Pros and Cons of Multiplexed PCR Analysis in Microbiology (& Infectious Diseases)

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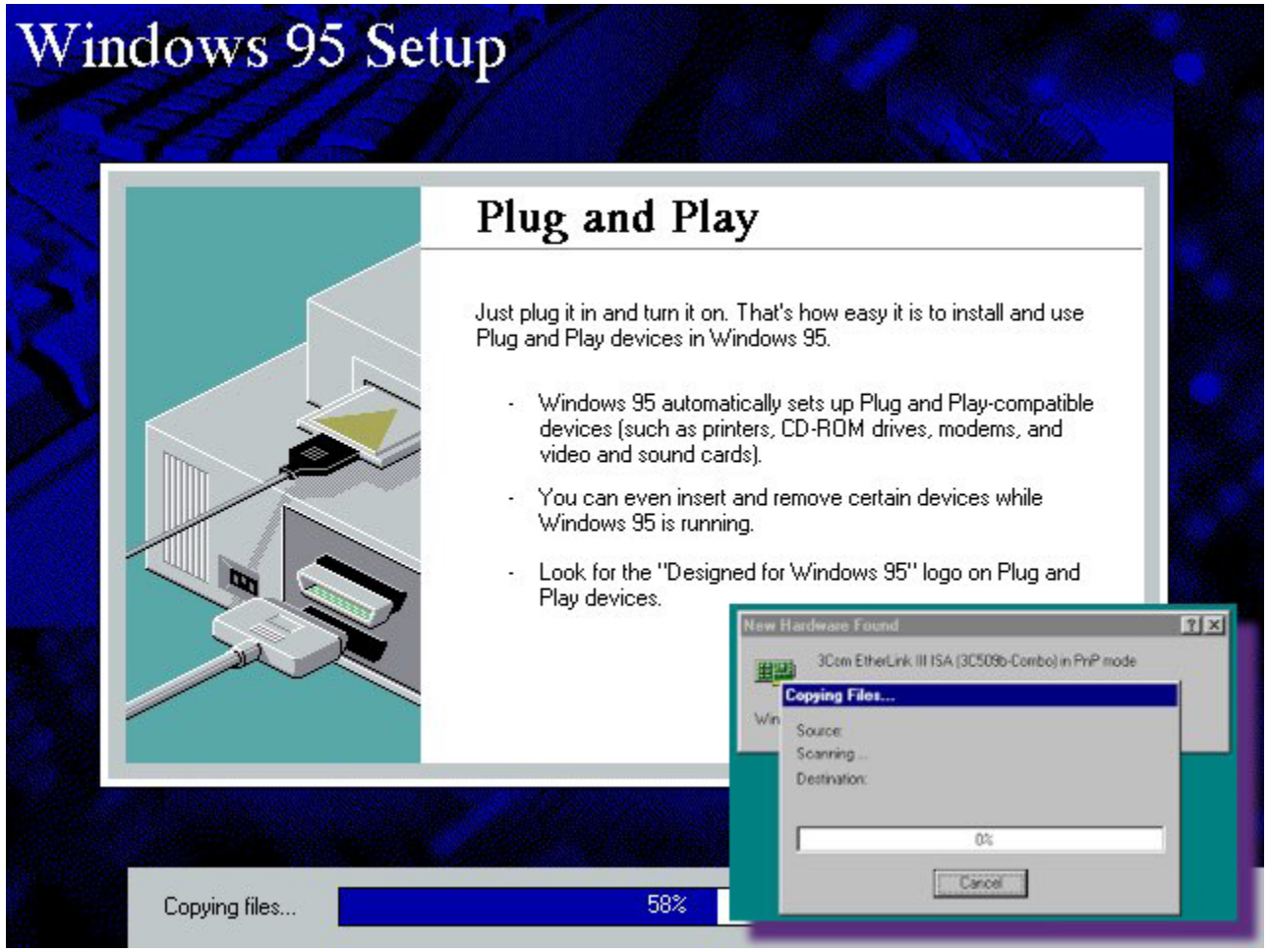
Molecular Diagnostics 2026 - Symposium - May 28 + 29, 2026, University-Children's Hospital Zurich

Agenda

- **Why Multiplex PCR Can Mislead**
  Limitations despite speed and sensitivity
- **Bayes' Theorem in Practice**
  Pre-test probability drives interpretation (Example: GI panels in clinical care)
- **Core Pitfalls of Multiplex Panels**
  False positives (low PPV, colonization, contamination), False negatives (timing, technical limits)
  Detection ≠ disease
- **Clinical Case-Based Pitfalls**
  GI panels – overdiagnosis in low-risk settings
  BCID panels – bloodstream infections & overtreatment
  Respiratory panels – colonization vs infection, adequate sample
  CNS panels – false positives (HHV-6, *H. influenzae*) / false negatives (HSV)
- **Practical Implications & Take-Home Messages**

