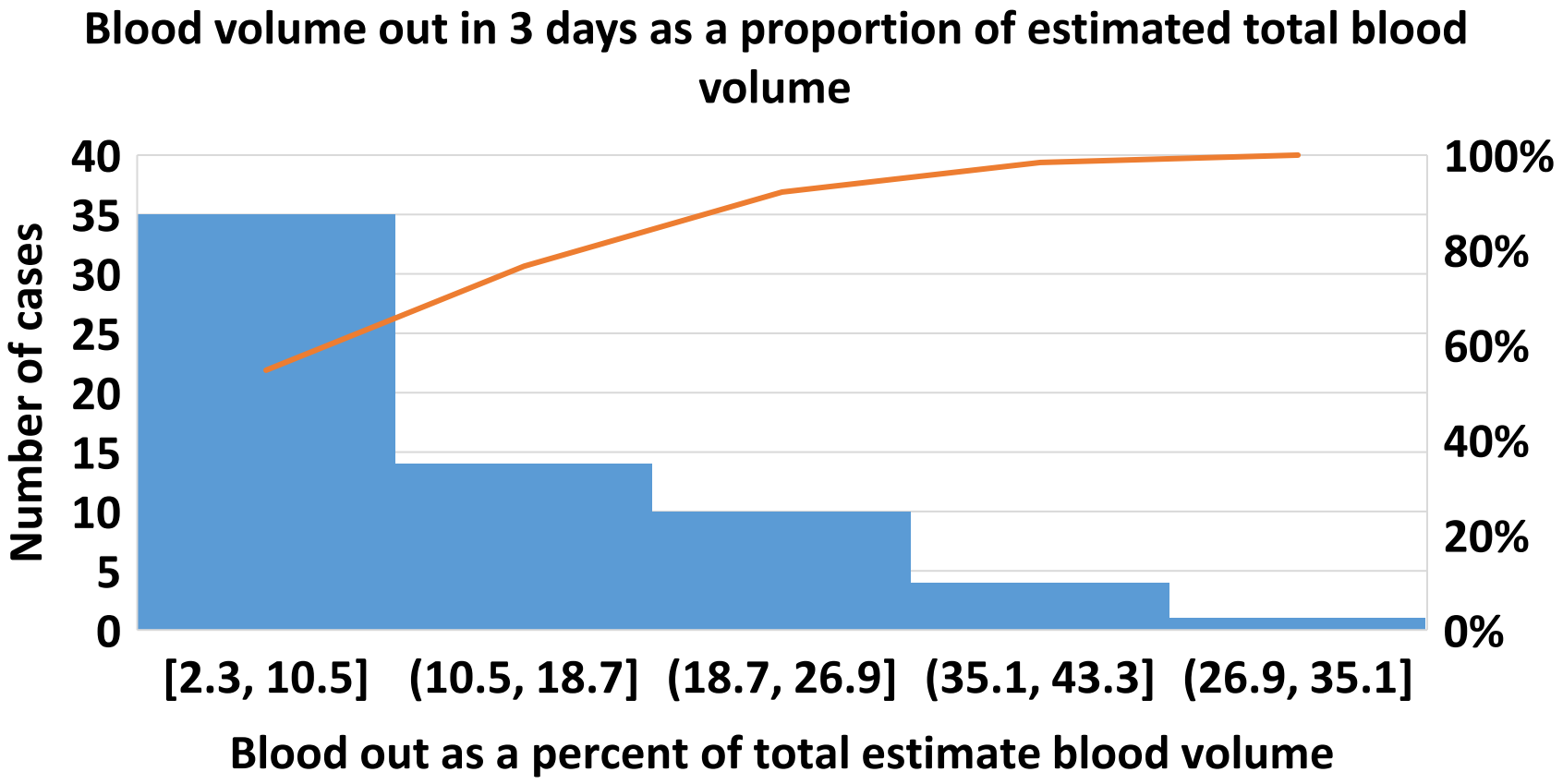
Lower inoculate in VLBW late-onset sepsis

Characteristics n (%) or median (IQR)	Not VLBW	VLBW & culture at ≤7 days	VLBW & culture at >7 days	p-value
Blood culture, n	380	60	104	
Total volume (mL) per set	2.1 (1.9-2.3)	2.0 (1.7-2.1)	1.8 (1.2-2.1)	<0.001
≥1 mL per set	362 (95.3)	57 (95.0)	89 (85.6)	0.002
<1 to 0.5 mL per set	17 (4.5)	2 (3.3)	14 (13.5)	0.002
<0.5 mL per set	1 (0.3)	1 (1.7)	1 (1.0)	0.32
At least one bottle with ≥1 mL	307 (80.8)	43 (71.7)	68 (65.4)	0.003

Reported minus weighed volume



What about blood loss?

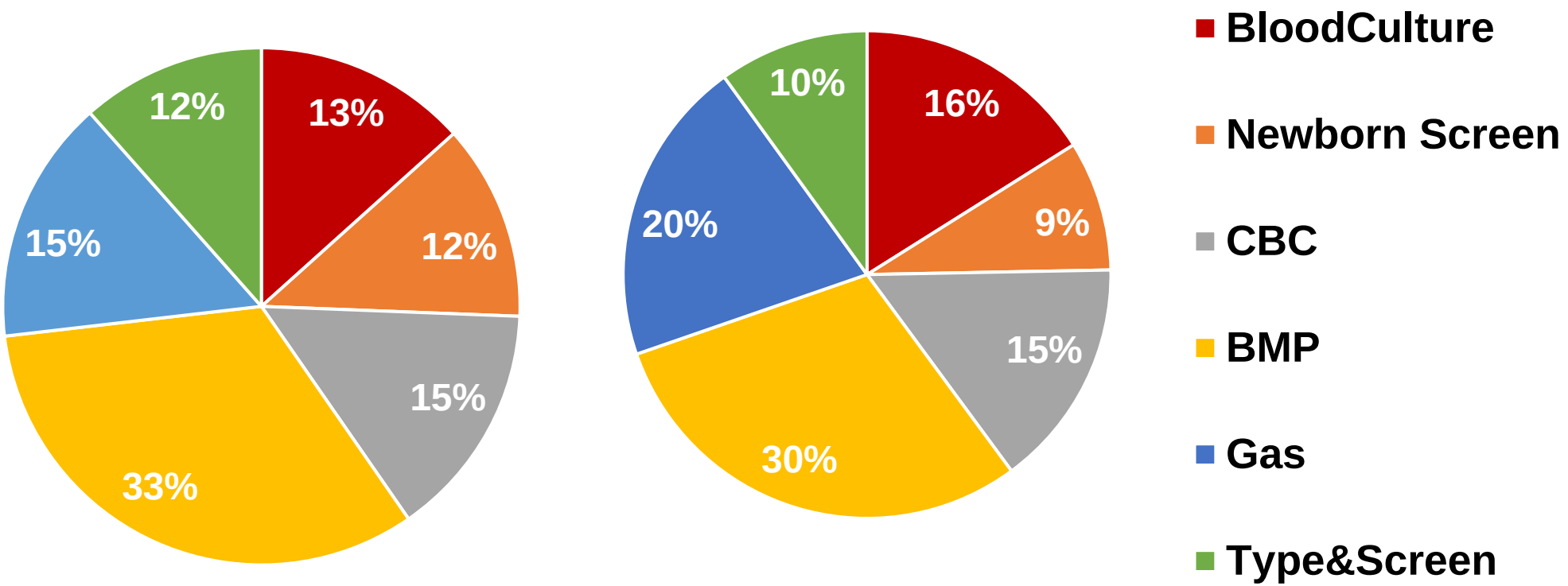


One year, 64 VLBW infants

May M, Dhudasia M, Mukhopadhyay S, PAS 2019

Tests contributing to total blood out in first 3 days

Among infants with a blood culture

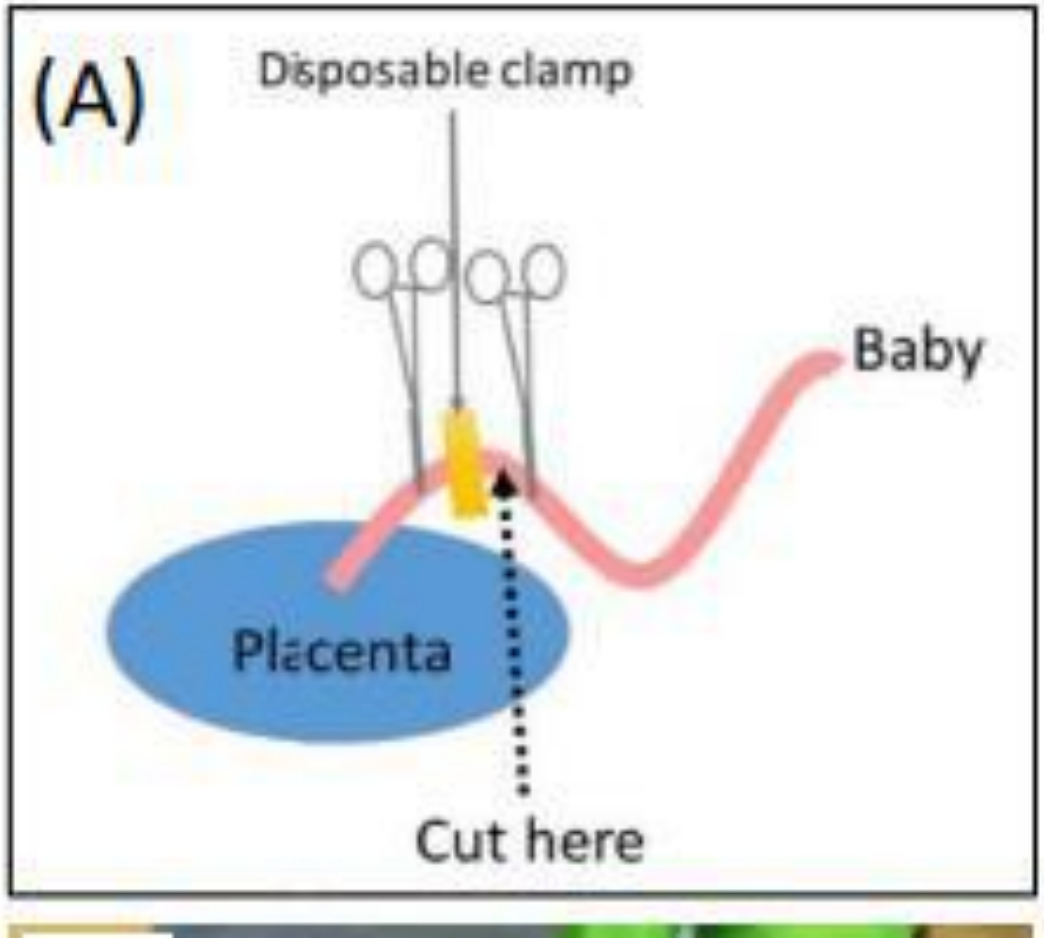


- Basic metabolic Panels (BMP, electrolytes), bilirubin was the highest contributor
- Complete blood count (CBC) ad blood gases were the second highest
- Blood culture was only the third largest contributor to blood loss

Addressing Blood Volume Loss

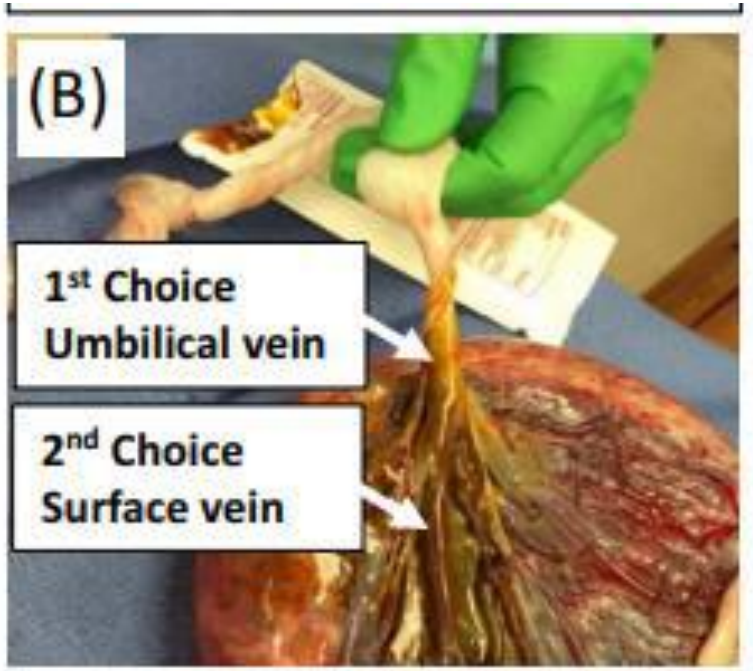
- **Optimize conducting sepsis evaluations**
  - **Stop sending culture on low-risk infants**
- **When doing it, do it well**
  - **Reduces contamination and need for repeats**
- **Optimize all testing including sending ‘routine labs’, serial biomarkers etc**
- **New approaches such as cord blood cultures**

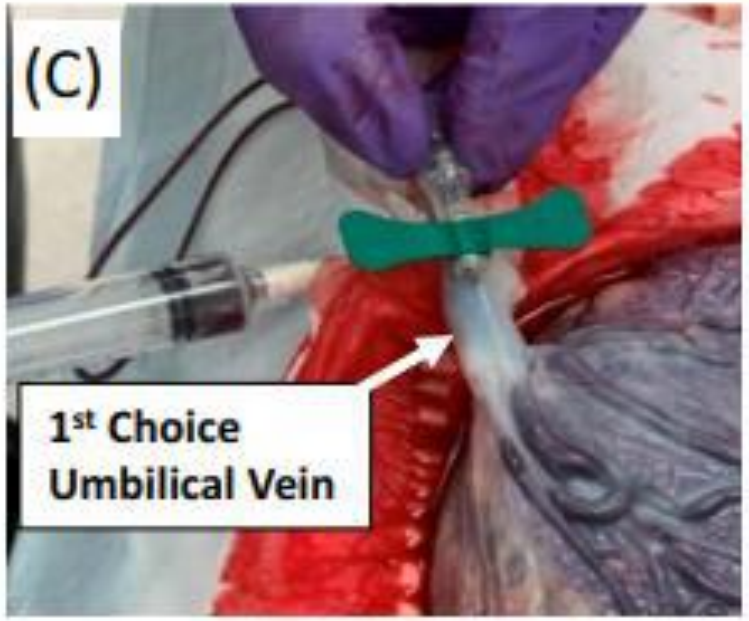
1	**Effectively clamp cord (A).** Leave cord segment clamped (after delayed cord clamping), replace with disposable clamp (optional), and place in sterile basin *-Prevents blood leakage form cord and placenta*
2	**Determine blood tests and blood volume needed.** *-Maximizes the number of tests that can be sent using cord blood to decrease infant blood loss. PCBS volume is about 5 to 10 mL.*
3	**Prepare work space.**
4	**Prep phlebotomy site.** -Dry site and remove coating first with sterile gauze to enhance antiseptic effectiveness. -Then apply antiseptic for 30 seconds (eg, chlorhexidine, povidone-iodine or isopropyl alcohol) and then let dry (takes ≥60 seconds). *-Creates sterility for blood cultures*



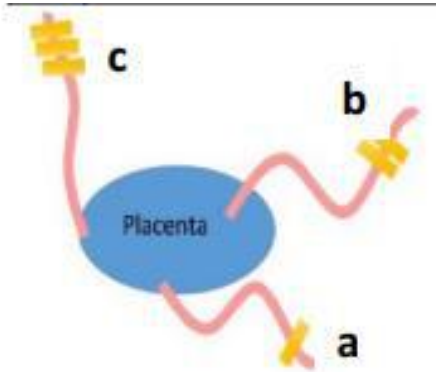
Kaufman et al. COFN, *Pediatrics* April 2025

5	**Draw laboratory sample** by puncturing the umbilical vein as 1st choice or a large surface vein as 2nd choice **(B and C).** -Limit venipuncture to 2 sites to decrease risk of contamination and of team member injury. -Bevel-down approach improves success by preventing vessel collapse. *-Prevents contamination and staff injury*

