

Performance of Sysmex UF-4000 research parameters in predicting urine culture outcomes

Critically Appraised Topic

15/09/2026

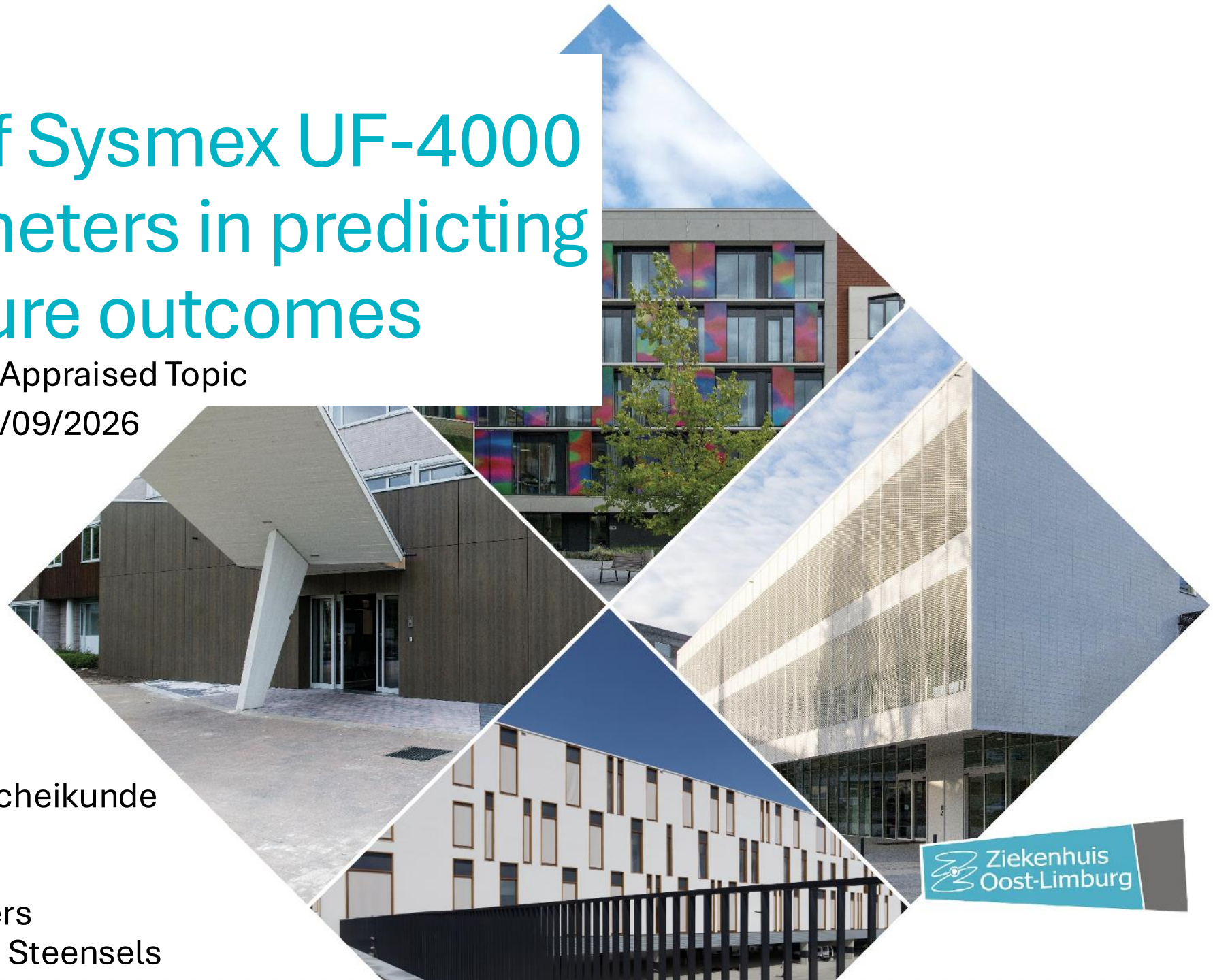
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 Ziekenhuis
Oost-Limburg