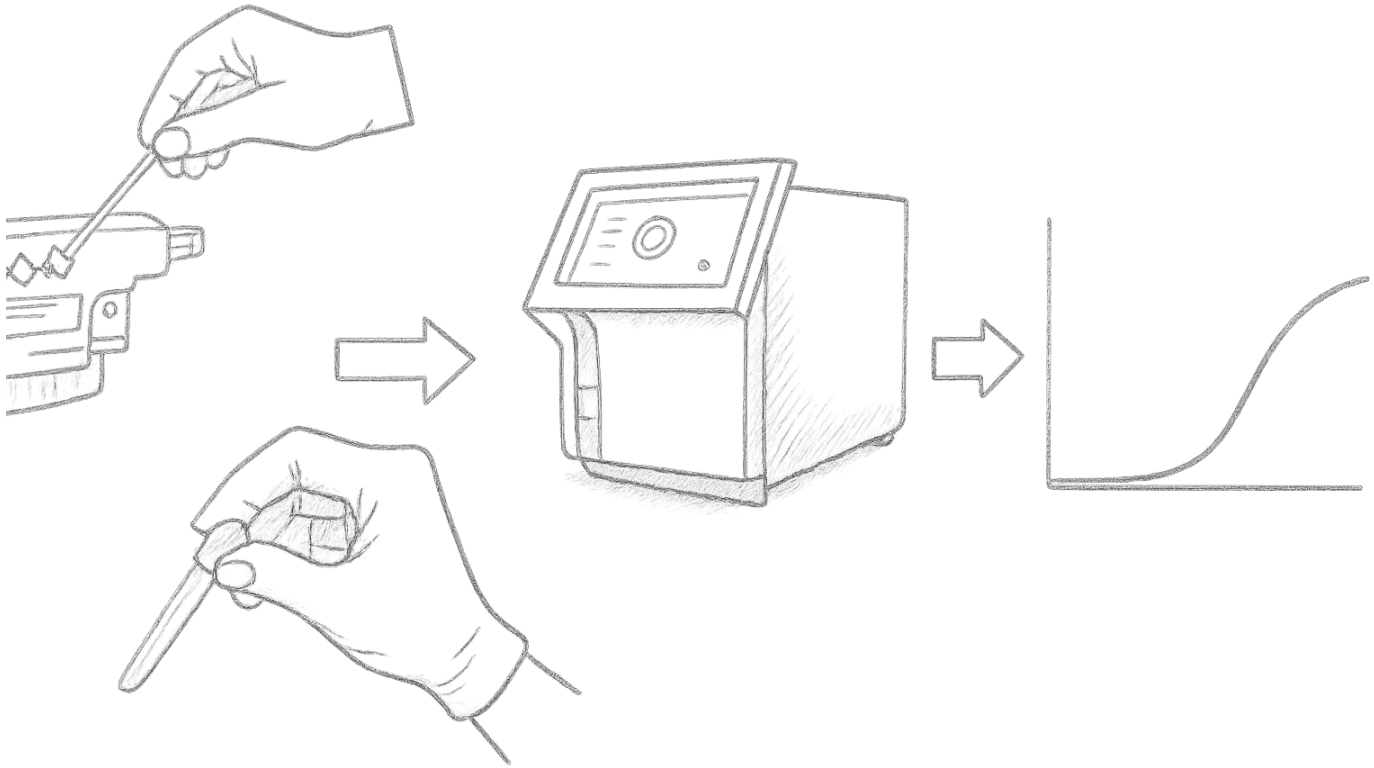
Multiplex PCR back then & nowadays

Multiplex-PCR back then



x.com/microsoft.com

Multiplex-PCR nowadays



Multiplex PCR panels in practice – friend or foe?

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GI Panels – Montezuma's revenge and other exotic diseaes

Case 1 – 20-year old traveler

A 20-year-old patient presents to the emergency department with slightly reduced general condition and diarrhea

- History:
  - Symptoms began four days ago with two days of fever and vomiting
  - For the past 2 days: watery diarrhea and abdominal cramps (approximately 10 episodes/day, including at night)
  - Was unable to tolerate food intake, about 3 L of fluids/d
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- Vital signs: GCS 15, SO_2 100%AA, BP 148/88 mmHg, P 90/min, T 37.2°C
- Clinical examination: Abdomen soft, non-tender, hyperactive bowel sounds, no palpable masses or organomegaly

What’s next?