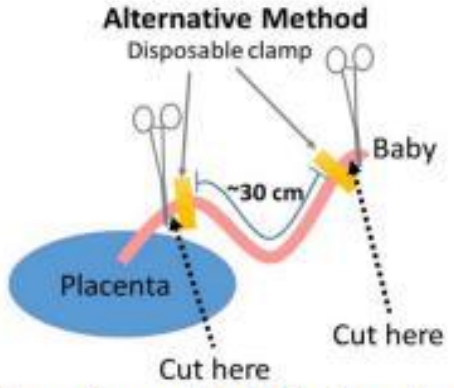




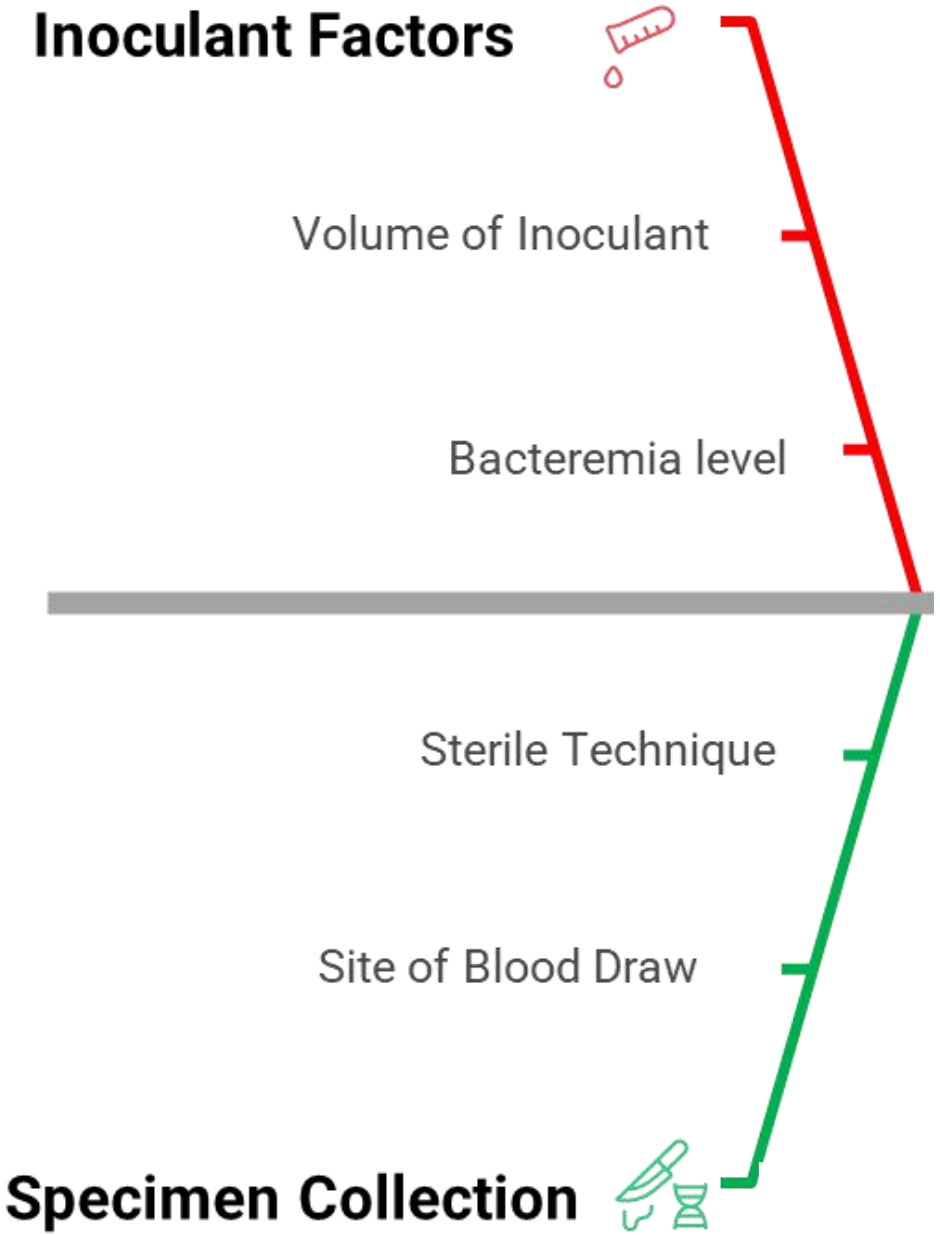
6	**Transfer blood to laboratory tubes.** For blood culture(s), alcohol top of bottle, transfer first with new sterile needle or transfer device. *-Prevents contamination and staff injury*
7	**Return placenta to container**. Do not return to the sterile field. *-Prevents contamination if sterile field present*
8	**Transport blood specimens** to NICU/Nursery. -Label each specimen and the transport bag. -Double bag samples for transport -Print and affix laboratory labels for specimens *-Ensures safe transport and proper assignment of specimens to the correct patient*
9	**Document the procedure.** Include blood culture volume if obtained. Document tests obtained and the disposition of specimens (sent to lab or location stored in some cases—eg, genetics)



For multiples: 2 clamps for cord of baby "b", 3 clamps on cord of baby "c"



Alternative method: Venipuncture either end of double-clamped blood filled umbilical cord segment (~30 cm)



- **Obtaining adequate volume is feasible**
- **Provider documentations encourage attention and accountability**
- **Address volume loss but not at the cost of compromising test performance**

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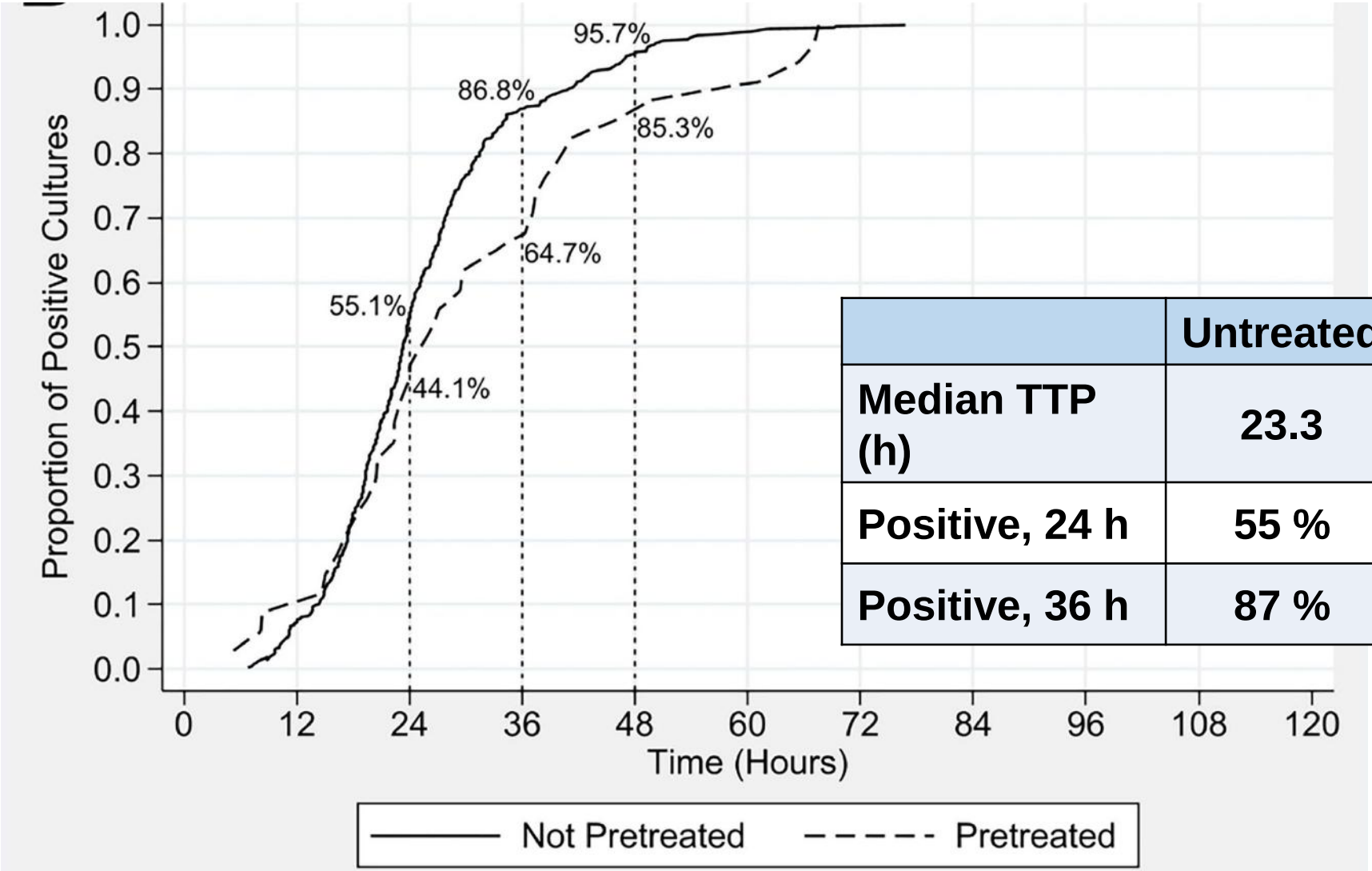
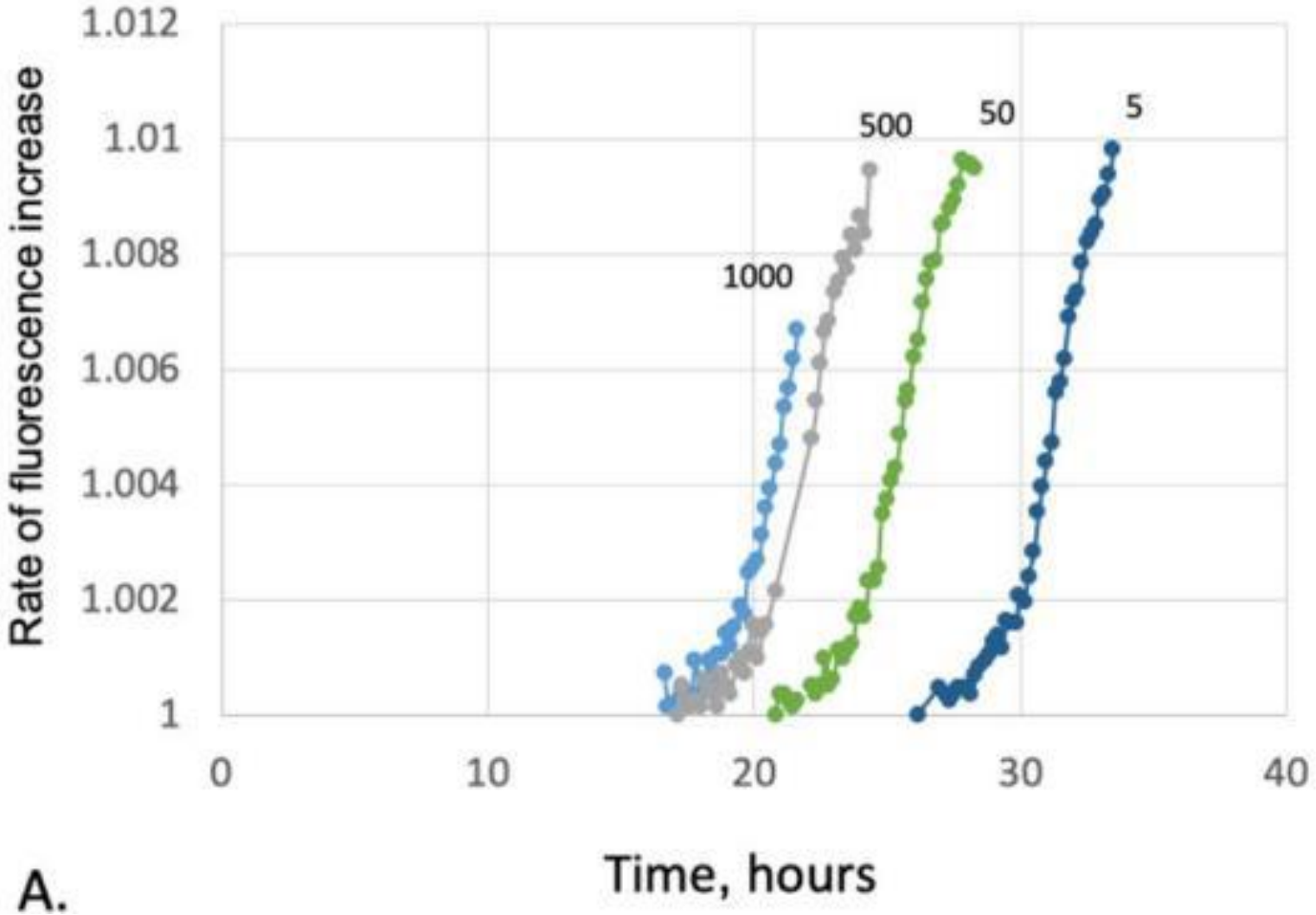
Pre-treated Blood Cultures



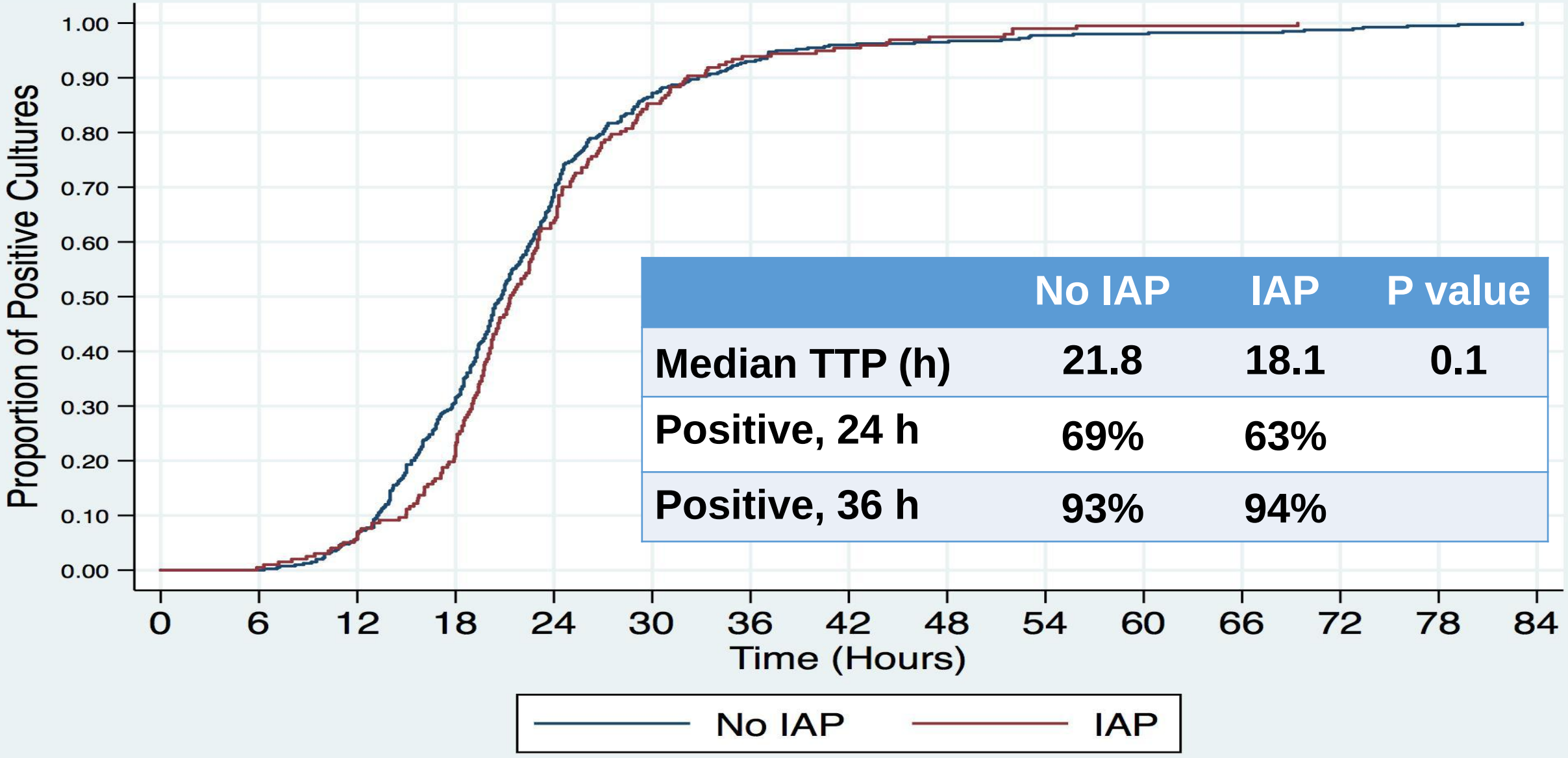
	Untreated	Pretreated	*P*
Median TTP (h)	23.3	25.8	0.12
Positive, 24 h	55 %	44 %	0.22
Positive, 36 h	87 %	65 %	0.001