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01

Clinical case & clinical need

Clinical case



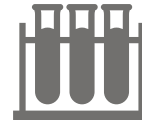
Patient

- ♀
- 72 years



Symptoms

- Acute dysuria
 - Pollakisuria
 - Fever
- Suspected UTI

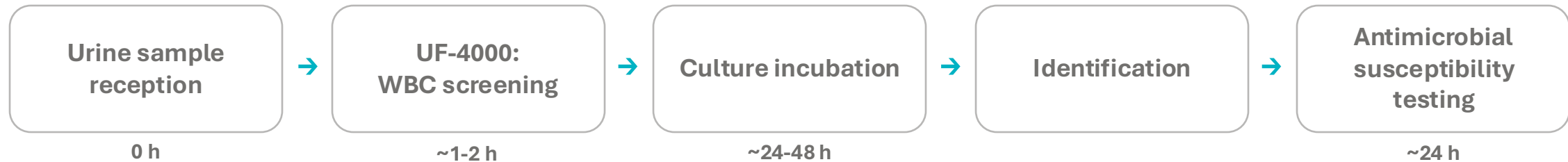


Urine sample

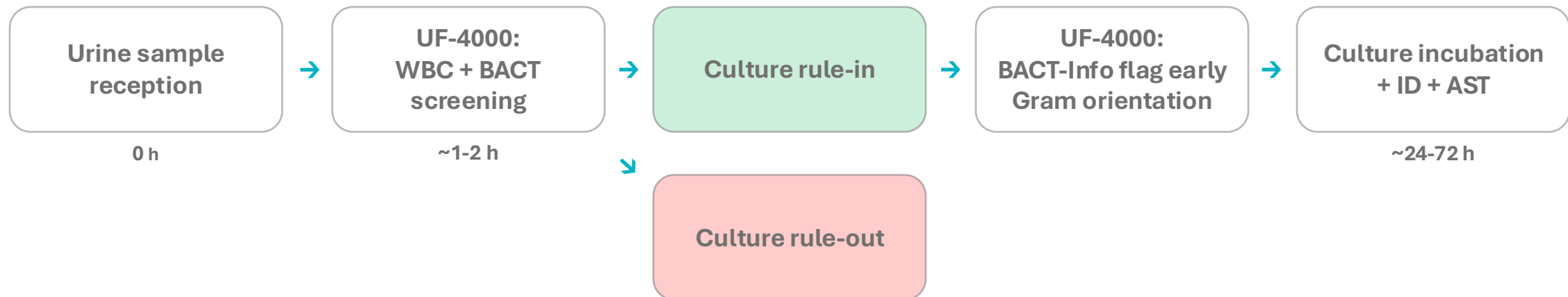
- WBC count: 180 WBC/ μ L
- BACT count: 2,400 BACT/ μ L
- BACT-Info flag: Gram-negative?

From sample collection to clinical decision

Conventional workflow



UF-4000-supported workflow



Why do we need optimisation?

**High volume of
urine cultures**

Substantial workload

Additional costs

**Long turnaround
times**

**Unnecessary or
suboptimal empirical
antibiotic therapy**

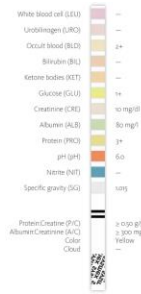
Rapid information could support a more efficient diagnostic workflow

- De Rosa R, et al. Clin Chim Acta. 2018.
- Korsten K, et al. J Clin Lab Anal. 2024.

02

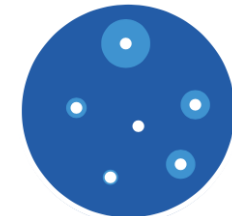
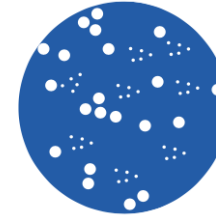
Background

Current diagnostic options



Urine test strip:

- Detection of infection-related parameters
 - Nitrites
 - Leucocyte esterase
- Rapid & low cost
- Limited specificity
- No information about antibiotics



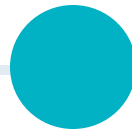
Urine culture and traditional AST:

- Two overnight cultures required
 - Day 1: urine culture
 - Day 2: antimicrobial susceptibility testing
- Identification of bacterial species
- Requires microbiology laboratory infrastructure & is labour-intensive

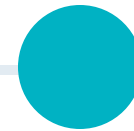
Sysmex urine flow cytometry analysers

1995**UF-100:**

First fully automated
urine flow cytometry

**2006****UF-1000i:**

Dedicated bacterial
channel

**2015****UF-4000/5000**

Blue laser, improved
bacterial differentiation,
BACT-Info flag

**2026****Current CAT:**

UF-4000 BACT count +
BACT-Info flag?

Sysmex UF-4000

- Connection UC-3500
- 17 diagnostic parameters – 15 research parameters
- UTI-related parameters:
 - **WBC**, EC, **BACT**, YLC, RBC
 - Flags: UTI-Info, **BACT-Info**
- 80 urine samples/hour
- 1 mL sampling volume
- 450 µL aspiration volume



Sysmex UF-4000: principle

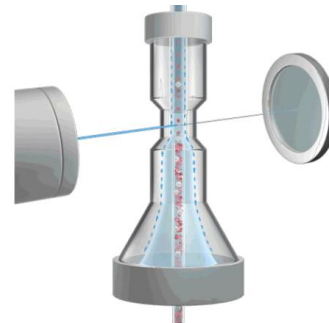
Fluorescence staining

- Channel-specific fluorochromes
- Staining of cell characteristics
 - Nucleic acids
 - Surface structures



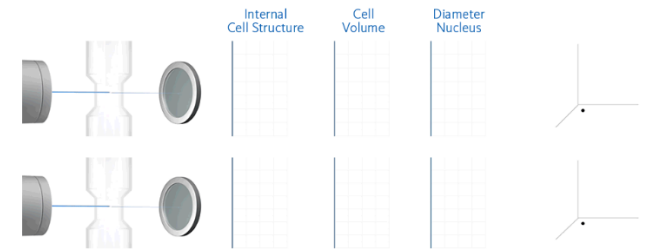
Hydrodynamic focussing

- Stained particles injected into flow cell
- Single-particle analysis
- Accurate particle counting



Particle characterisation

- 488 nm blue semiconductor laser
- Detection of optical signals
- Unique optical fingerprint



Sysmex UF-4000: principle

Forward scattered light (FSC):

Particle size/length and light permeability

Side scattered light (SSC):