A — Rule out malaria

B — Complete blood count (CBC), C-reactive protein (CRP), electrolytes, blood cultures

C — Stool culture

D — Multiplex-PCR „GI Panel“

E — Uncomplicated traveler’s diarrhea (no red flags) – symptomatic treatment

There you go

Probenmaterial Stuhl mit Transportmedium

Entnahme	25.11.2025 / 14:55

Molekulare Diagnostik

Campylobacter spp. DNA	**positiv**
Dieser Befund löst eine Labormeldung an BAG und Kantonsarzt aus. Campylobacter spp.: Typisierung erfolgt mittels Kultur.	
Clostridioides difficile Toxin A/B DNA	negativ
Plesiomonas shigelloides DNA	**positiv**
Salmonella spp. DNA	negativ
Vibrio cholerae DNA	negativ
Vibrio parahaemolyticus DNA	negativ
Vibrio vulnificus DNA	negativ
Yersinia enterocolitica DNA	negativ
Enteroaggregative E. coli (EAEC) DNA	**positiv**
Enteropathogene E. coli (EPEC) DNA	**positiv**
Enterotoxische E. coli (ETEC) DNA	negativ
Enterohämorrhagische E. coli (EHEC) DNA	negativ
Shigella spp. / Enteroinvasive E. coli (EIEC) DNA	negativ
Cryptosporidium DNA	negativ
Cyclospora cayetanensis DNA	negativ
Entamoeba histolytica DNA	negativ
Giardia lamblia DNA	negativ
Adenovirus F 40/41 DNA	negativ
Astrovirus RNA	negativ
Norovirus RNA	negativ
Rotavirus A RNA	negativ
Sapovirus RNA	negativ

Now what?

A Ciprofloxacin

B Azithromycin

C Ciprofloxacin & Azithromycin

D Uncomplicated traveler's diarrhea (no red flags) – symptomatic treatment

E Insist on stool culture

F ID consultation

Diagnostics: what, when, and why to investigate?

Pros

- May influence treatment decisions
  - Species will determine necessary spectrum of antibiotics
  - Antibiotics are (generally) contraindicated if EHEC (enterohemorrhagic *E. coli*) is detected
- Surveillance of outbreaks/epidemics
  - Reporting to the FOPH (Federal Office of Public Health, Switzerland) for notifiable pathogens such as EHEC, *Campylobacter*, *Salmonella*, *Shigella*

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Cons

- No therapeutic consequence:
  - Antibiotics are rarely indicated for the most common pathogens
- Costs: Consider financial implications of additional diagnostic testing (119.7+4x47.7 TP)

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Acute diarrhea lasting <7 (–13) days without fever or blood in the stool does not require diagnostic workup or antibiotics in the outpatient setting in most cases

Case 2 – fellow on the phone with the cantonal medical service

Untersuchungen	Flag	Resultat	Einheit	Referenzbereich
Material 1				
Material Diagnostik		Stuhl nativ		
Gastrointestinale Viren und Bakterien (Blockanalyse)				
Adenovirus F40/41, RT-PCR ql (BioFire)		negativ		
Astrovirus, RT-PCR ql (BioFire)		negativ		
Norovirus GI/GII, RT-PCR ql (BioFire)		negativ		
Rotavirus A, RT-PCR ql (BioFire)		negativ		
Sapovirus (I/II/IV/V), RT-PCR ql (BioFire)		negativ		
C. jejuni/coli/upsal., RT-PCR ql (BioFire)		negativ		
C. difficile (Toxin A/B), RT-PCR ql (BF)		negativ		
P. shigelloides, RT-PCR ql (BioFire)		negativ		
Salmonella, RT-PCR ql (BioFire)		negativ		
Y. enterocolitica, RT-PCR ql (BioFire)		negativ		
V. parah./vulnif./choler., RT-PCR ql (BF)		**positiv**		
V. cholerae, RT-PCR ql (BioFire)		**positiv**		
E. coli (EAEC), RT-PCR ql (BioFire)		negativ		
E. coli (EPEC), RT-PCR ql (BioFire)		negativ		
E. coli lt/st (ETEC), RT-PCR ql (BioFire)		negativ		
E. coli stx1/stx2 (STEC), RT-PCR ql (BF)		negativ		
E. coli O157, RT-PCR ql (BioFire)		negativ		
Shigella/E. coli (EIEC), RT-PCR ql (BF)		negativ		

Legende:

* nicht akkreditiertes Verfahren (markiert ab 12.11.2025); nnwb = nicht nachweisbar; LDT = laboratory developed test

Case 2 – fellow on the phone with the cantonal medical service

- 50-year-old patient with metastatic ovarian cancer and multiple abdominal surgeries
- 11/2025: ileostomy; currently on 3rd-line systemic therapy
- Symptoms: recurrent vomiting, watery stools, postprandial upper abdominal pain for one day
- Clinical exam: afebrile, no tenderness, active bowel sounds
  Labs: mildly elevated CRP, no leukocytosis
- One day later: Minimal symptoms, soft stool via stoma, no nausea, no fever
- History / exposure:
  - No seafood or fish consumption, no sick contacts
  - Travel: 20 Apr–04 May (Bochum, Germany)
  - 04 May: Düsseldorf airport (coffee), flight to Zurich ate quiche with “unusual taste”

Cholera in Düsseldorf?