Lower the bacteremia longer the TTP



Turkletaub et al, Eur Clin Mic ID 2024

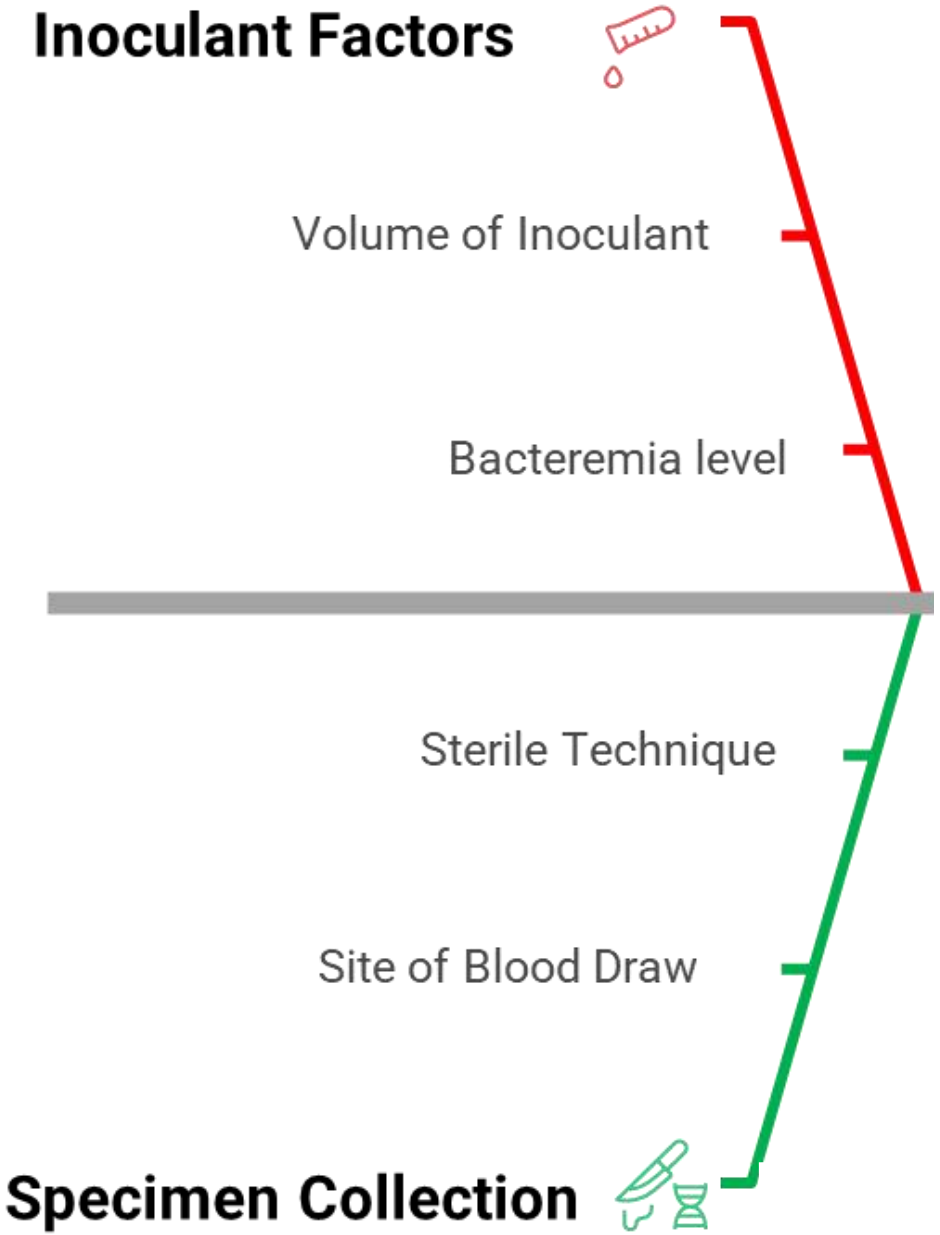
TTP: No Effect of Maternal Antibiotics



	No IAP	IAP	P value
Median TTP (h)	21.8	18.1	0.1
Positive, 24 h	69%	63%	
Positive, 36 h	93%	94%	

Maternal vs. Infant pre-treated blood culture

Characteristic	Maternal treatment	Infant treatment
Indication	Symptoms in mother	Symptoms in Infant
Administration	Indirect	Direct
Antibiotic Intention	Prophylactic for the infant	Treatment



- **Pre-treatment impacts culture performance only if administered to child directly**

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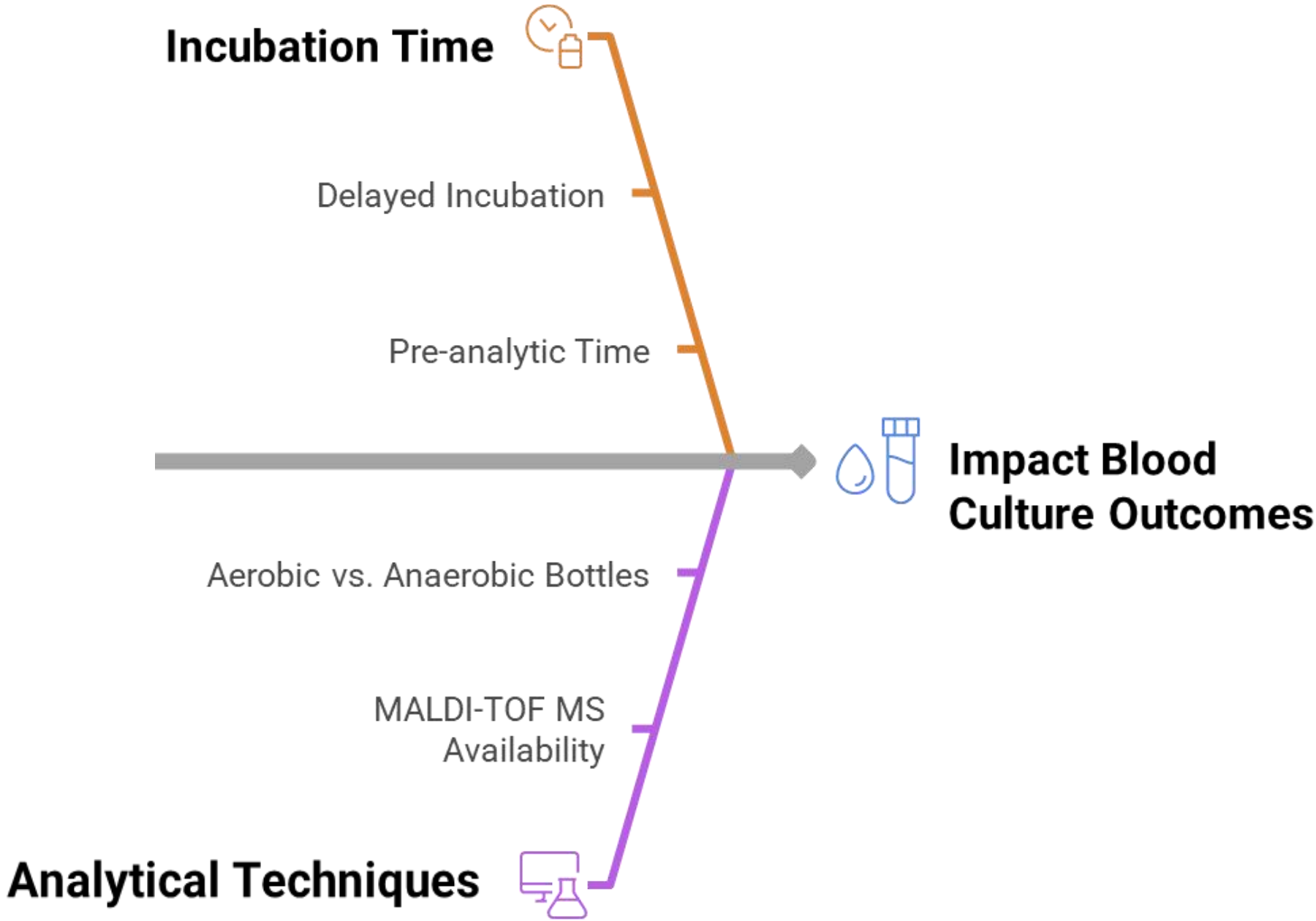

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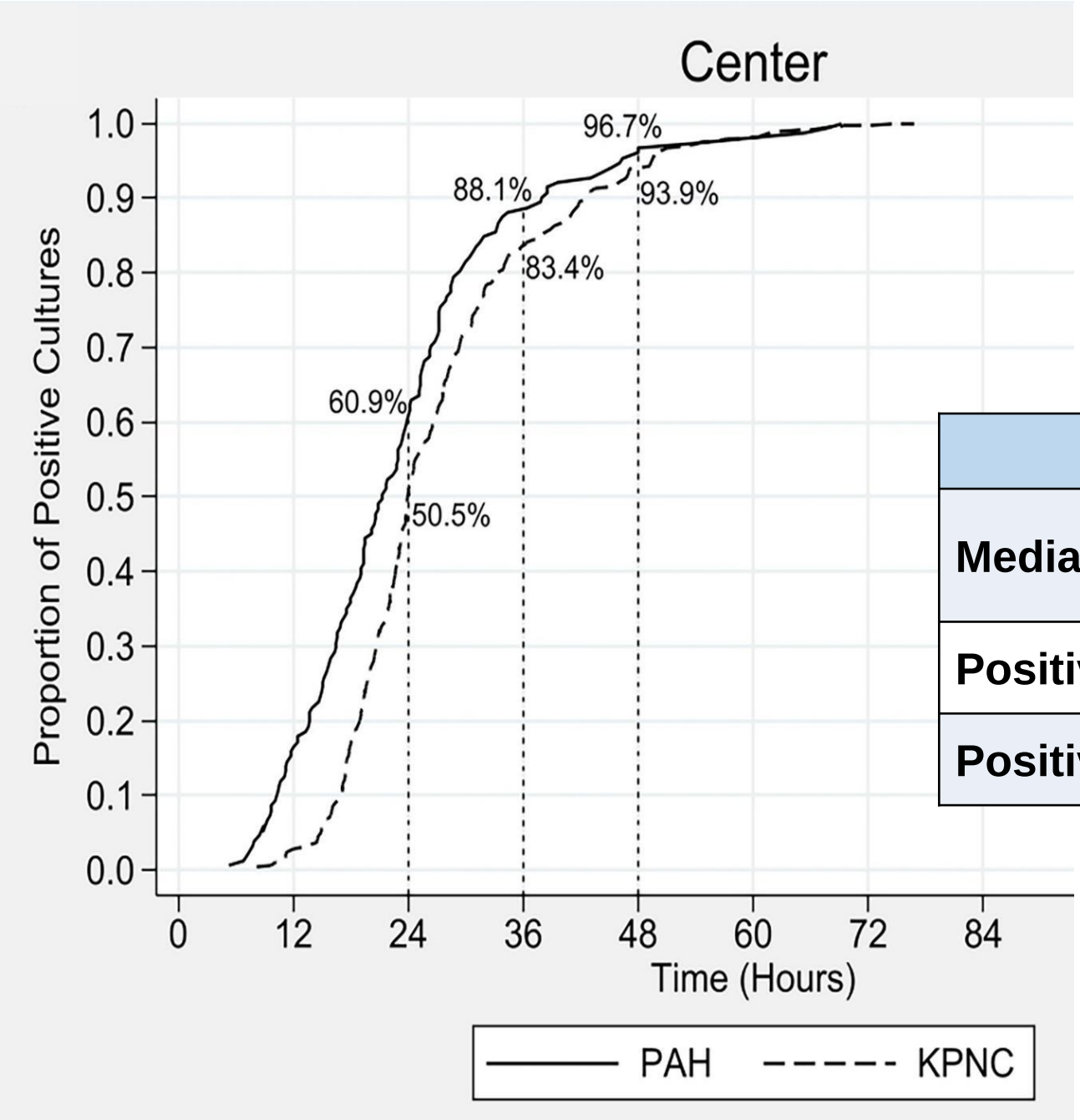
Delayed Incubation delays TTP



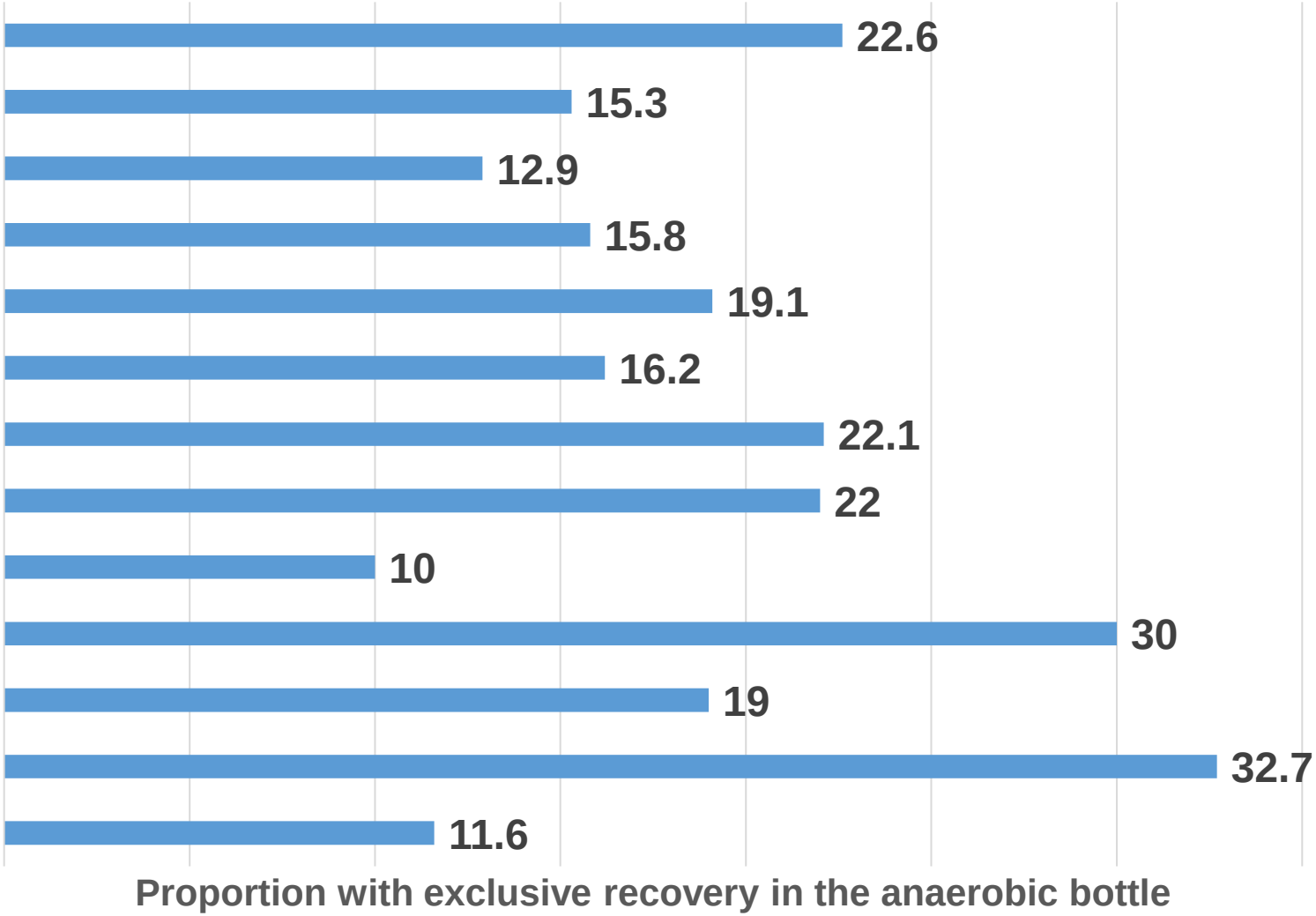
	Penn	KPNC	*P*
Median TTP (h)	21.3	24.0	<0.001
Positive, 24 h	61%	51%	0.04
Positive, 36 h	88%	83%	0.19