Internal complexity of particles

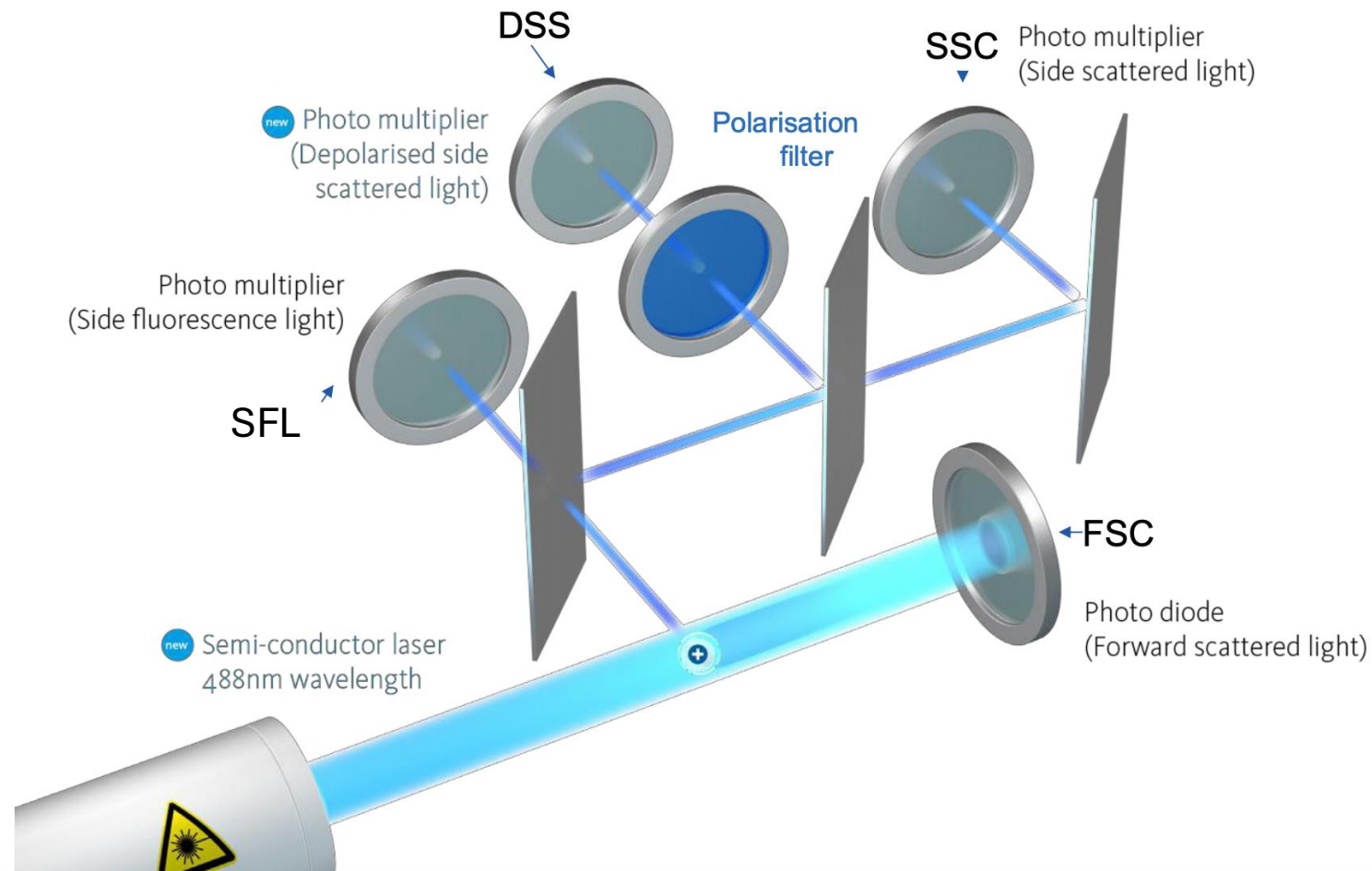
Side fluorescence light (SFL):

Degree of fluorescence staining

Depolarised side scattered light (DSS):

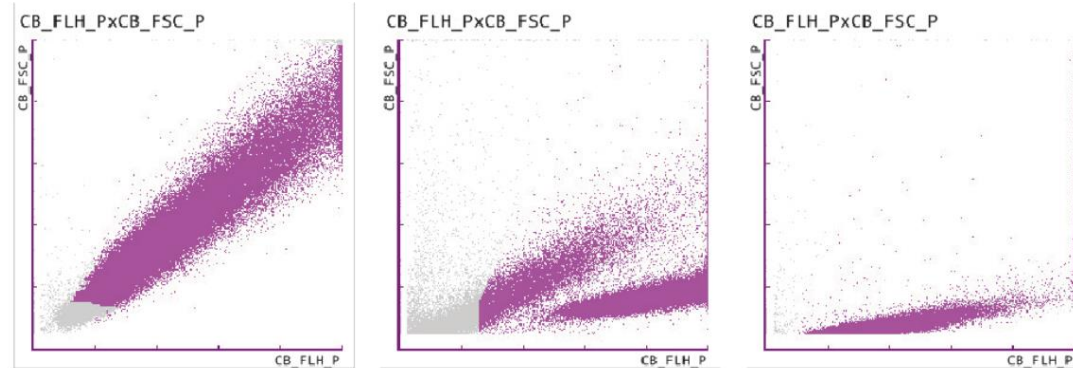
Differentiation of RBCs and crystals

Sysmex UF-4000: principle

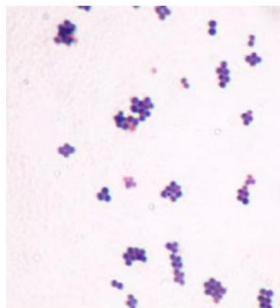


Sysmex UF-4000: BACT-Info flag

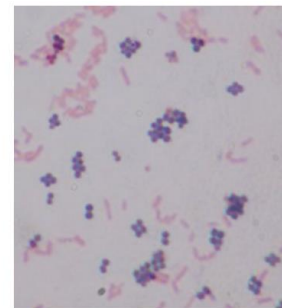
- Prediction of bacterial Gram type
- Differences in scattergram patterns reflect bacterial optical properties