- Very low reproducibility of positive results: only 37.5% are confirmed on repeat testing
- Many PCR-positive cases cannot be confirmed by culture
- Detection of *Vibrio cholerae* in the certain GI Panel shows a high false-positive rate

- Assess epidemiological plausibility
- Lack of typical clinical-epidemiological features in false-positive cases:
  - no seafood or water exposure
  - no travel to endemic regions
  - atypical clinical presentation (typical cholera symptoms: profuse watery “rice-water” diarrhea)
- High risk of overdiagnosis in low-prevalence settings
- Diagnostic restraint (“diagnostic stewardship”) is required

Decuir, OFID, 2021; Hitchcock, DiagnMicroID, 2019

Diagnostic and Clinical Performance of Multiplex GI Panels

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Performance

Compared to standard methods, most studies show very good performance:

- Meta-analysis of multiplex PCR GI panels (FilmArray and Luminex xTAG) for detecting pathogens causing acute gastroenteritis
- 11 studies including a total of 7,085 stool samples (systematic literature search up to end of 2019)
- Multiplex PCR results compared with established gold standards (culture, PCR, EIA, microscopy)
- High diagnostic accuracy: specificity ≥ 0.98, AUROC ≥ 0.97 (except *Yersinia enterocolitica* with AUROC 0.91)

BUT: clinical utility has often not been evaluated

- False-positive results have been described, depending on the specific panel (e.g., norovirus, *Campylobacter* in BioFire FilmArray)
- In some studies, very high rates of detection of more than one pathogen were observed (up to >50%, depending on the study)
- Detection does not necessarily correlate with disease (e.g., *Clostridioides difficile*)
  - In some cases, Ct-value correlation may be helpful (e.g., Ct < 28 suggesting higher likelihood of true infection)

Sanchez, JClinMicro, 2025; Meltzer, JACEP Open, 2022; Caza, JClinViro, 2024
Chang, BMJOpGastro, 2021; Polage, JAMA Intern Med, 2015

PPV depends highly on prevalence with high-performance tests

Table 3 The calculation of post-test probabilities

Pathogens	Study(n)	Pre-test probability (%)	Test kit	Likelihood ratio	Post-test probability (%)
Campylobacter	11	5.6	Luminex	LR+: 55 (21-140)	77
				LR-: 0.06 (0.03-0.12)	0
	5	3.6	FilmArray	LR+: 120 (47-330)	82
				LR-: 0.05 (0.02-0.13)	0
Entamoeba histolytica	6	0.2	Luminex	LR+: 59 (18-160)	11
				LR-: 0.30 (0.08-0.68)	0

$$P(D \mid T) = \frac{P(T \mid D)\,P(D)}{P(T)}$$

Chang, BMJOpGastro, 2021, Khare, JClinMicro, 2014; Yoo, Diagnostics, 2021; Binnicker, JClinMicro, 2015; Connor IJID, 2017

PPV depends highly on prevalence with high-performance tests

- Clinical scenario: Primary care patient with mild diarrhea → Low pre-test probability for bacterial pathogen (~5%)
- Test characteristics (typical multiplex PCR): Sensitivity: 95% & Specificity: 95%

	Disease Present	No Disease	Total
Positive test	48 (true +)	48 (false +)	**96**
Negative test	2 (false –)	902 (true –)	904
Total	50	950	1000

- Positive Predictive Value = ~50% → Every second “positive” is false
- High sensitivity + low prevalence = misleading positives even when excellent test performance

Clinical consequences of widespread use of panels can be significant

- Positive results should always be interpreted in the clinical context
  - Confirmation by 2nd test to be considered, especially when clinical probability is low
  - Ct value-based thresholding
- Introduction of multiplex panels might results in overdiagnosis and overtreatment with all its consequences

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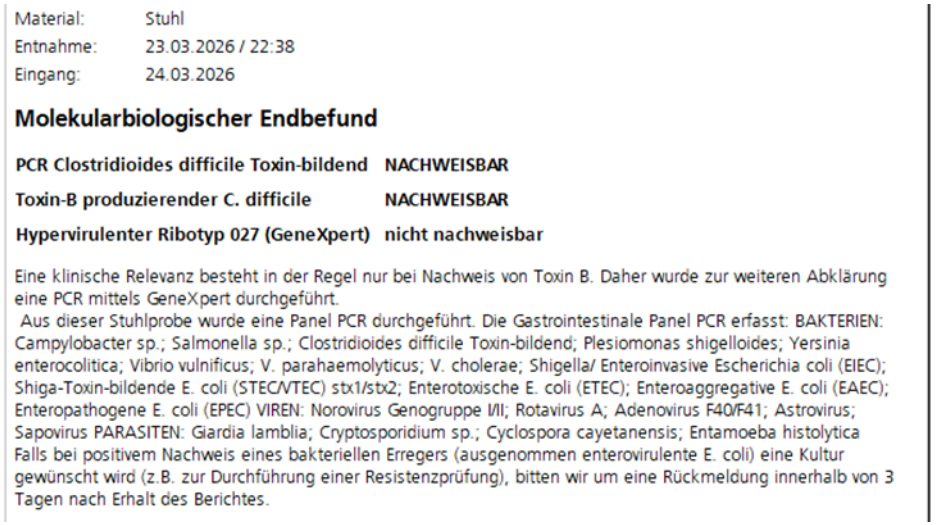