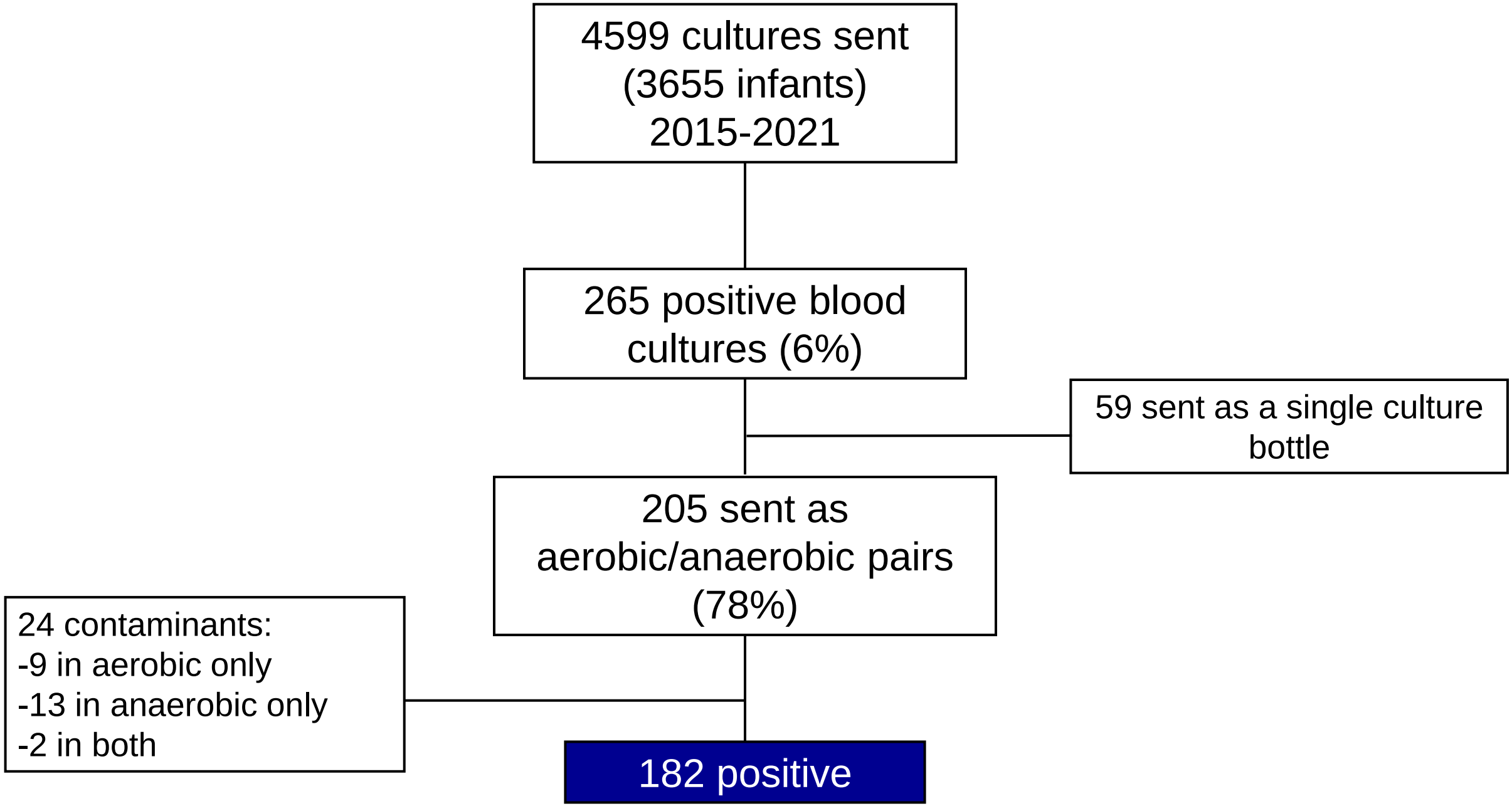
Anaerobic blood culture bottle

- Many units do not obtain anaerobic culture due to:
  - Obligate anaerobes rare pathogens
  - Blood loss
  - Non availability of anaerobic facilities
- Does sending anaerobic blood cultures add clinically useful information?

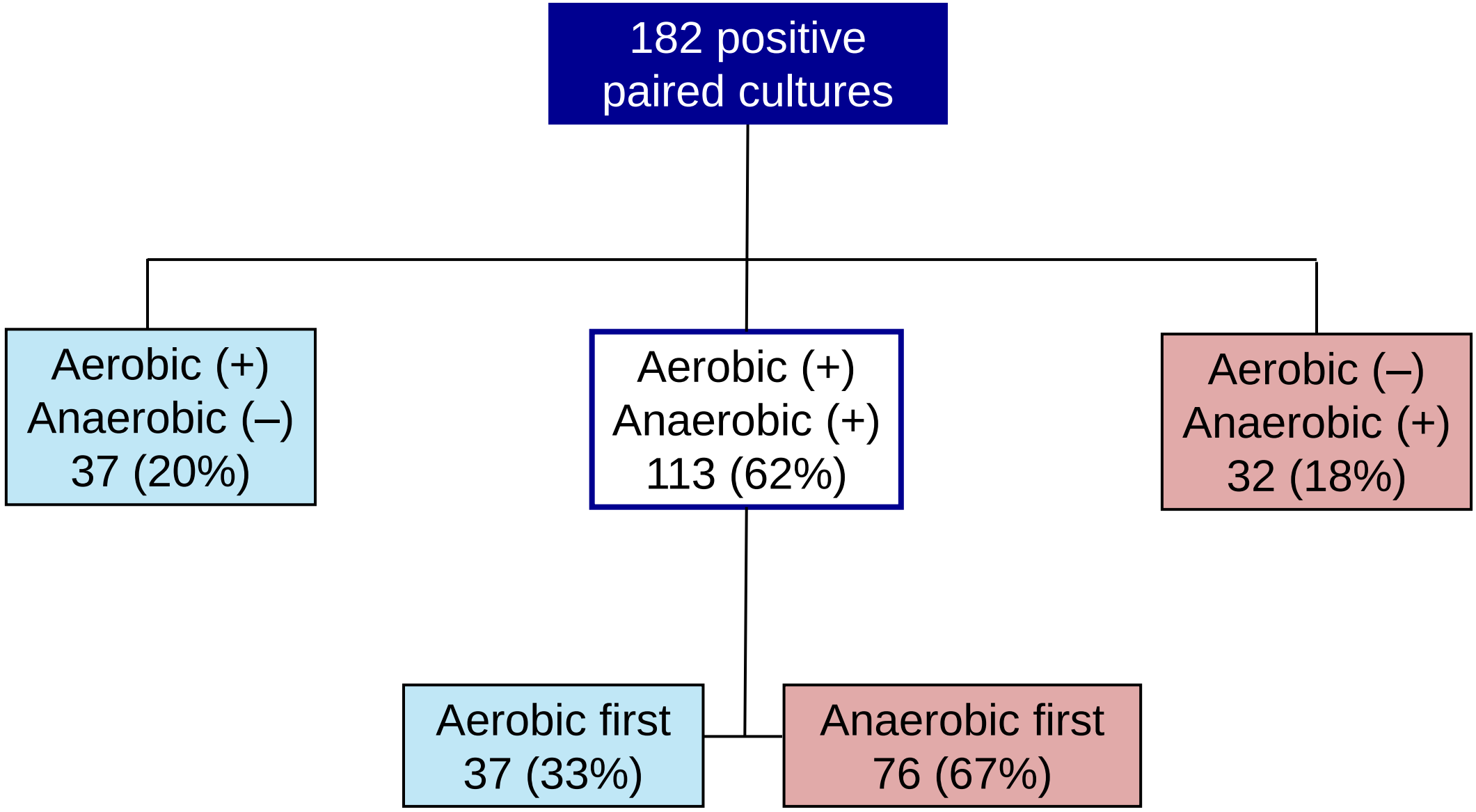
Exclusive recovery in anaerobic bottles

Gross I, *et al*. 2018	Neonates
Messbarger *et al*. 2018	Neonates
Créixems MR, *et al*. 2002	Pediatric
Dien Bard J, *et al*. 2020	Pediatric
Dunne WM Jr, *et al*. 1994	Pediatric
Gross I, *et al*. 2018	Pediatric
Kato H, *et al*. 2023	Pediatric
Chiueh TS, *et al*. 2013	Adult
Grohs P, *et al*. 2007	Adult
Lafaurie M, *et al*. 2020	Adult
Passerini R, *et al*. 2014	Adult
Wen H, *et al*. 2022	Adult
Ransom EM, *et al*. 2022	All





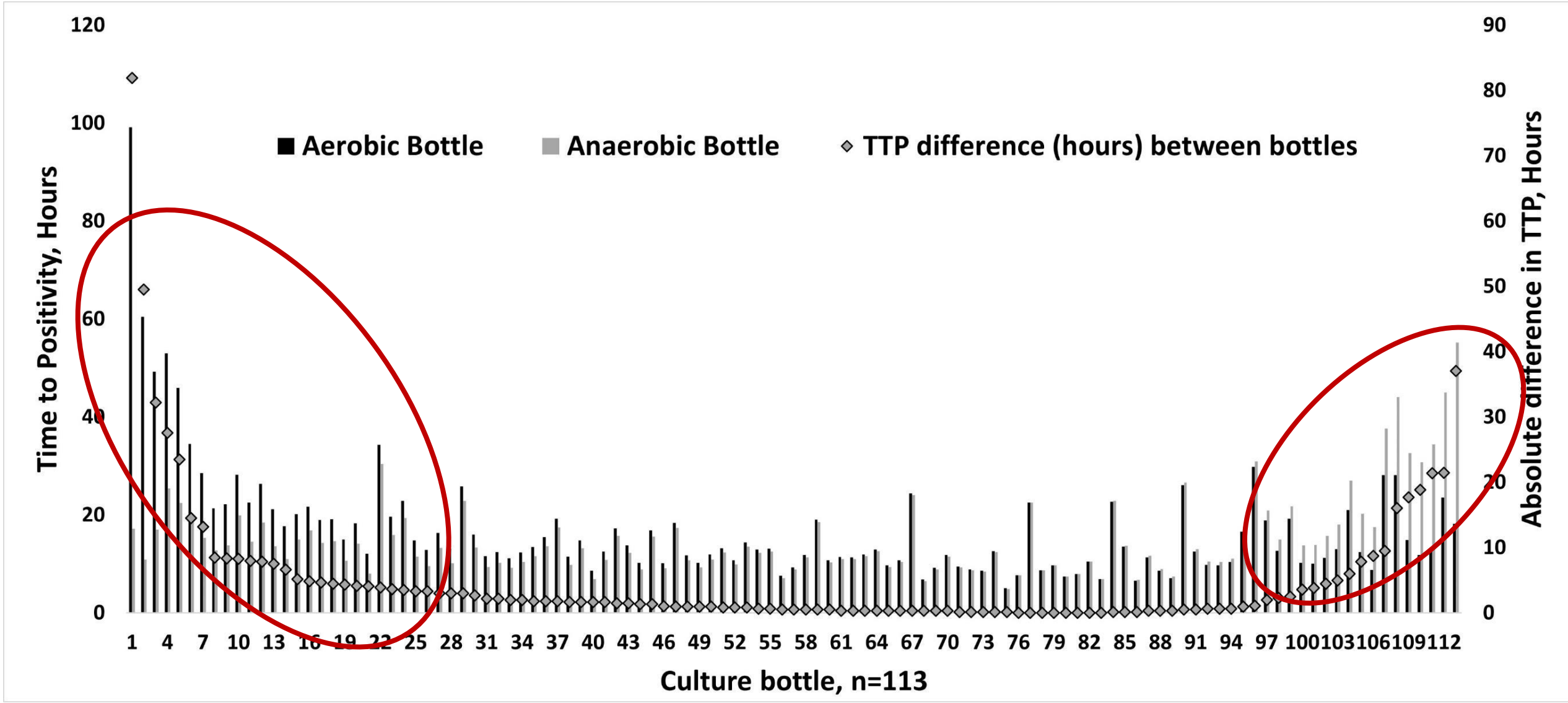
Gottschalk A..Mukhopadhyay S, [in print], JPIDS, 2024



18% of all positive cultures recovered only in the anaerobic bottle

When recovered in both, 67% recovered first in the anaerobic bottle

Anaerobic bottles often have a shorter TTP

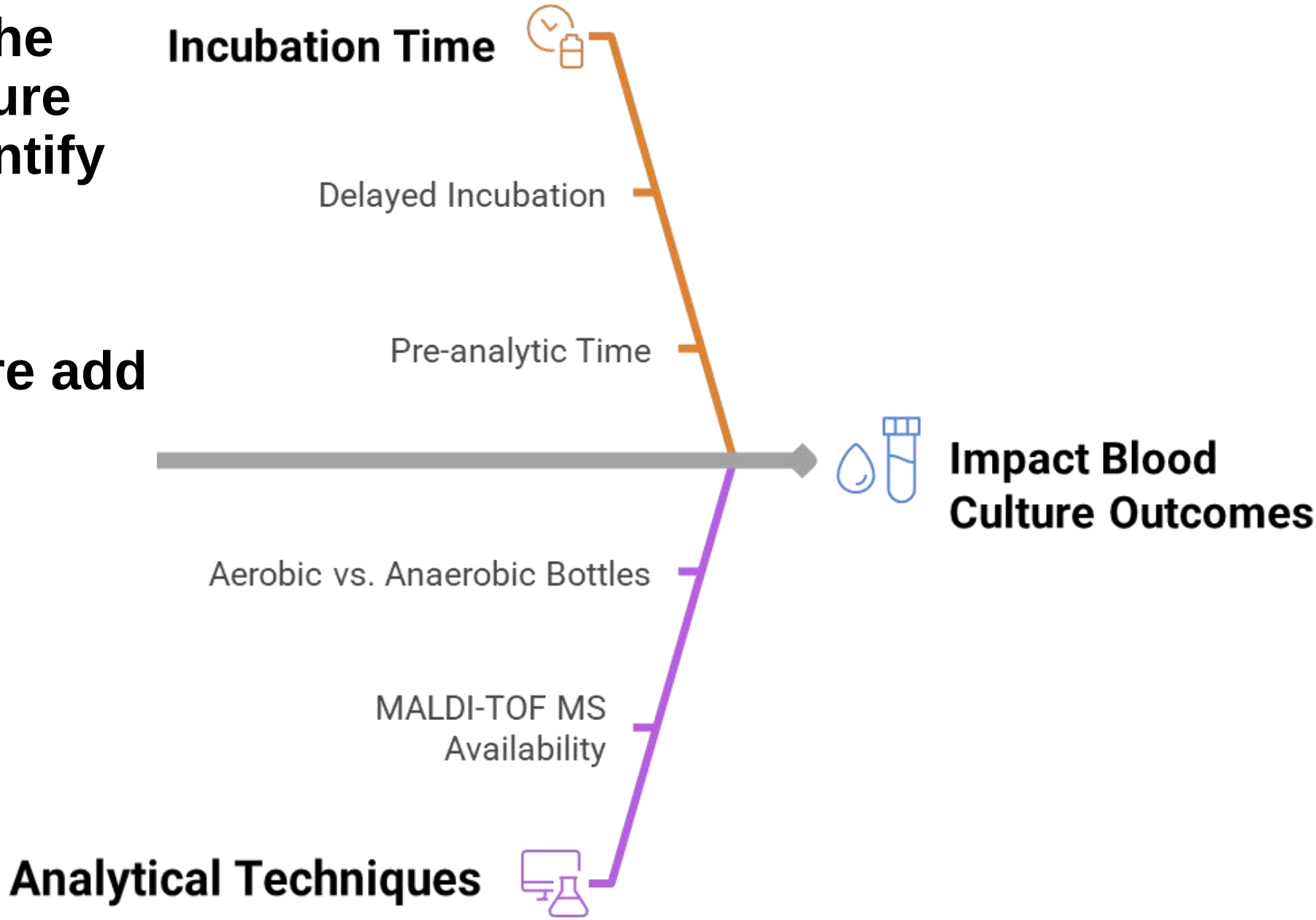


Facultative Anaerobes Detected in Anaerobic Only

	Total N = 182	Anaerobic Only N = 32	Aerobic Only N = 37
Gram positive (not CoNS)	**68**	**8 (11.8)**	**14 (20.6)**
Staphylococcus aureus[1]	36	5 (13.9)	7 (19.4)
Streptococcus agalactiae	16	1 (6.3)	1 (6.3)
Enterococcus faecalis	8	2 (25.0)	2 (25.0)
Other streptococci	8	0	4 (50.0)
CoNS	**35**	**12 (34.3)**	**3 (8.6)**
Gram negative	**63**	**11 (17.5)**	**17 (27.0)**
Escherichia coli	45	5 (11.1)	12 (26.7)
Klebsiella sp.	6	1 (16.7)	2 (33.3)
Serratia sp.	4	0	2 (50.0)
Enterobacter sp.	3	1 (33.3)	0
Bacteroides fragilis	2	2 (100.0)	0
Haemophilus influenzae	1	0	1 (100.0)
Morganella morganii	1	1 (100.0)	0
Prevotella loescheii	1	1 (100.0)	0
Fungus (*Candida albicans*)	**4**	**0**	**3 (75.0)**