No bacteriuria



„Gram positive?“



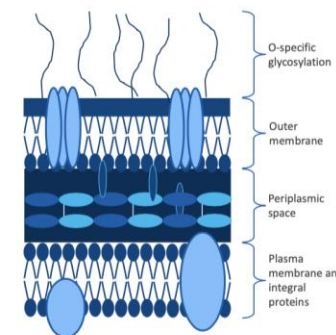
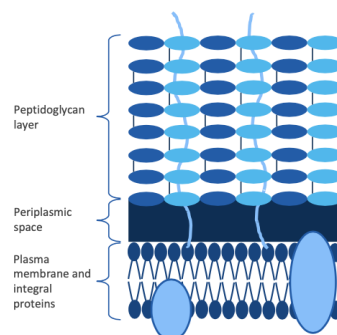
„Gram pos/neg?“



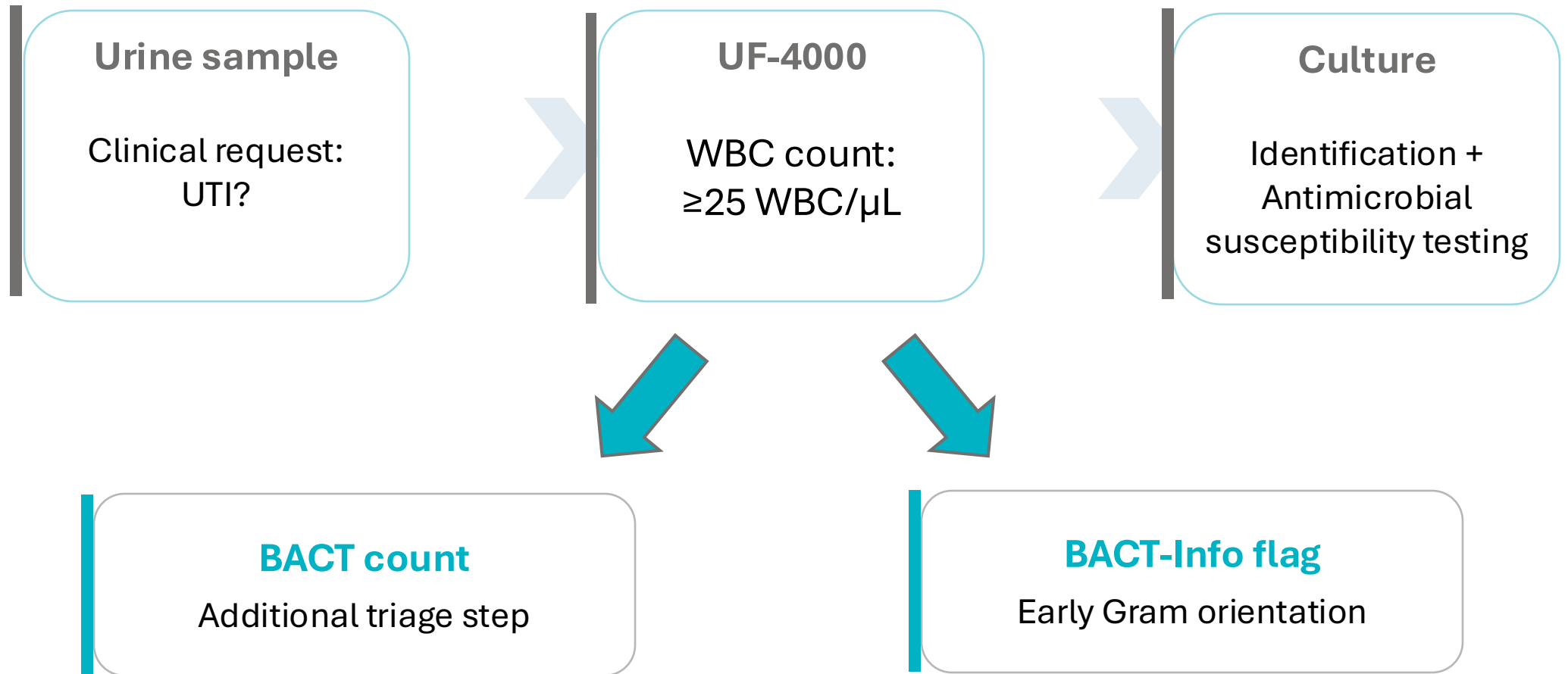
„Gram negative?“

Sysmex UF-4000: BACT-Info flag

Properties	Gram-positive bacteria	Gram-negative bacteria
Peptidoglycan layer	Thick (multilayered)	Thin (single layer)
Outer membrane	-	+
LPS layer	-	+
Teichoic acids	+	-
Lipid content	Low	High
Optical signals	↑ FSC, ↓ SFL	↓ FSC, ↑ SFL, ↑ SSC



Where does this fit in the current workflow?