Material: Stuhl
Entnahme: 23.03.2026 / 22:38
Eingang: 24.03.2026

Molekularbiologischer Endbefund

PCR Clostridioides difficile Toxin-bildend	**NACHWEISBAR**
Toxin-B produzierender C. difficile	**NACHWEISBAR**
Hypervirulenter Ribotyp 027 (GeneXpert)	**nicht nachweisbar**

Eine klinische Relevanz besteht in der Regel nur bei Nachweis von Toxin B. Daher wurde zur weiteren Abklärung eine PCR mittels GeneXpert durchgeführt.
Aus dieser Stuhlprobe wurde eine Panel PCR durchgeführt. Die Gastrointestinale Panel PCR erfasst: BAKTERIEN: Campylobacter sp.; Salmonella sp.; Clostridioides difficile Toxin-bildend; Plesiomonas shigelloides; Yersinia enterocolitica; Vibrio vulnificus; V. parahaemolyticus; V. cholerae; Shigella/ Enteroinvasive Escherichia coli (EIEC); Shiga-Toxin-bildende E. coli (STEC/VTEC) stx1/stx2; Enterotoxische E. coli (ETEC); Enteroaggregative E. coli (EAEC); Enteropathogene E. coli (EPEC) VIREN: Norovirus Genogruppe I/II; Rotavirus A; Adenovirus F40/F41; Astrovirus; Sapovirus PARASITEN: Giardia lamblia; Cryptosporidium sp.; Cyclospora cayetanensis; Entamoeba histolytica
Falls bei positivem Nachweis eines bakteriellen Erregers (ausgenommen enterovirulente E. coli) eine Kultur gewünscht wird (z.B. zur Durchführung einer Resistenzprüfung), bitten wir um eine Rückmeldung innerhalb von 3 Tagen nach Erhalt des Berichtes.

Clinical significance vs. overdiagnosis in antibiotic use

Study:

Investigation of the influence of a multiplex gastrointestinal PCR panel (GI PCR) on physicians' antibiotic treatment decisions compared with standard testing in patients with acute infectious diarrhea in the emergency department (ED)

Methods:

- Prospective, single-center, randomized controlled trial
- Patients with moderate to severe suspected infectious diarrhea and signs of dehydration, inflammation, or persistent symptoms

Primary endpoint:

- Antibiotic prescription

Results:

- 74 patients: 38 in the GI PCR group, 36 in the control group
- Antibiotic administration when a pathogen was detected:
  - GI PCR group: 87% (13/15)
  - Control group: 46% (6/13)
  - ($p < 0.05$)

In a nutshell

- Acute diarrhea lasting <7 (–13) days without fever or blood in the stool does not require diagnostic workup or antibiotics in the outpatient setting in most cases
- Molecular tests detect pathogens more rapidly and are usually more sensitive; however, they only detect NA
  - No phenotypic susceptibility testing is possible
  - Positive signal does not necessary mean infection (i.e., colonization, false pos.)
- Clinical correlation is required to avoid unnecessary treatment
- There is no evidence of improved clinical outcomes in traveler’s diarrhea

Other panels – less problems?

Blood Culture Identification (BCID) panel – BCID2

Fast. Easy. Comprehensive.

Syndromic testing provides a streamlined workflow and fast, comprehensive results.