- **Understanding the workflow of culture analysis can identify gaps**

- **Anaerobic culture add clinical value**



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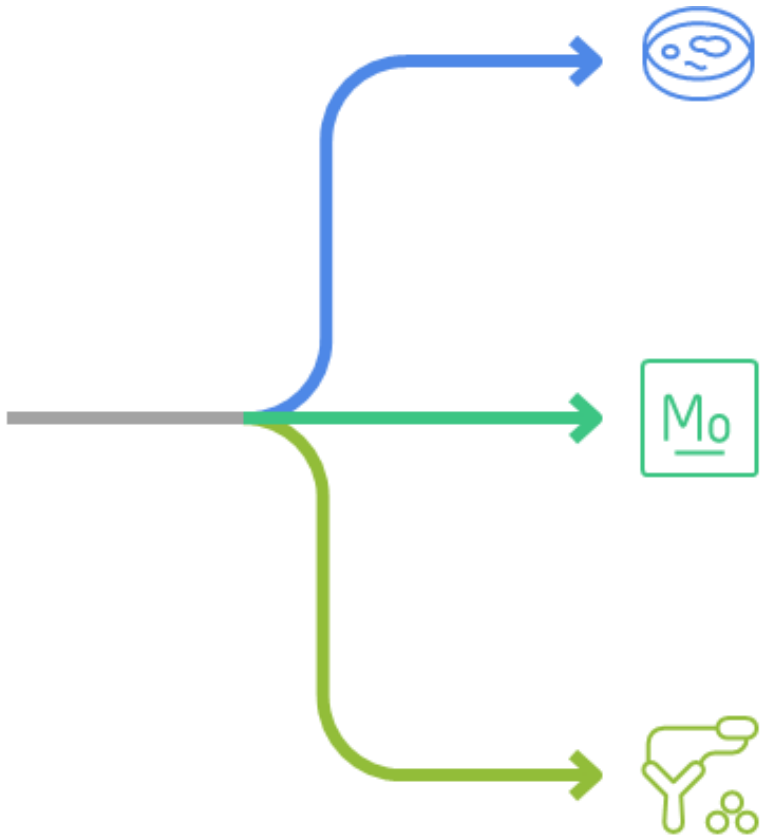
Reducing time from positive culture to pathogen identification



Traditional Methods

Involves Gram staining and phenotypic analysis, providing detailed results within an hour.

MALDI-TOF MS

Offers rapid identification using mass spectrometry, ideal for quick results.

PCR-based BCID

Accelerates identification through nucleic acid analysis, enabling earlier treatment adjustments.

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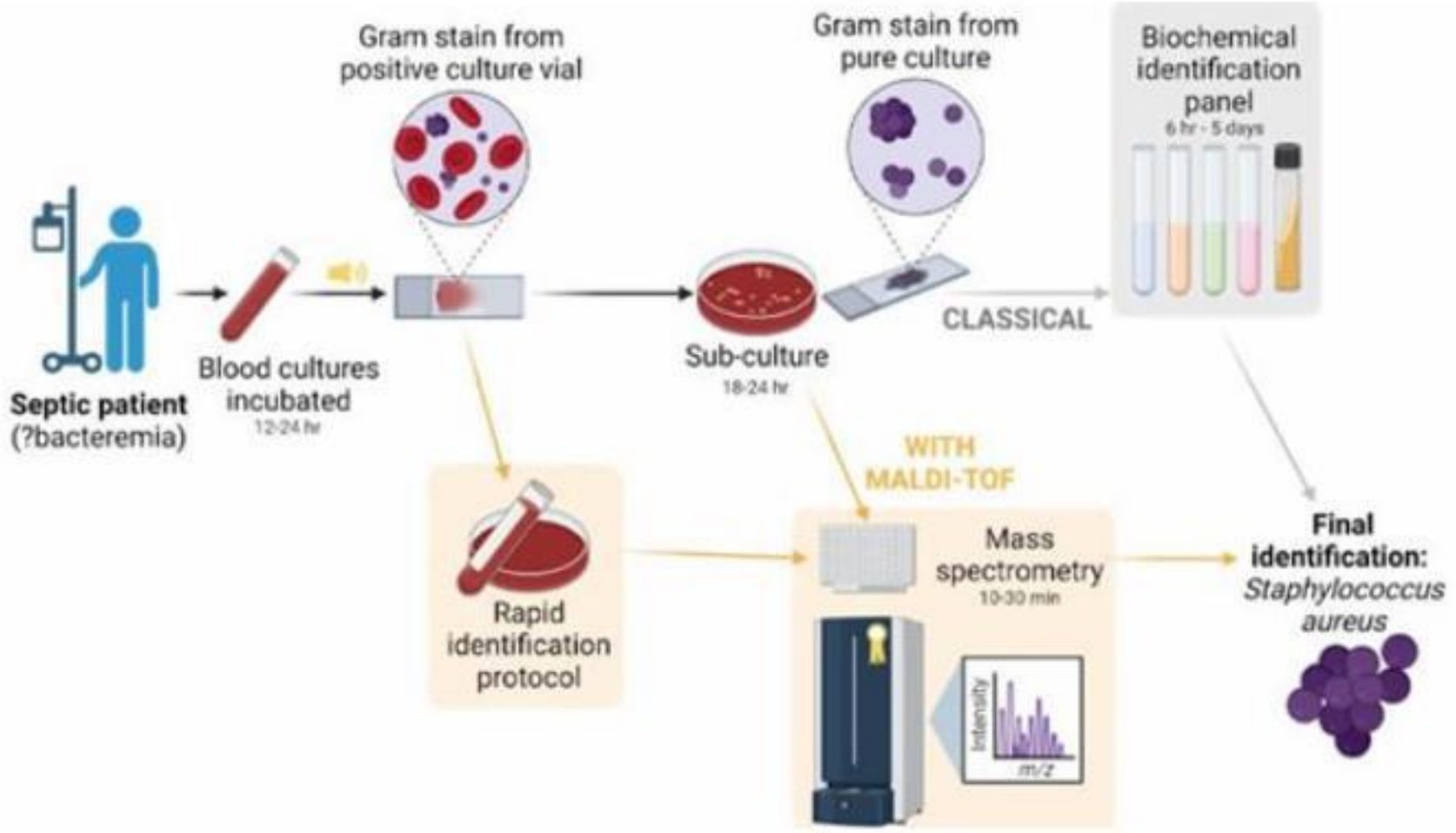
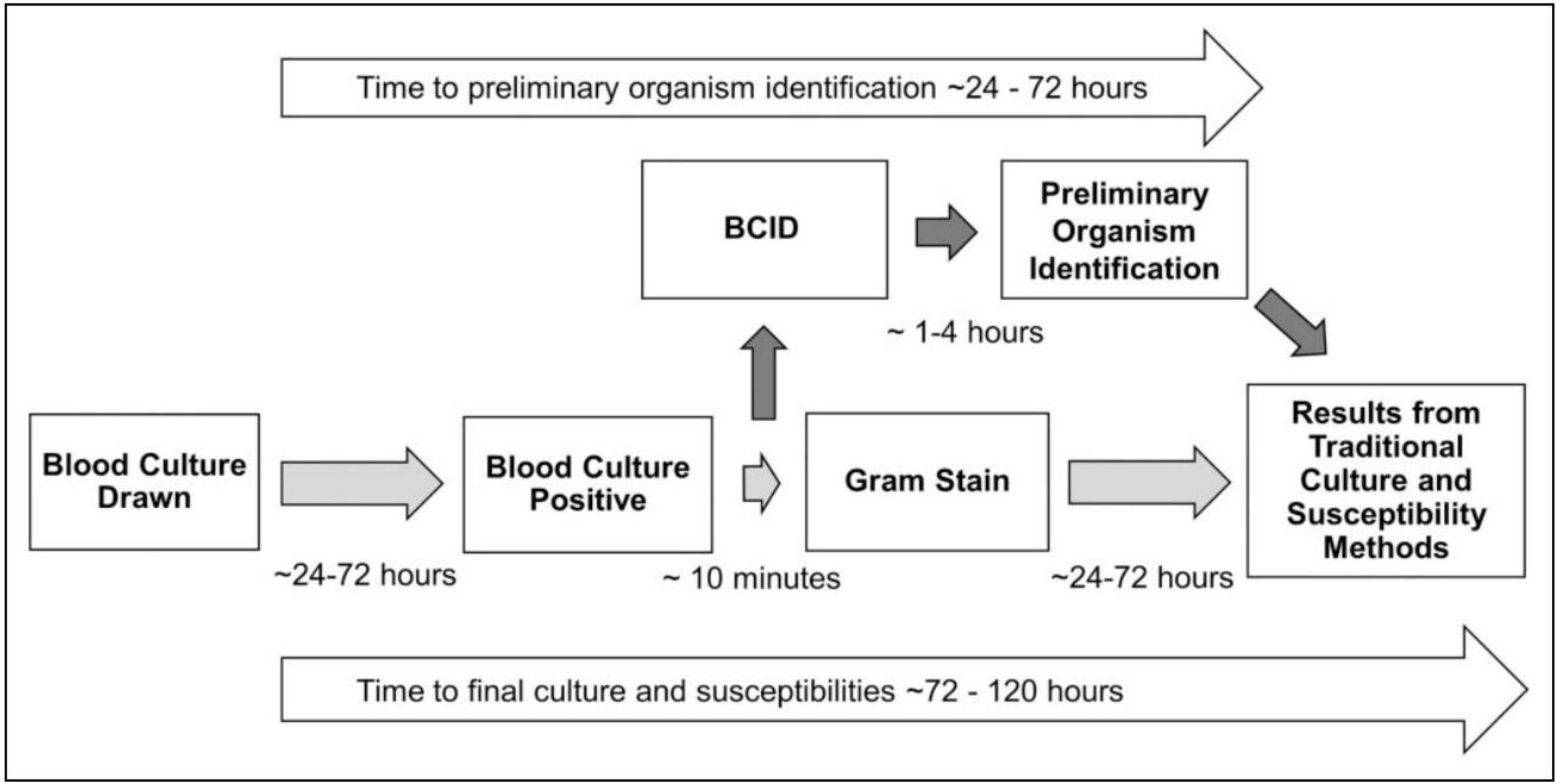
Matrix-Assisted Laser desorption/Ionization – Time of Flight



Workflow and timing for bacterial identifications (classical vs. MALDI).

Source: Created with BioRender.com by Natalie Marshall

Beyond the Matrix: Mass Spec as a Clinical Microbiology Tool



Chiasson JM, et al, *Journal of Pharmacy Practice*. 2021

S

Rapid pathogen identification

Faster results

- Faster turnaround time
- Broad pathogen coverage
- Antimicrobial resistance detection
- High agreement with cultures

W

Workflow and interpretation

Requires skilled clinical integration

- Workflow variability
- Genotypic Phenotype mismatch
- Neonatal-specific considerations
- Gram-stain dependency

O

Point-of-care testing

Expanding access, improving outcomes

- Off-label use potential
- Early antibiotic discontinuation
- Workflow standardization

T

Clinical interpretation needed

Requires careful result analysis

- Variable accuracy
- Polymicrobial infection challenges
- High costs
- Not all pathogens detected

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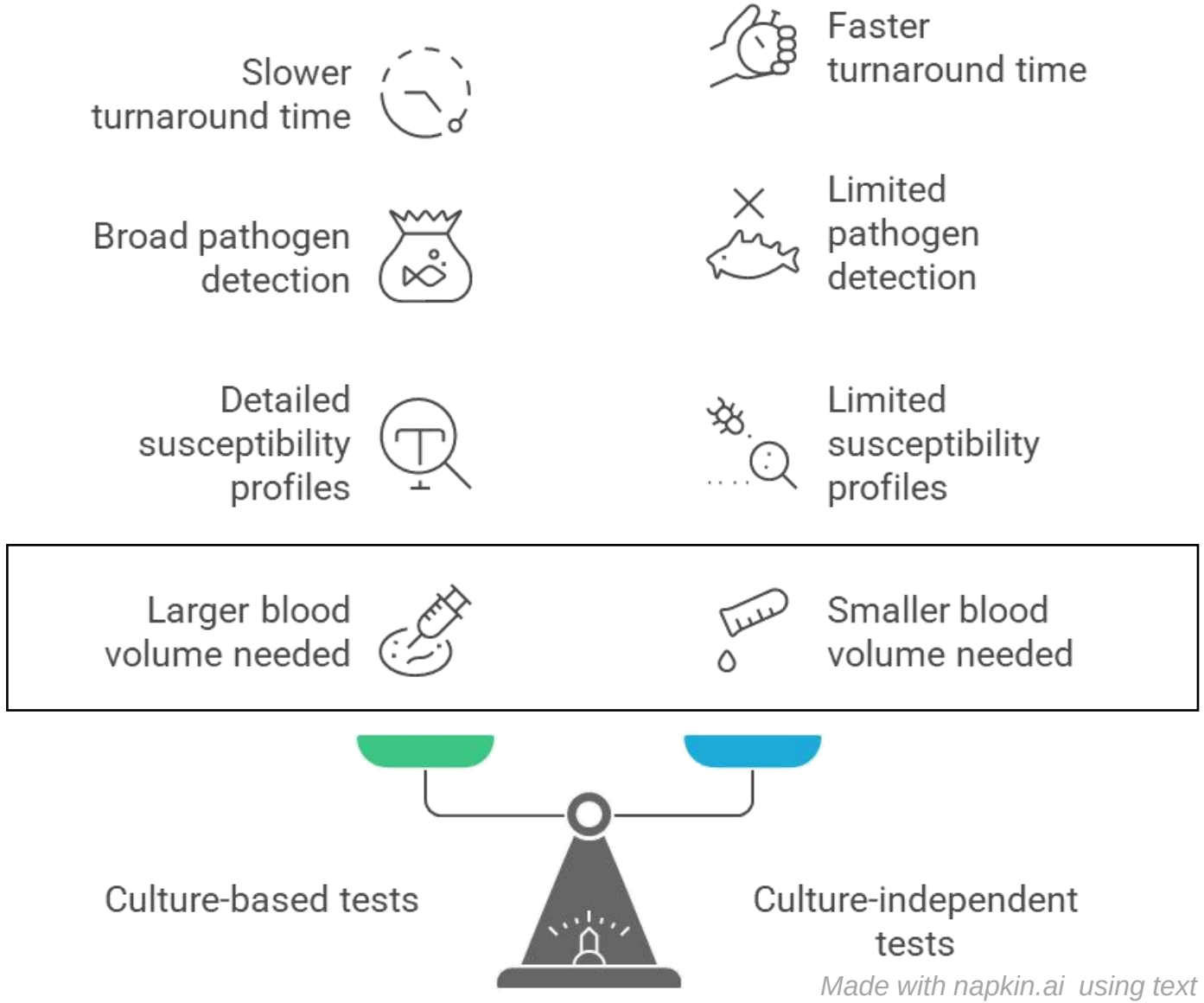
Culture-Independent Blood Testing