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Research questions

Research questions

1. BACT count

What is the added value of incorporating the Sysmex UF-4000 BACT count into the current WBC-based urine culture screening algorithm for the detection of culture-positive urine samples?

2. BACT-Info flag

What is the clinical and analytical value of the UF-4000 BACT-Info flag for bacterial classification?

a. Literature review

What is the clinical utility of the BACT-Info flag for early bacterial classification in suspected UTI, based on the available literature?

b. Retrospective analysis

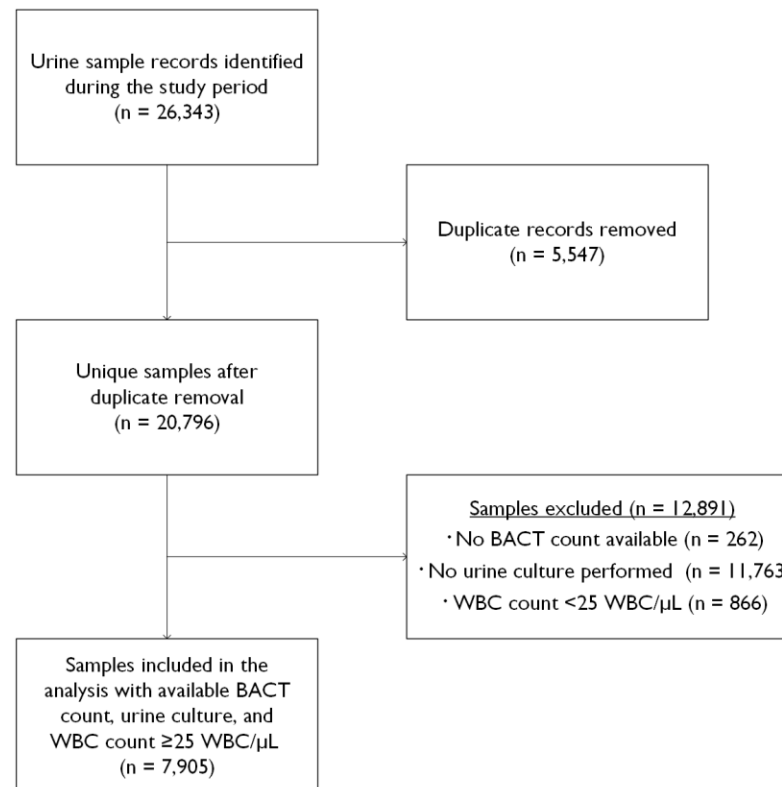
What is the diagnostic performance of the Sysmex UF-4000 BACT-Info flag in routine clinical practice, using culture-based results as the reference standard?

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Research question 1: BACT count

Study design

- Single-centre, retrospective study at Ziekenhuis Oost-Limburg
- Samples selected for culture based on WBC count or irrespective of WBC count



Study population

66 years

Median age

66.3%

Female

82.6%

Midstream urine

90.2%

WBC-triage rule

Culture outcome

- Negative: 12.7%
- Contamination/mixed flora: 41.7%
- ≥ 1 uropathogen: 45.5%

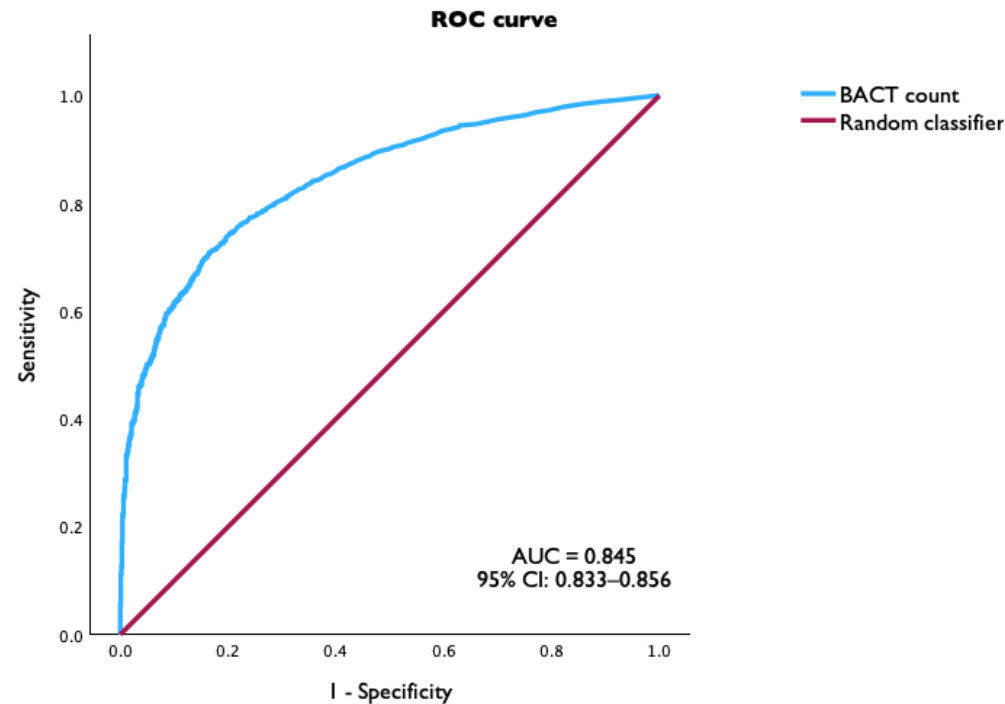
Uropathogens identified

4,551 uropathogen occurrences

1. *Escherichia coli*: 33.25%
2. *Enterococcus faecalis*: 16.52%
3. *Klebsiella pneumoniae/variicola*: 6.88%