0.2mL of positive blood culture

2 minutes hands-on time

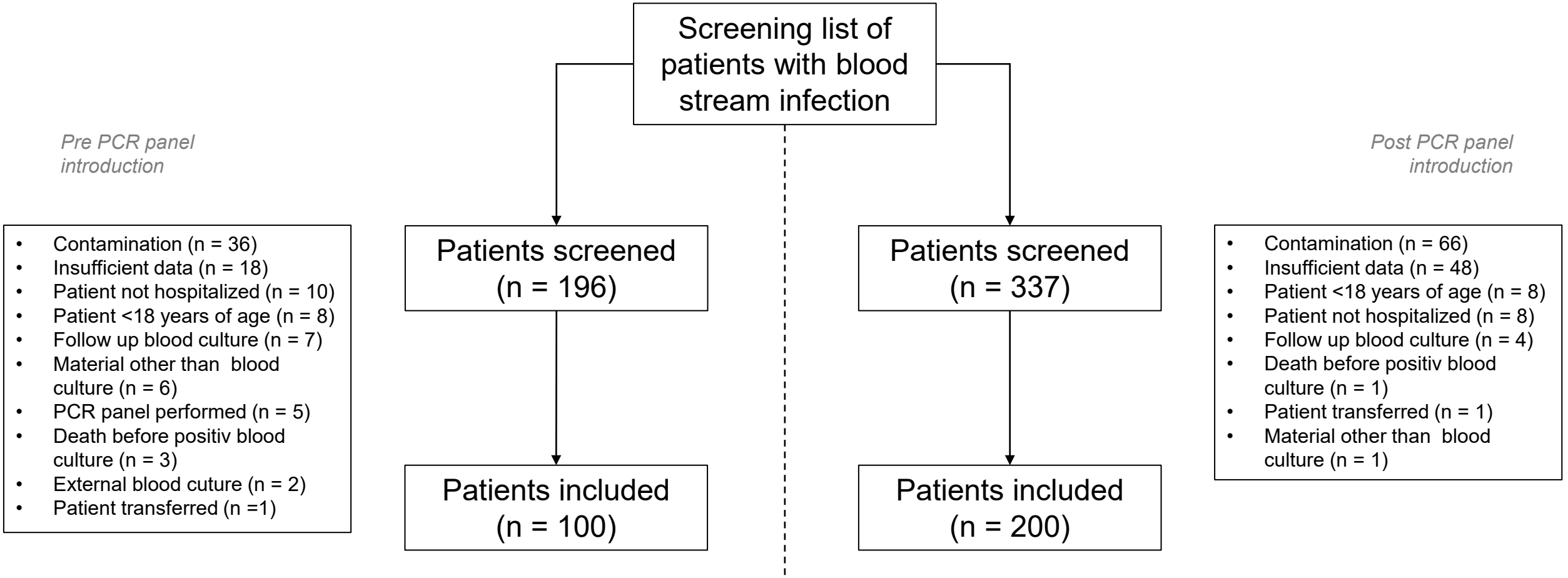
Results in about an hour

Comprehensive results in a single report

↓70.0%* Reduction in Time to ID[2]

Overall Performance: 99.0% sensitivity and 99.7% specificity[1]

Blood Culture Identification (BCID) panel – BCID2



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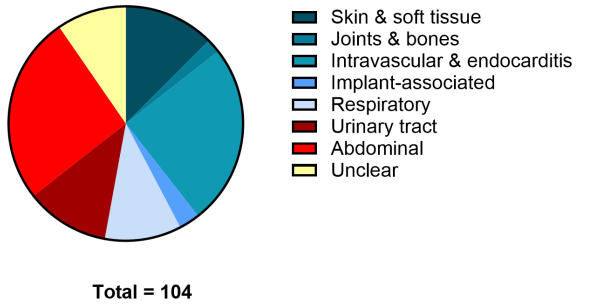
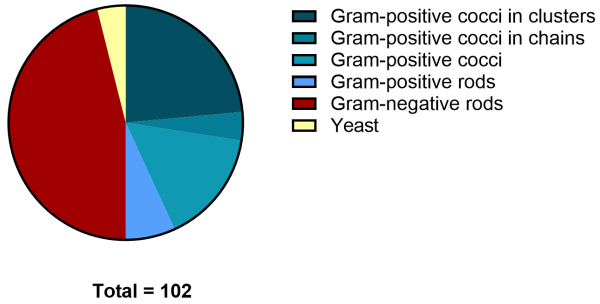
Blood Culture Identification (BCID) panel – BCID2

Pre PCR panel introduction



Total = 104



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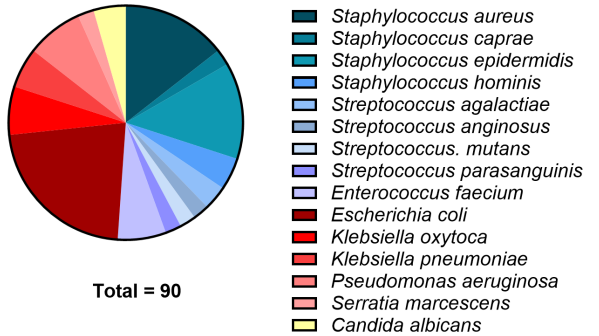
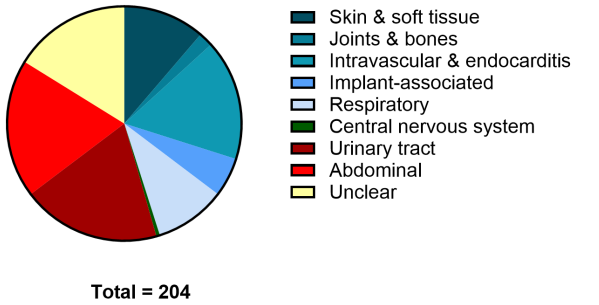