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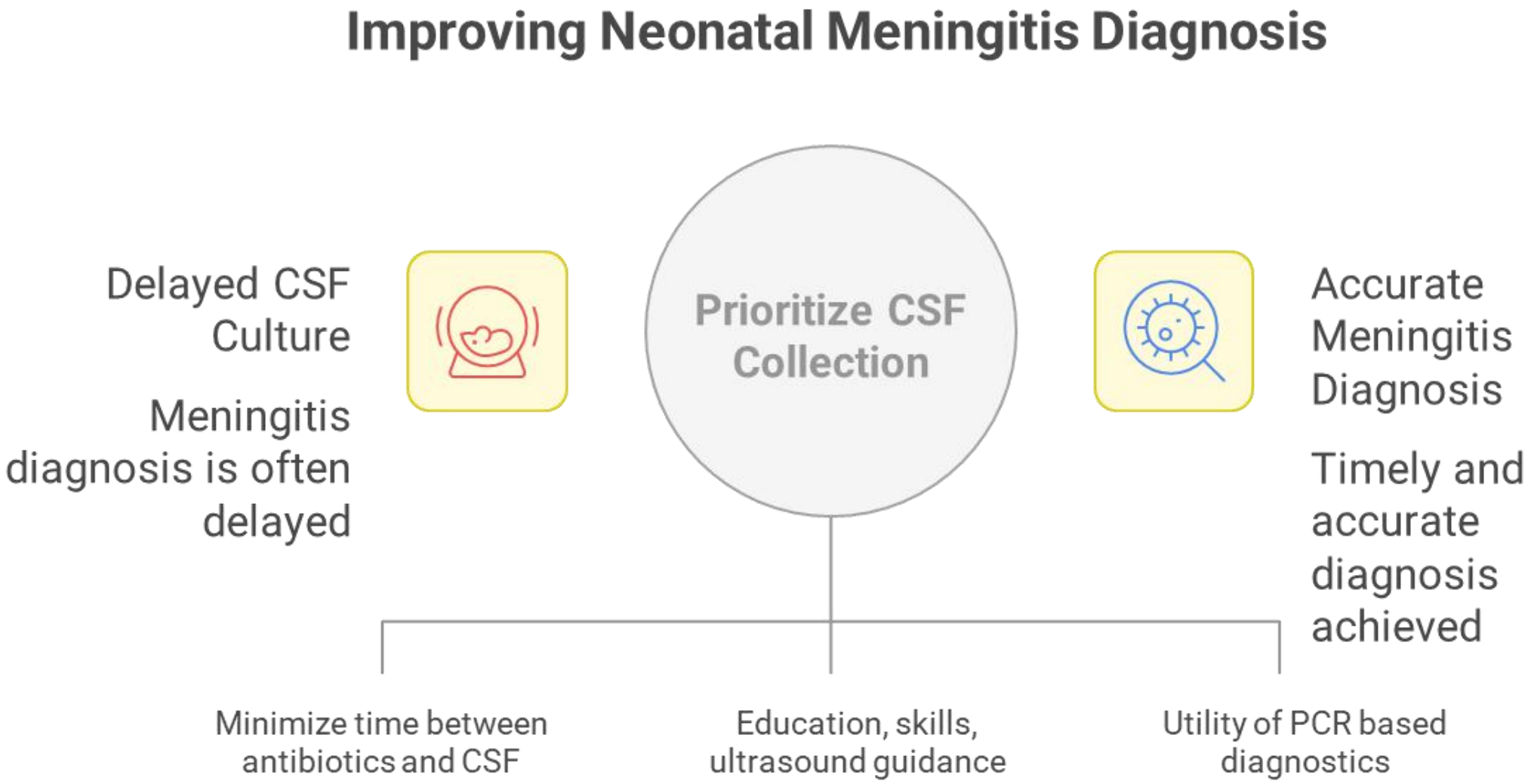
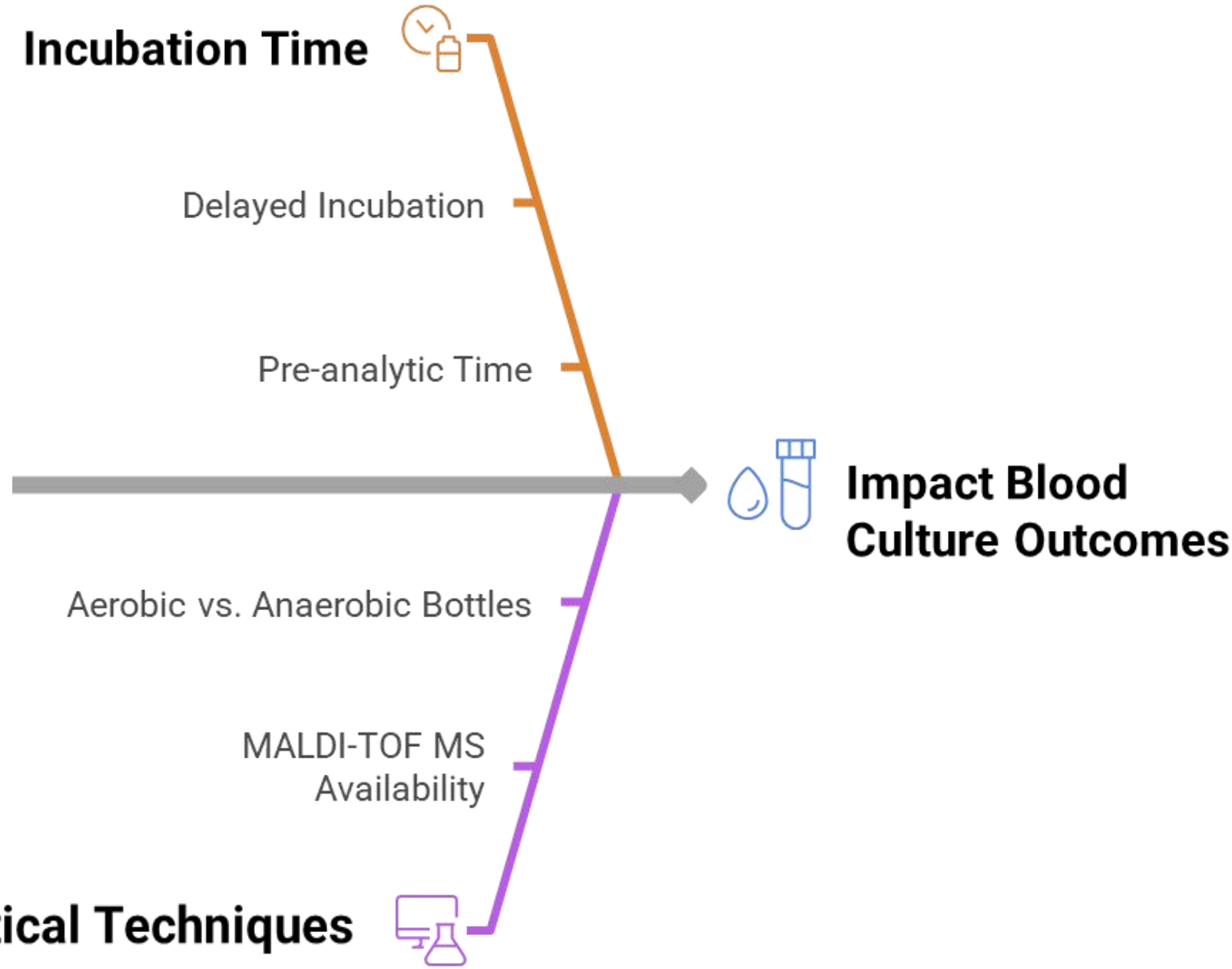
Culture-Independent Testing of Other Specimen



Made with napkin.ai using text from Rub et al, Seminars in Perinatology, 2020

- **Understanding the workflow of culture analysis can identify gaps**
- **Anaerobic culture add clinical value**
- **Combining with molecular diagnostics can reduce time to pathogen identification**

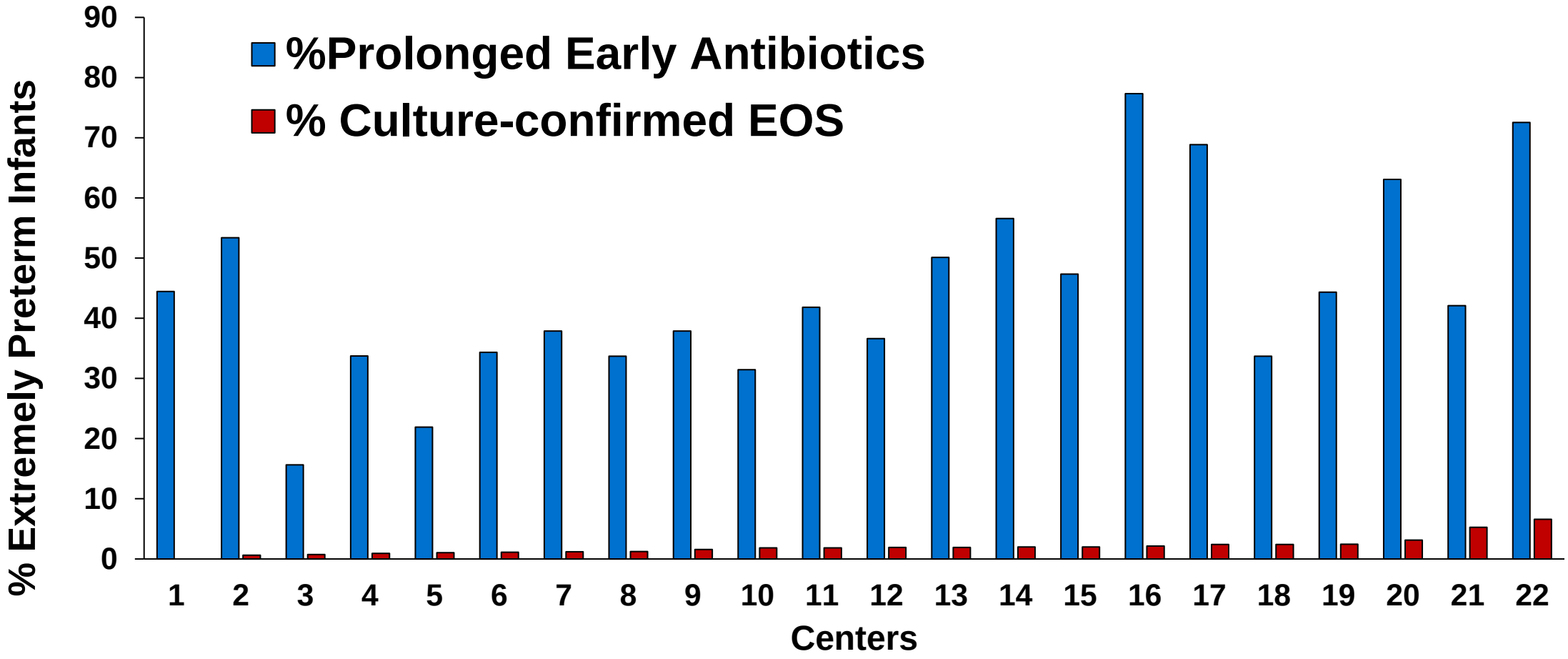


Made with napkin.ai using text from Rub et al, Seminars in Perinatology, 2020

Culture Negative Infection (CNI)

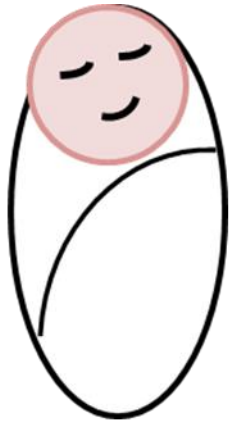
- **Preterm infants are frequently administered prolonged antibiotic therapy in the absence of culture-confirmed infection**
- **Rationale of therapy is that the infant has bacterial infection not isolated in culture**
- ***Goal of therapy is to prevent death or morbidity from untreated infection***

Wide Variation in Diagnosis of CNI



Puopolo KM, Mukhopadhyay S et al. *Pediatrics* 2017

Approach to Understanding CNI

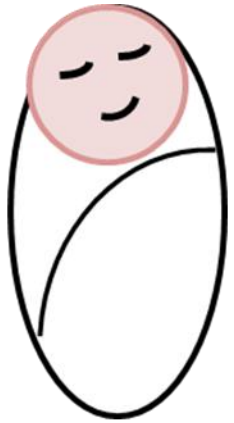


Blood Culture Negative

Antibiotics

No Antibiotics

Outcomes different?



Complete Antibiotic Course

Blood culture +

Blood Culture -

Outcomes same?

Retrospective Study Design

- **Study population: Infants born 2006-2014 at 24 participating centers of the Neonatal Research Network (NRN)**
  - **Gestational age 22 0/7-26 6/7 weeks**
  - **Birth weight 401-1000 grams**
  - **Survival >12 hours and without major birth defects**

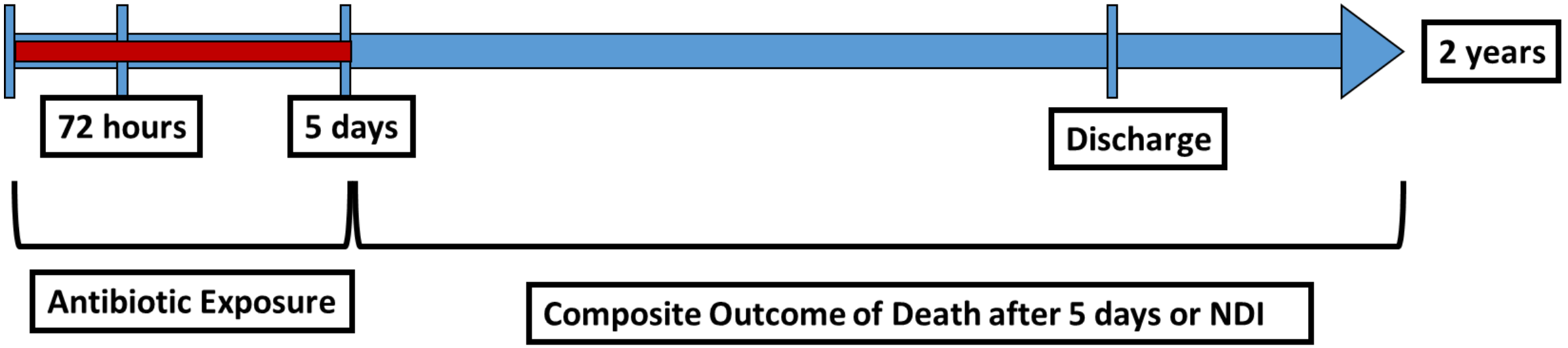
Definitions

- **Standard indications for antibiotics: culture-confirmed infection; necrotizing enterocolitis (NEC); spontaneous intestinal perforation (SIP)**
- **Prolonged Antibiotics: antibiotics continued for ≥5 days without standard indications**

Outcomes

- **Death: After exposure till hospital discharge**
- **NDI: 18-26 months corrected age, one or more of**
  - **Bilateral blindness**
  - **Hearing impairment ± amplification**
  - **Gross motor function classification system (GMFCS) level ≥2, including moderate-severe cerebral palsy**
  - **Bayley-III cognitive composite score <85**