ROC analysis

- Contamination/mixed flora = culture-positive
- AUC = 0.845 (95% CI: 0.833 – 0.856) → considerable discriminative ability
- Youden index: optimal cut-off 166 BACT/μL



Cut-off 166 BACT/ μ L

		Culture			Total
		Negative	Contamination	Positive	
Cut-off (166 BACT/ μ L)	Negative	840 (10.6%)	1,199 (15.2%)	809 (10.2%)	2,848 (36.0%)
	Positive	166 (2.1%)	2,100 (26.6%)	2,791 (35.3%)	5,057 (64.0%)
Total		1,006 (12.7%)	3,299 (41.7%)	3,600 (45.5%)	7,905 (100%)

Contamination/mixed flora = positive	TP	TN	FP	FN	SE	SP	PPV	NPV
Cut-off (166 BACT/ μ L)	4,891	840	166	2,008	70.9%	83.5%	96.7%	29.5%

Additional analyses



Two definitions of the reference standard

- 1) Contamination/mixed flora = culture-positive
- 2) Contamination/mixed flora = culture-negative



Three BACT counts

- 1) $\approx 95\%$ sensitivity
- 2) Youden index optimal cut-off
- 3) $\approx 95\%$ specificity

Additional analyses

Contamination/mixed flora = positive		TP	TN	FP	FN	SE	SP	PPV	NPV
Cut-off (BACT/ μ L)	15	6,567	319	687	332	95.2%	31.7%	90.5%	49.0%
	166	4,891	840	166	2,008	70.9%	83.5%	96.7%	29.5%
	798	3,450	956	50	3,449	50.0%	95.0%	98.6%	21.7%
Contamination/mixed flora = negative		TP	TN	FP	FN	SE	SP	PPV	NPV
Cut-off (BACT/ μ L)	19	3,419	627	3,678	181	95.0%	14.6%	48.2%	77.6%
	166	2,791	2,039	2,266	809	77.5%	47.4%	55.2%	71.6%
	1,828	1,972	3,525	780	1,628	54.8%	81.9%	71.7%	68.4%
	12,638	1,198	4,088	217	2,402	33.3%	95.0%	84.7%	63.0%

BACT count rule-out cut-offs

Contamination/mixed flora = positive

- 15 BACT/ μ L
- Sensitivity: 95.2%
- **Potential culture reduction: 8.2%**
- 141/651 (21.7%) < 15 BACT/ μ L contained a confirmed uropathogen
→ **3.9% of all uropathogen-positive samples**

Contamination/mixed flora = negative

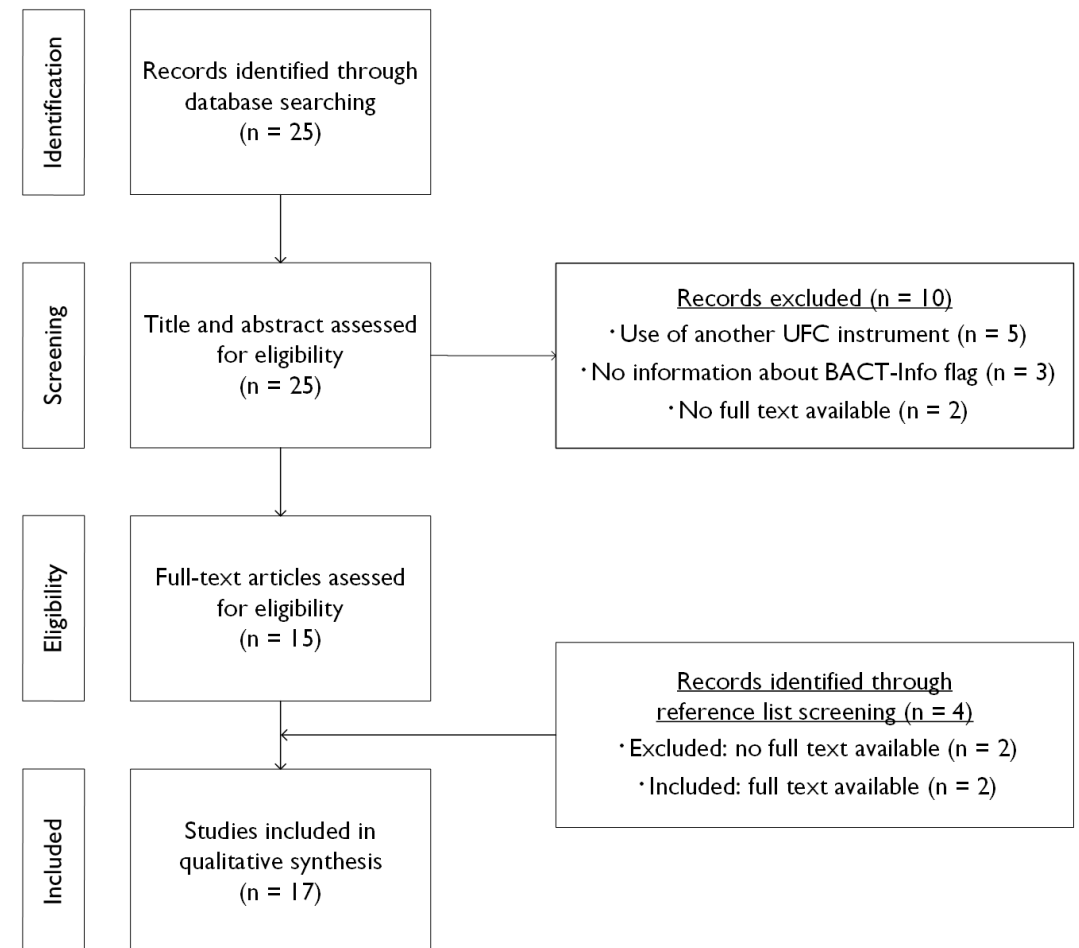
- 19 BACT/ μ L
- Sensitivity: 95.0%
- **Potential culture reduction: 10.2%**
- 181/808 (22.4%) < 19 BACT/ μ L contained a confirmed uropathogen
→ **5.0% of all uropathogen-positive samples**