Total = 90

Post PCR panel introduction



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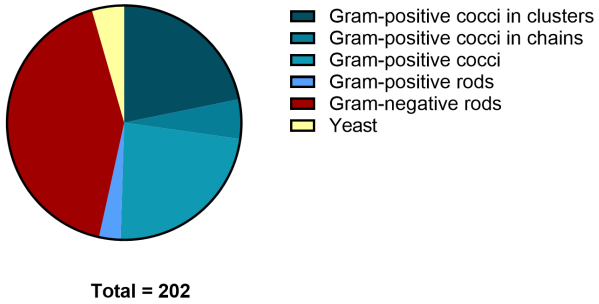

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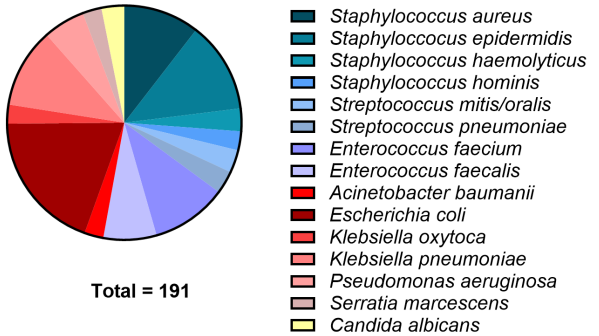

Total = 191

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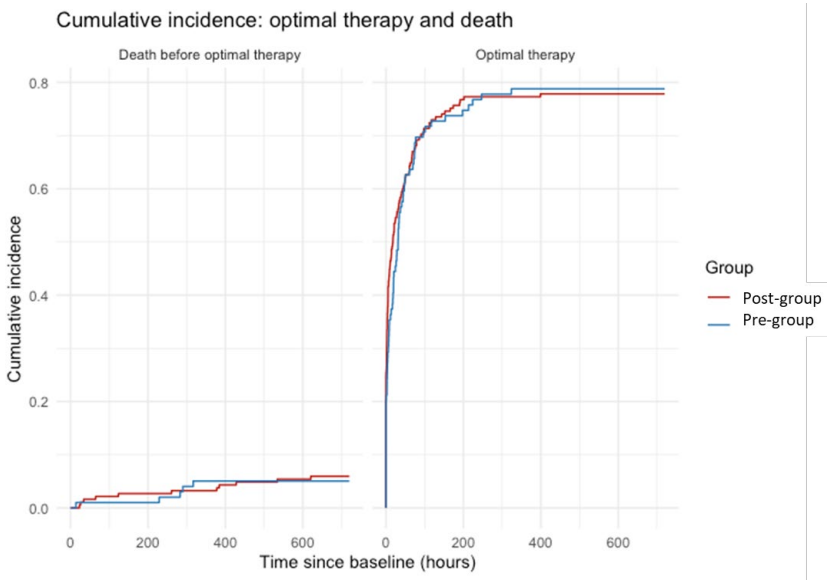
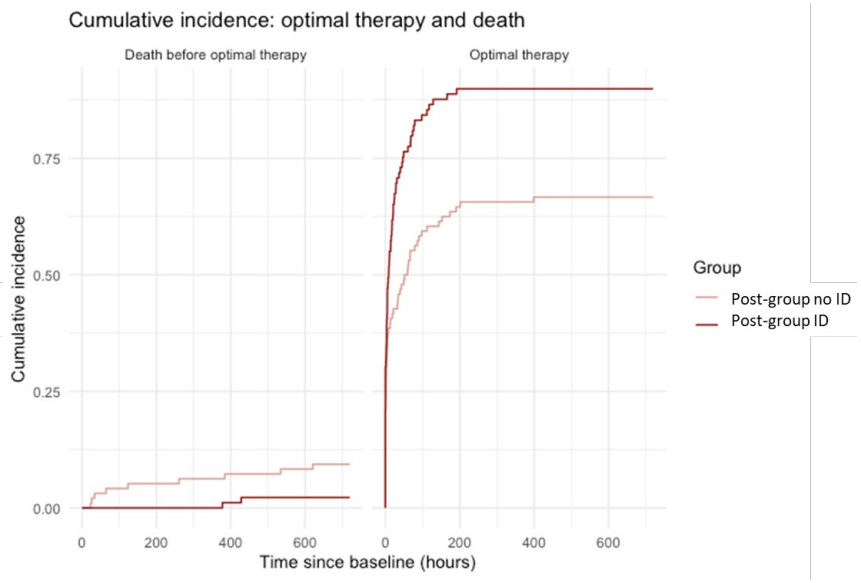
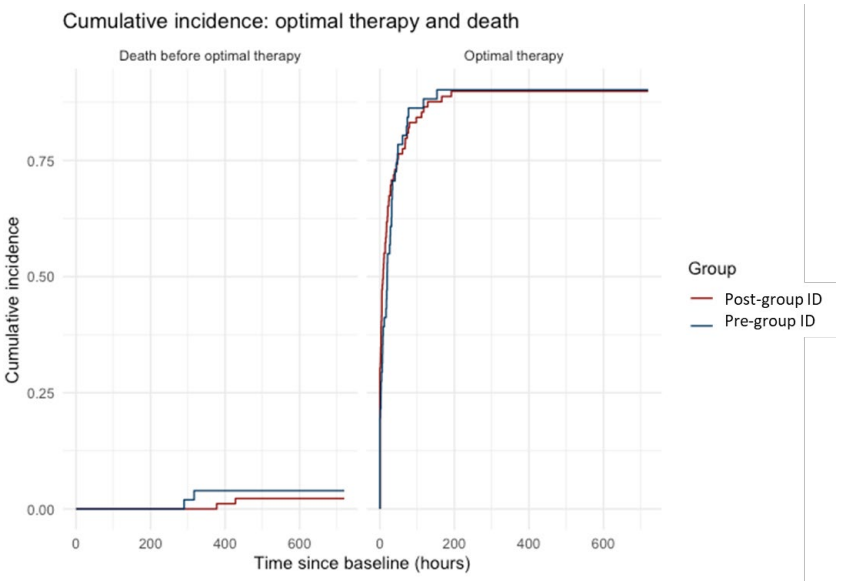
Clinical performance