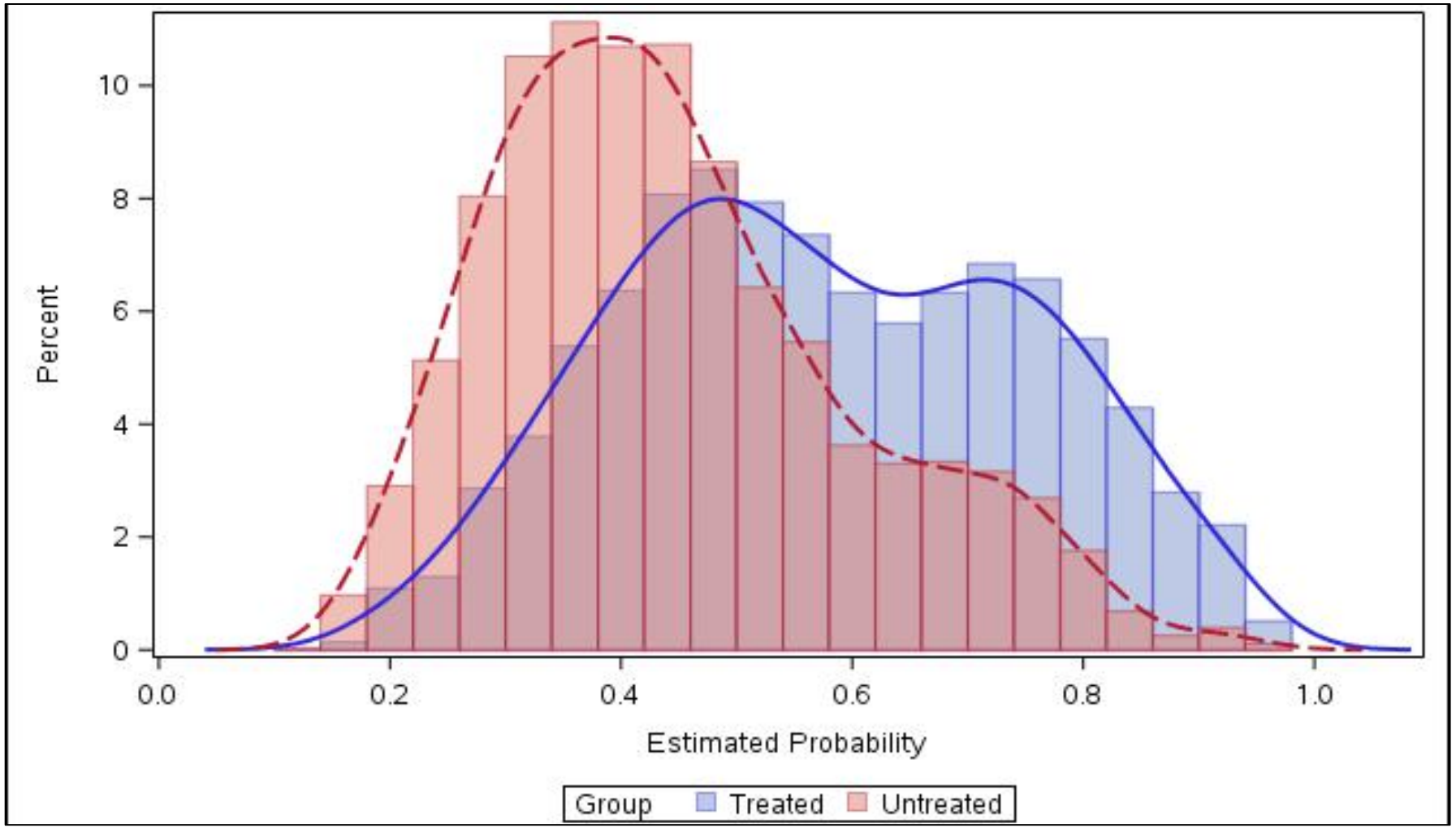
Early-Onset CNI Treatment



Predictors of Propensity Score

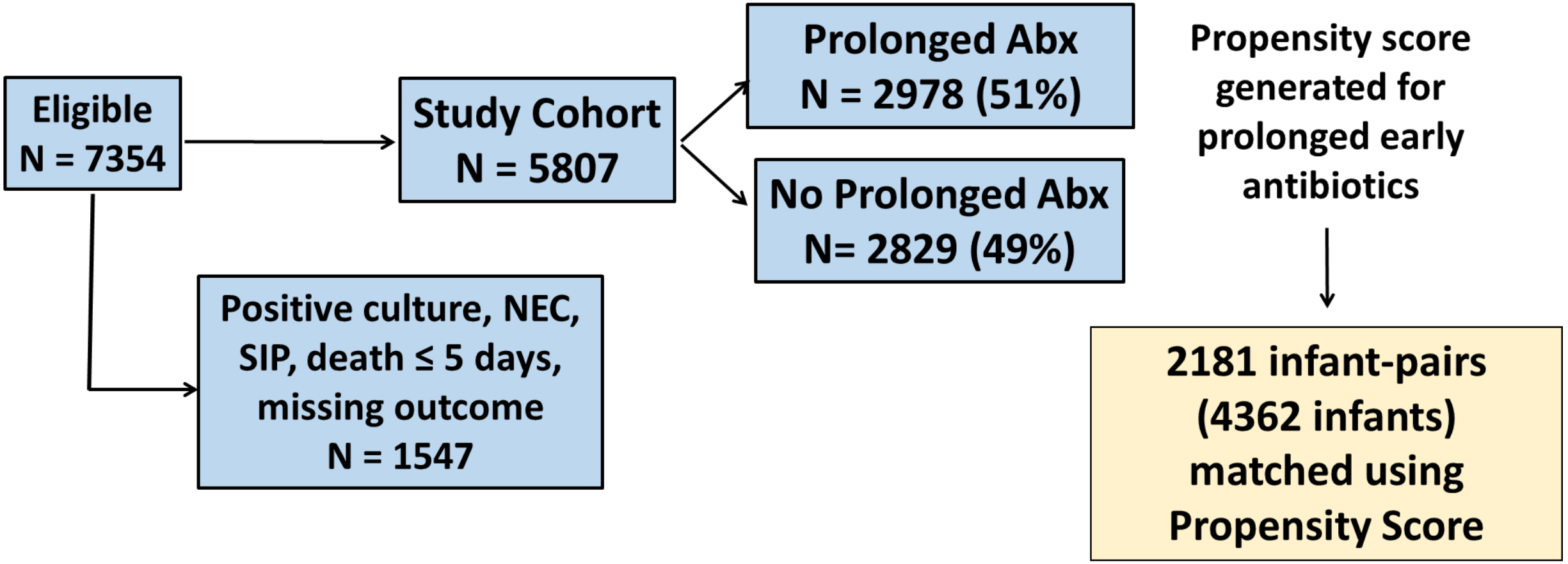
- GA, BW, Sex
- Chorioamnionitis
- Maternal antibiotics
- Antenatal steroids
- Cesarean
- Membrane rupture >18 hours
- Intubation at birth
- First temperature
- Respiratory support at 24 h
- Severe IVH at ≤ 7 days
- Enteral feeds at ≤3 days
- Maternal hypertension
- Antepartum hemorrhage
- Race/ethnicity
- Maternal education
- Maternal insurance

Proportion Score Matching



75% of the cohort matched

Study Population



Abx = Antibiotics

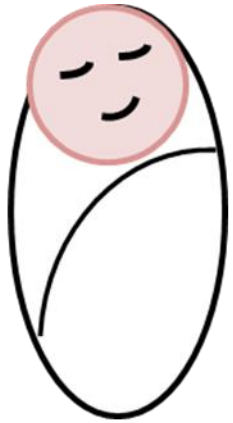
Results

	Prolonged Abx n=2181	No Prolonged Abx n=2181	RR (95% CI)	P-value
Death/NDI	1,091 (50.0)	1,047 (48.0)	1.04 (0.98-1.11)	0.18
Death	512 (23.5)	469 (21.5)	1.09 (0.98-1.22)	0.12
NDI	579 (34.7)	578 (33.8)	1.03 (0.94-1.13)	0.57

No difference in Death/NDI with Prolonged Antibiotics

Adjusted RR (95% CI)	EOS vs. Prolonged Abx	EOS vs. No Prolonged Abx	Prolonged Abx vs. No Prolonged Abx
Death/NDI	1.18 (1.06-1.32)	1.23 (1.10-1.37)	1.04 (0.99-1.08)
Death	1.16 (0.97-1.40)	1.16 (0.96-1.40)	1.00 (0.93-1.07)
NDI	1.26 (0.99-1.60)	1.34 (1.05-1.71)	1.06 (0.98-1.16)

Approach to Understanding CNI



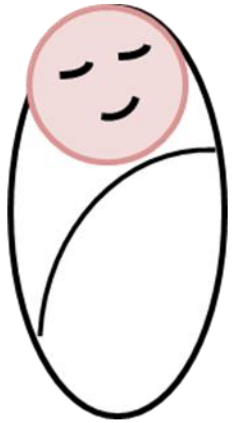
Blood Culture Negative

Antibiotics

No Antibiotics

Outcomes different?

No



Complete Antibiotic Course

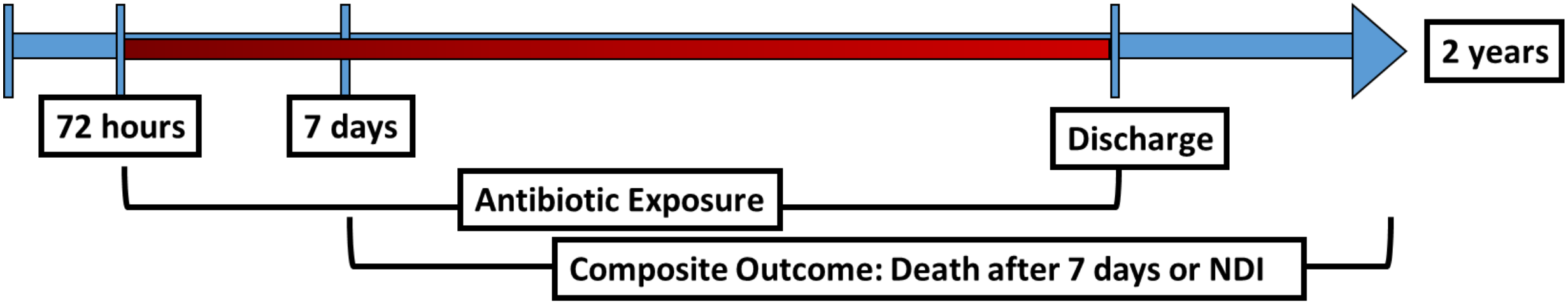
Blood culture +

Blood Culture -

Outcomes same?

No

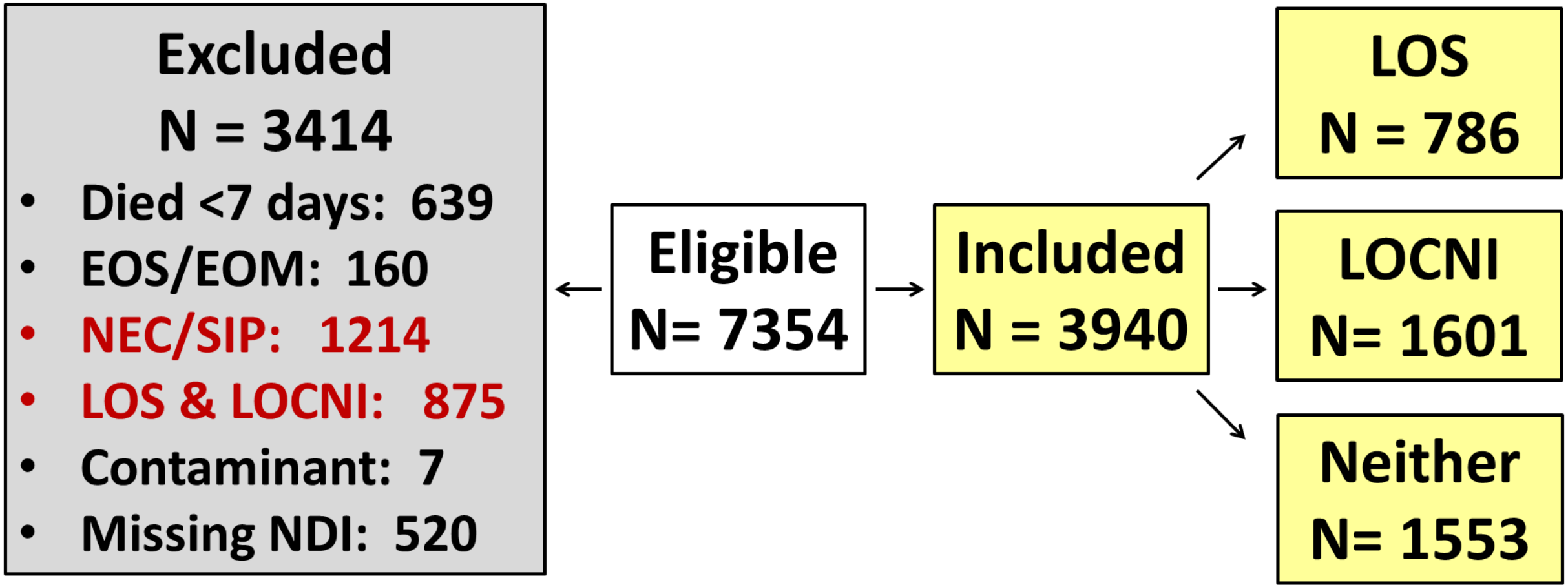
Late-Onset CNI Treatment



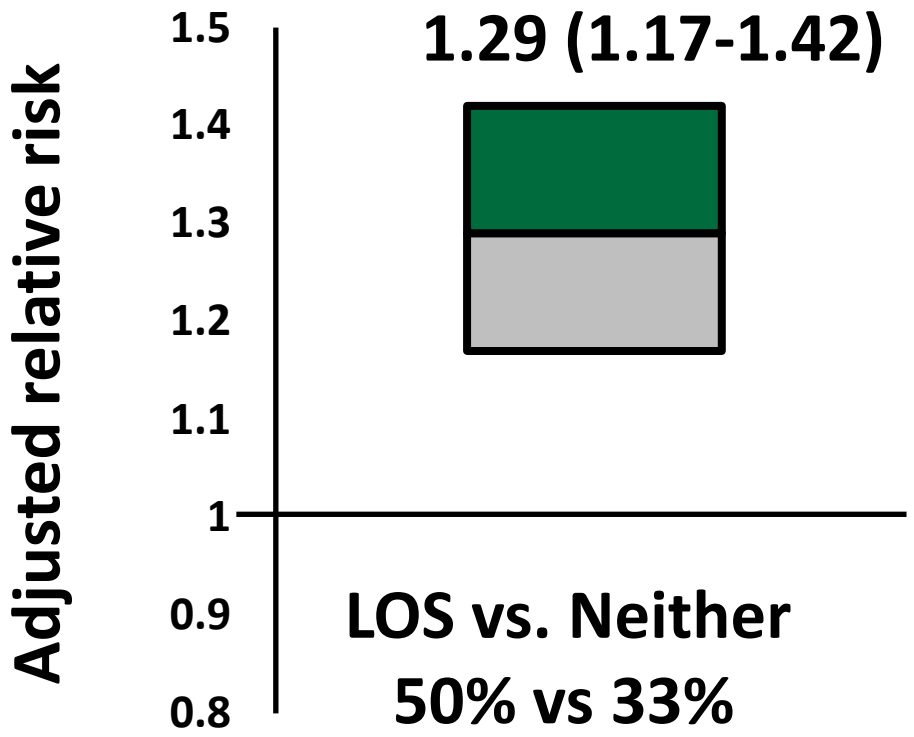
Analysis

- **Not by Propensity Score as LOCNI not timed in database**
- **Poisson regression models with robust variance estimators**
- ***A priori* covariates included**
  - **study center**
  - **maternal education**
  - **insurance**
  - **race/ethnicity**
  - **antepartum hemorrhage**
  - **maternal antibiotics**
  - **antenatal steroids**
  - **GA, BW, sex**
  - **intubation at birth**
  - **first temperature**
  - **respiratory support at 24 hr**
  - **enteral feeds at ≤3 days of age**
  - **early prolonged antibiotics**
  - **severe IVH ≤ 7 days**

Study Population



Results: Composite Death/NDI



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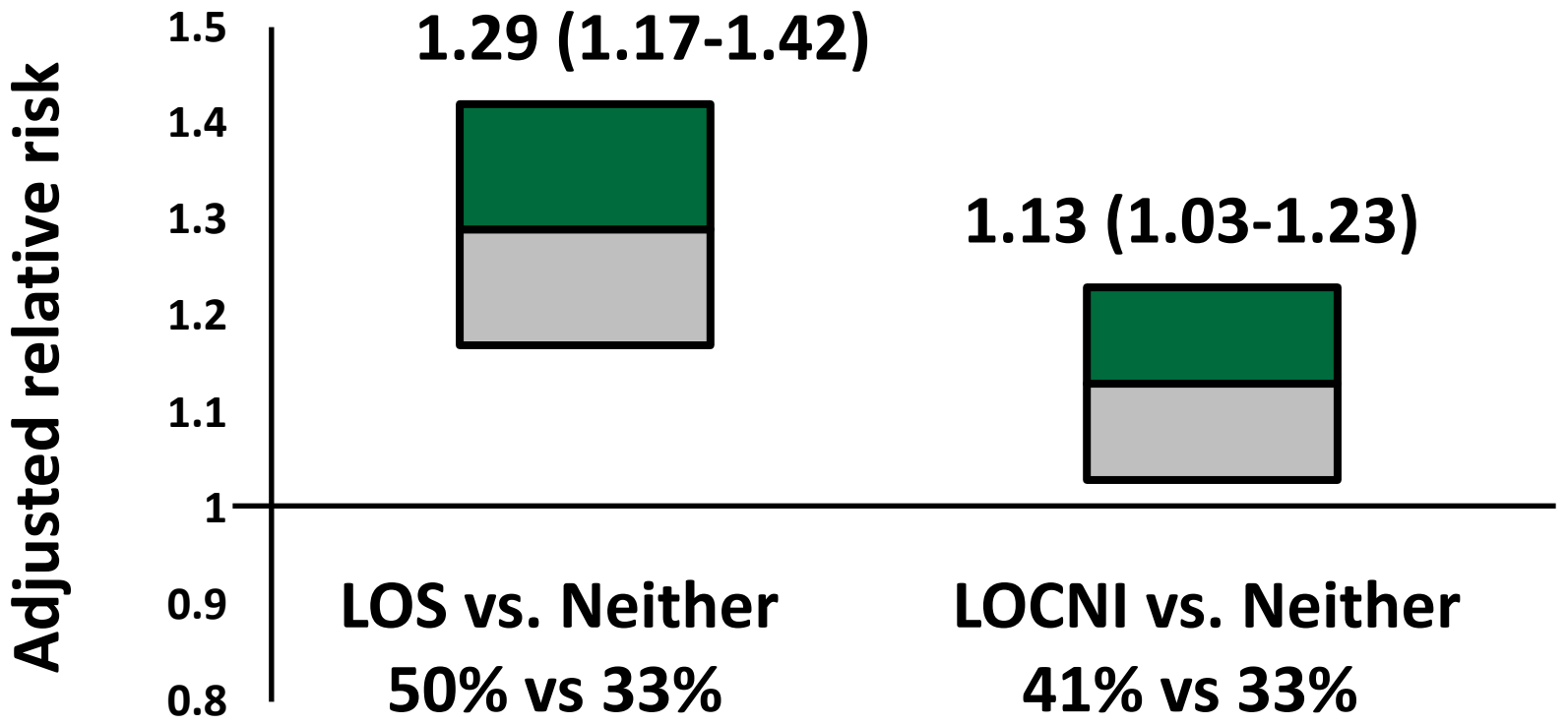