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Research question 2: BACT-Info flag

Literature review: study selection

- PubMed (MEDLINE)
- Studies published up to 31 May 2026



Literature review: results

Gram-negative flag

- More consistent than GP flag
- Concordance: 62.5–96%
- PPV: high, often >90%

Gram-positive flag

- More variable across studies
 - Concordance: 20–86.8%
 - PPV: generally lower than GN flag
- ➔ Strongly affected by contamination/mixed flora

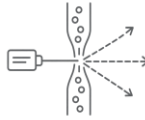
Clinical utility: rapid Gram orientation, particularly for likely GN bacteria

Literature review: GP classification



Microbiology

- GP organisms frequently occur in skin/perineal flora
- Contamination/mixed flora → GP signal ≠ confirmed GP uropathogen



Flow cytometry

- Cell-wall structure + fluorochrome uptake → influence the optical signature



Clinical consequence

- GP flag ≠ GP UTI
- BACT-Info flag: orientation – not identification

Literature review: Chang et al.

18 studies

Systematic review and
meta-analysis

25,337 urine samples

$\kappa = 0.52$

Pooled moderate
agreement

**GN flag performance
> GP flag performance**

BACT count: AUC 0.932
WBC count: AUC 0.867

Retrospective analysis: results

- 5,057 samples ≥ 166 BACT/ μ L
- Overall concordance: 67.0% $\rightarrow \kappa = 0.484$ (moderate agreement)

		Culture				Total
		Negative	GN	GP	Mixed	
BACT-Info flag	Unclassified	2	7	32	4	45
	GN	31	1,121	83	409	1,644
	GP	120	179	1,964	326	2,589
	Mixed	13	310	151	305	779
Total		166	1,617	2,230	1,044	5,057

Retrospective analysis: results

BACT-Info flag	TP	TN	FP	FN	SE	SP	PPV	NPV
GN	1,121	2,917	523	496	69.3%	84.8%	68.2%	85.5%
GP	1,964	2,202	625	266	88.1%	77.9%	75.9%	89.2%

Apparent GP performance > GN performance

Retrospective analysis: GP classification

Gram-positive cultures

- n = 2,230
- 602 (27.0%) confirmed GP uropathogens
- 1,628 (73.0%) contamination/mixed flora without confirmed GP uropathogen

Gram-negative cultures

- n = 1,617
- 1,611 (99.6%) confirmed GN uropathogens
- 6 (0.4%) contamination/mixed flora without confirmed GN uropathogen

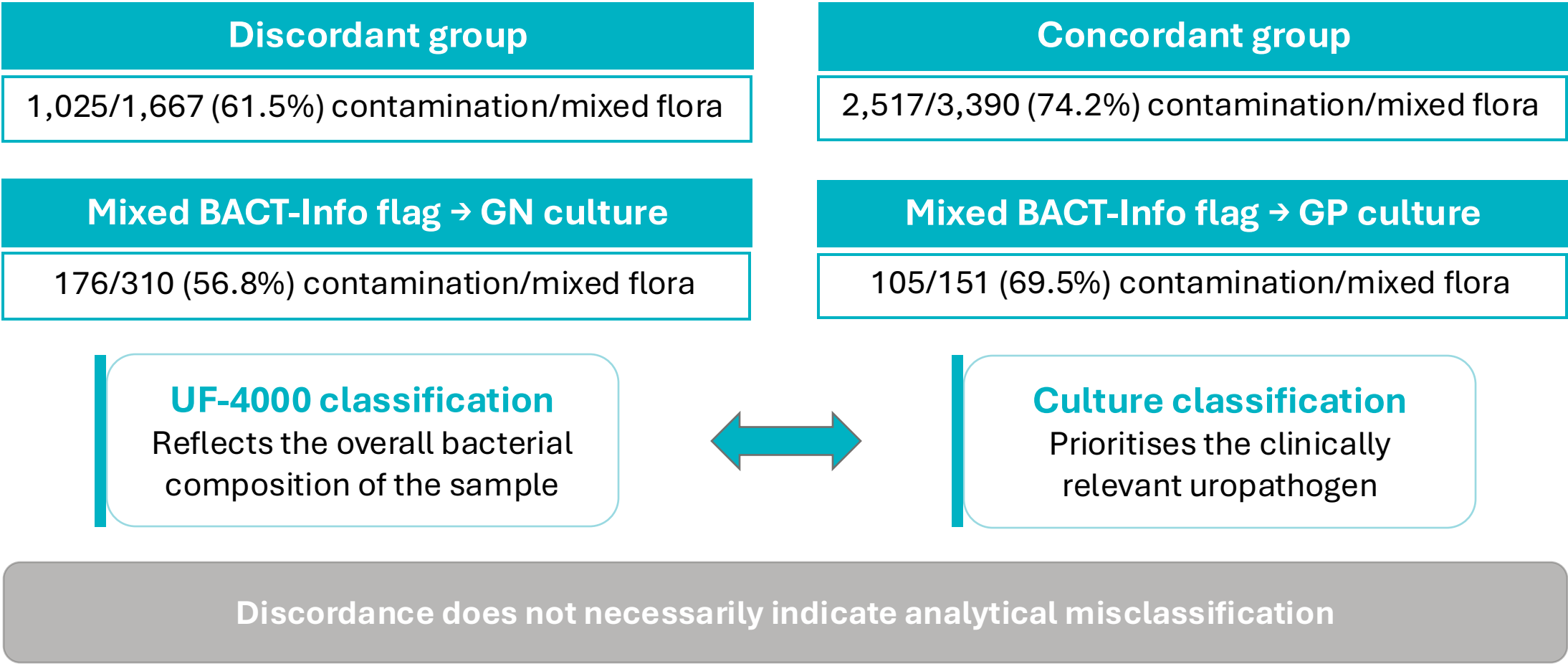
GP and GN culture groups differ substantially in composition