«Time to optimal therapy». Subgroup analyses accounting for competing risk of death comparison for: (A) pre and post introduction of BCID2; (B) post introduction with and without followed ID recommendation; (C) pre and post introduction with followed ID recommendation.

Comparison	**Adjusted sHR (95% CI)**	**p**
Post vs pre BCID2	1.15 (0.88–1.50)	0.31
ID involvement	**1.69 (1.23–2.33)**	**0.001**
Post- vs pre-BCID2 in ID cases	1.11 (0.78–1.56)	0.56

Adjusted Fine-Gray competing risk models accounting for age, sex, and SOFA score yielded comparable effect estimates.

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But what about AMR?

In 33/200 (16.5%) of BSI in the post-group, the PCR panel tested positive for resistance genes

- 34/235 (14.5%)

mecA/C (n = 22):

- Coagulase-negative staphylococci (CoNS) (*S. epidermidis* n = 20; *Staphylococcus hominis* n = 2).
- *S. aureus: mecA/C* and MREJ in two isolates => adjustment of therapy

KPC (*K. pneumoniae* n =1), CTX-M (*E. coli* n = 5; *K. pneumoniae* = 4) => In 8 (1 KPC, 7 CTX-M) adjustment of anti-infective treatment regimen

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But what about AMR?

- The routine use of blood culture PCR panels showed no clinical benefit when testing was performed without structured involvement of infectious disease specialists.
- Fifty percent of PCR panels were performed in bloodstream infections caused by gram-positive cocci, which are generally well covered by amoxicillin/clavulanic acid in low MRSA-prevalence area.
- Within the current implementation framework and local epidemiological context, the benefit to patients does not justify the considerable cost of this diagnostic method.

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CNS – pitfalls

False positives / overcalling

- *Haemophilus influenzae, Streptococcus* spp.
  - Low-prevalence setting → ↓ PPV
  - Possible contamination / transient NA
  - Risk: unnecessary antibiotics
- HHV-6 DNA detection
  - Chromosomally integrated HHV-6 (ciHHV-6)
  - Latent/reactivation ≠ disease
  - Especially problematic in immunocompetent patients → Detection ≠ encephalitis

False negatives

- HSV PCR false negative
  - Early sampling (<72h symptoms)
  - Low viral load / partially treated
  - Technical limits of multiplex format
  - → Do NOT rule out HSV solely on panel result

Pellet, RevMedVirol, 2012; Leber, JClinMicro, 2016; Tunkel, IDSA, 2008; De Gryse, BelJPed, 2025

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→ Always interpret with: Pre-test probability, CSF profile (cells, protein, glucose) and clinical syndrome

Pellet, RevMedVirol, 2012; Leber, JClinMicro, 2016; Tunkel, IDSA, 2008; De Gryse, BelJPed, 2025

Conclusions

Multiplex PCR in CM/ID: Powerful Tool, Imperfect Test

1. **Speed ≠ diagnostic certainty**
- Rapid turnaround improves workflow
- But clinical accuracy depends on context
2. **Bayes matters more than technology**
- Low pretest probability → false positives rise
- High pretest probability → false negatives matter most
3. **Detection ≠ disease**
- DNA/RNA may represent: Colonization (GI, respiratory), latency/reactivation (HHV-6)or nonviable organisms
4. **Panels change behavior**
- Risk of: Over-treatment, anchoring bias & ignoring clinical data
5. **When to use panels?**
- Severe illness / time-critical decisions
- Reasonable pretest probability
- Clear management consequence

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Multiplex PCR shortens time to results - but not the need for thinking

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sdb-lab.org