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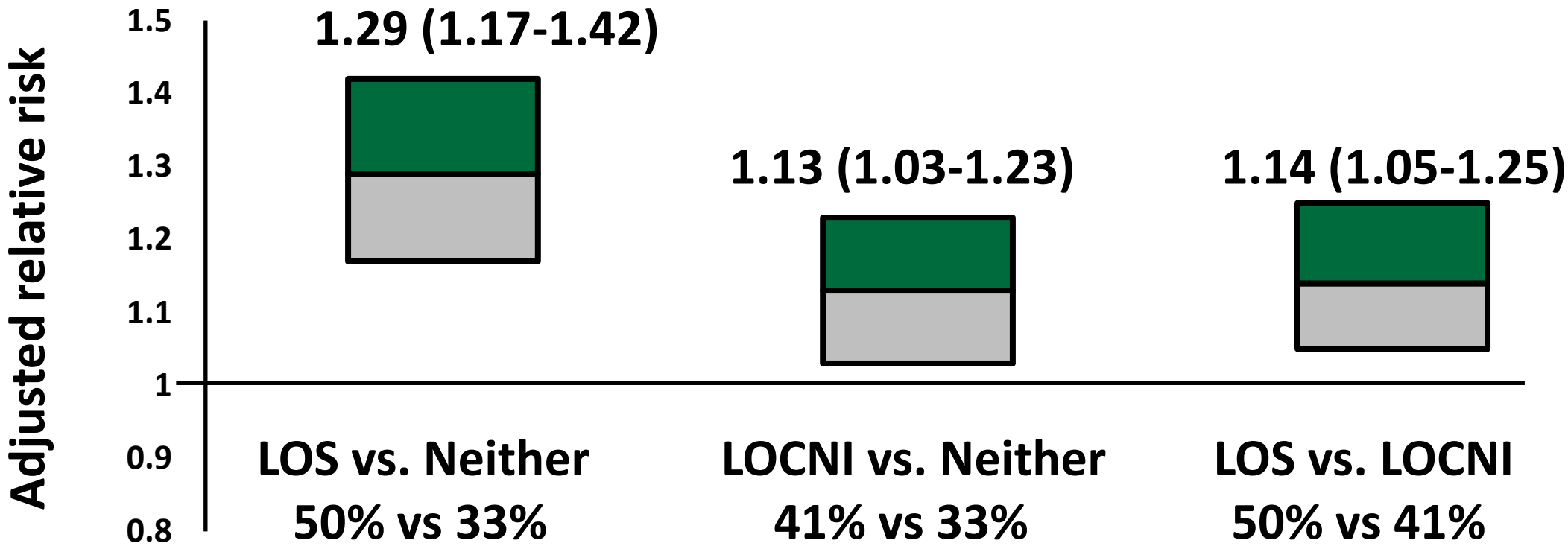
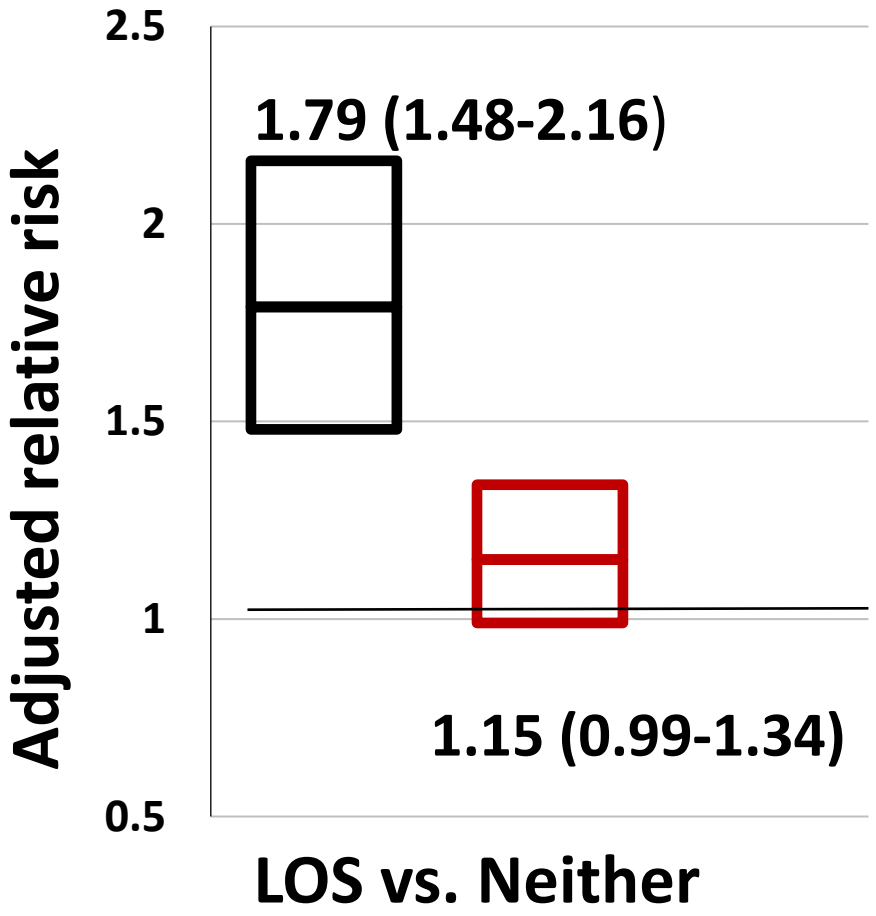
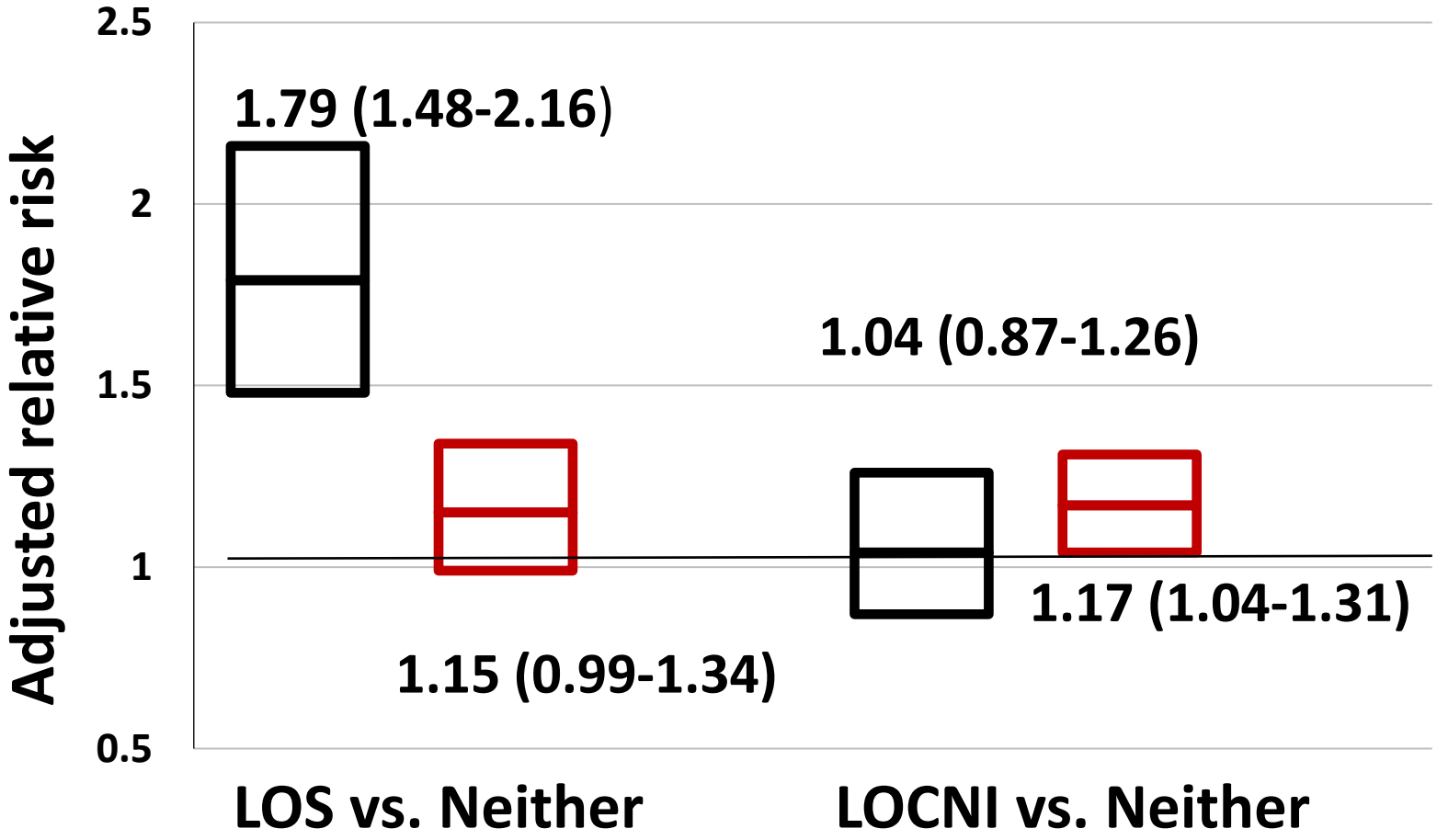


Results: Death or NDI



Death
NDI

Results: Death or NDI



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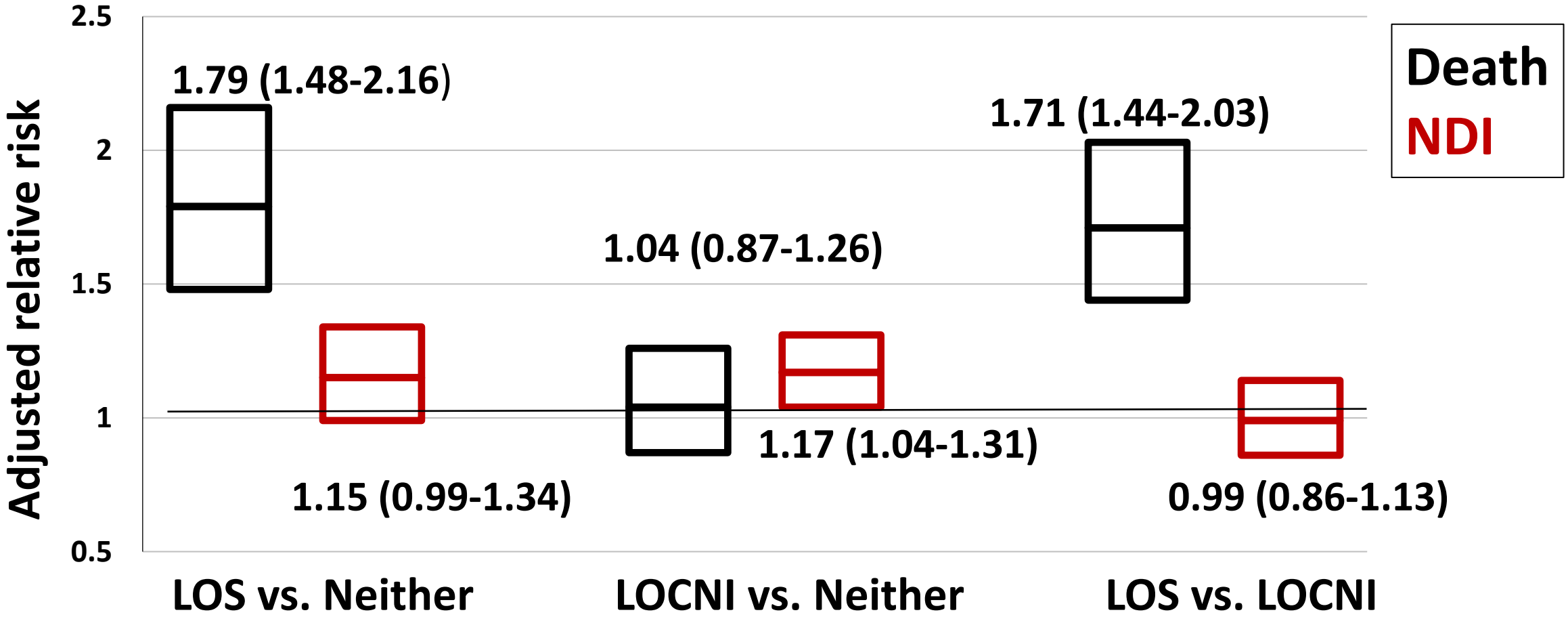
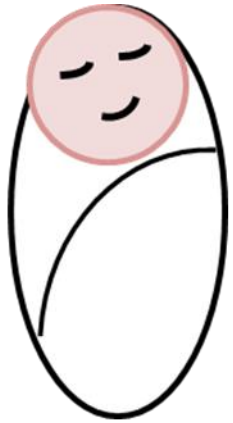


Approach to Understanding CNI



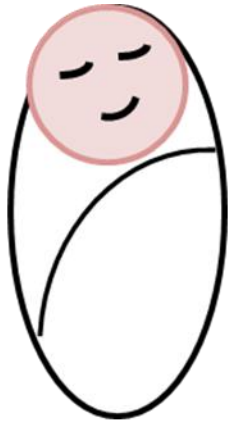
Blood Culture Negative

Antibiotics

No Antibiotics

Outcomes different?

Yes - NDI



Complete Antibiotic Course

Blood culture +

Blood Culture -

Outcomes same?

No

Implications for Early Antibiotic Use

- **There is a large proportion of very premature infants who receive prolonged antibiotic without standard indications**
- **75% of our cohort could be matched on propensity for treating presumed, culture-negative EOS -- meaning that *the care for this issue is essentially mediated by the toss of the proverbial coin***
- **We could find *no benefit* of coming up heads or tails**

Implications for Late Antibiotic Use

- **We found no evidence for benefit of antibiotics for LOCNI for the combined outcome of death/NDI**
- **Yet we estimate that >70% of antibiotic use in study cohort was for LOCNI**
- ***In the absence of benefit, prolonged antibiotic use exposes the infant only to the potential risks of antibiotics***

Strategies Targeting Major Contributors

General Stewardship and Infection Prevention Practices

Empiric "rule outs"

- Start less: Risk-stratification
- Stop early: Reduce empiric therapy duration
- Select wisely: Narrow the spectrum of empiric therapy

Culture-negative therapy

- **Avoid prolongation**
  - **Monitor reliability of diagnostic tests**
  - **Question the risk-benefit of prolonged antibiotics**

Acknowledgements

- Karen M Puopolo, MD, PhD
- Research Team
  - Miren Dhudasia, MBBS, MPH
  - Erica Hartman, MA
  - Emily Woodford, BA
  - Alvaro Z Barboza, MPH
- Collaborators:
  - Jeffery Gerber, MD, PhD, MSCE
  - Kyle Bittinger, Phd
  - Robert Grundmeier, MD
  - Scott Lorch, MD, MSCE
  - Dustin Flannery, DO, MSCE
  - Sam Garber, MD
  - Amanda Gottschalk, MD
  - Mikayala Galloway, MPH
- Supporting Institutes
  - Eric Eichenwald, MD. Division Chief, Neonatology, CHOP
  - Center's of Pediatric Clinical Effectiveness (CPCE), CHOP
  - PennCHOP Microbiome Program
- Sponsors
  - NICHD - K23HD088753
  - AHRQ - 1R01HS029500
  - Neonatal Research Network
  - Society of Pediatric Research
  - Centers for Disease Control & Prevention:
  - Institute for Translational Medicine and Therapeutics
  - Gerber Foundation

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Questions?