Retrospective analysis: GP classification

BACT-Info flag	TP	TN	FP	FN	SE	SP	PPV	NPV
GN	1,121	2,917	523	496	69.3%	84.8%	68.2%	85.5%
GP (all GP cultures)	1,964	2,202	625	266	88.1%	77.9%	75.9%	89.2%
GP (confirmed GP uropathogens only)	489	2,355	2,100	113	81.2%	52.9%	18.9%	95.4%

Restricting to confirmed GP uropathogens only: PPV 75.9% → 18.9%

Retrospective analysis: additional analyses

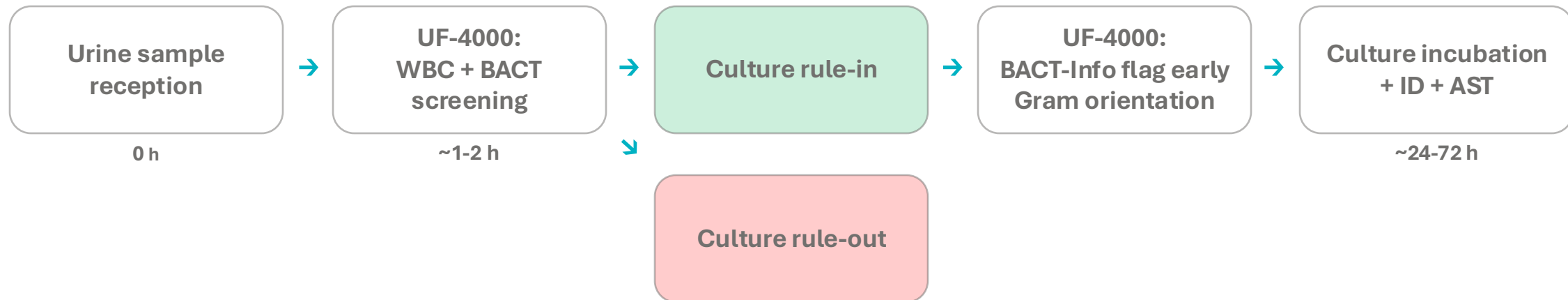


06

Conclusions

How could the workflow evolve?

UF-4000-supported workflow



What the CAT supports:

- BACT count adds diagnostic information beyond current WBC-based triage
- BACT-Info flag provides early bacterial orientation

What the CAT doesn't support:

- No validated rule-out cut-off is ready for routine implementation
- BACT-Info flag cannot replace culture or AST

Strengths and limitations

Strengths

- Large real-world cohort
- Routine laboratory setting
- Literature evidence integrated with local data

Limitations

- Single-centre, retrospective design
- Local population, workflow, and culture definitions → limits generalizability
- Potential culture reduction estimated retrospectively

To do's

Prospective evaluation of
a new workflow

Implement and prospectively evaluate a
new clinical laboratory workflow for UTIs

Clinical reporting of
BACT-Info flag

Define how the BACT-Info flag should
be interpreted and reported

Serial BACT count
monitoring

Evaluate changes in BACT count during antibiotic
therapy as an early indicator of treatment response

Take-home messages

- 1 BACT count** Promising additional parameter to rule out unnecessary cultures
- 2 BACT-Info flag** Useful for rapid Gram orientation – interpret GP results cautiously
- 3 Culture** Still required for definitive identification and antimicrobial susceptibility-guided treatment

References

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- Chang KW, Tsai CY, Wong SM, Chueh JSC, Chang SJ. Diagnostic accuracy of urine flow cytometry (UF) in urinary tract infection (UTI) detection and management: a systematic review and meta-analysis. Diagnostics (Basel). 2026;16(9):1275. <https://doi.org/10.3390/diagnostics16091275>.

