
Supplementary Materials

Table S1. Samples excluded based on isolated micro-organisms other than *Enterobacterales* spp, *Staphylococcus* spp or *Enterococcus* spp.

Achromobacter xylosoxidans (3)

Acinetobacter baumannii (1)

Acinetobacter spp. (1)

Haemophilus influenzae (1)

Micrococcus luteus (2)

Neisseria meningitidis (1)

Pseudomonas aeruginosa (4)

Streptococcus agalactiae (1)

Streptococcus constellatus (milleri gr.) (1)

Streptococcus dysgalactiae (1)

Stenotrophomonas maltophilia (1)

Streptococcus milleri group (1)

Streptococcus vestibularis (salivarius gr.) (1)

Table S2. Specifications of errors occurred.

Preparation errors SpecifAST®	
Leaks	9 b/d in 4 blood cultures
Bacterial contamination	2 b/d in 1 blood culture
Incorrect number of antibiotic dilutions	2 b/d in 2 blood cultures
Technical errors SpecifAST®	
No signal	12 b/d in 4 blood cultures
Software problem	11 b/d in 4 blood cultures
Instrument error	3 b/d in 1 blood culture
No bacterial growth in SpecifAST® and VITEK®2	2 b/d in 1 blood culture

b/d: bug/drug combinations.

Table S3. Minimal inhibitory concentrations of *Enterobacterales*, *Staphylococcus* and *Enterococcus* spp. and corresponding tested antimicrobials measured by SpecifAST® and VITEK®2.

A. Ciprofloxacin, *Enterobacterales* spp.

		VITEK®2					
		Susceptible	ATU	Resistant			
		≤0.25	0.5	1	2	>2	Total
SpecifAST®	≤0.25	35	0	0	0	0	35
	0.5	0	0	0	0	0	0
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	>2	0	0	0	0	9	9
	Total	35	0	0	0	9	44

B. Cefotaxime, *Enterobacterales* spp.

		VITEK®2									
		Susceptible			I	Resistant					
		≤0.25	0.5	1	2	4	8	16	32	>32	Total
SpecifAST®	≤0.25	37	0	0	0	0	0	0	0	0	37
	0.5	0	1	0	0	0	0	0	0	0	1
	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
	16	0	0	0	0	1	0	0	0	0	1
	32	0	0	0	0	0	0	1	0	1	2
	>32	0	0	0	0	0	0	1	0	3	4
	Total	37	1	0	0	1	0	2	0	4	45

C. Meropenem, *Enterobacterales* spp.

		VITEK®2							
		Susceptible				I		Resistant	
		≤0.25	0.5	1	2	4	8	>8	Total
SpecifAST®	≤0.25	43	0	0	0	0	0	0	43
	0.5	1	0	0	0	0	0	0	1
	1	1	0	0	0	0	0	0	1
	2	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0
	>8	0	0	0	0	0	0	0	0
	Total	45	0	0	0	0	0	0	45

D. Oxacillin, *S. aureus*, *S. lugdunensis* and *S. saprophyticus*.

		VITEK®2					
		Susceptible				Resistant	
		≤0.25	0.5	1	2	>2	Total
SpecifAST®	≤0.25	6	3	0	0	0	9
	0.5	0	1	0	0	0	1
	1	1	0	0	0	0	1
	2	0	0	0	0	0	0
	>2	1	0	0	0	0	1
	Total	8	4	0	0	0	12

E. Oxacillin, coagulase negative staphylococci other than *S. lugdunensis* and *S. saprophyticus*.

		VITEK®2					
		Susceptible	Resistant				
		≤0.25	0.5	1	2	>2	Total
SpecifAST®	≤0.25	10	0	0	0	1	11
	0.5	0	0	0	0	0	0
	1	1	0	1	0	0	2
	2	1	0	0	0	4	5
	>2	1	0	0	0	8	9
	Total	13	0	1	0	13	27

F. Cefoxitin (6 mg/L), *Staphylococcus* spp.

		VITEK®2		
		Susceptible	Resistant	
		Negative	Positive	Total
SpecifAST®	Negative	22	4	26
	Positive	0	12	12
	Total	22	16	38

G. Vancomycin, *S. aureus*.

		VITEK®2							
		Susceptible			Resistant				
		≤0.5	1	2	4	8	16	>16	Total
SpecifAST®	≤0.5	4	0	0	0	0	0	0	4
	1	1	4	0	0	0	0	0	5
	2	2	0	0	0	0	0	0	2
	4	1	0	0	0	0	0	0	1
	8	0	0	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	0
	>16	0	0	0	0	0	0	0	0
	Total	8	4	0	0	0	0	0	12

H. Vancomycin, coagulase negative *Staphylococcus* spp.

		VITEK®2							
		Susceptible				Resistant			
		<=0.5	1	2	4	8	16	>16	Total
SpecifAST®	<=0.5	0	1	0	0	0	0	0	1
	1	6	3	1	0	0	0	0	10
	2	3	3	1	0	0	0	0	7
	4	3	3	2	0	0	0	0	8
	8	0	0	1	0	0	0	0	1
	16	0	0	0	0	0	0	0	0
	>16	0	0	0	0	0	0	0	0
	Total	12	10	5	0	0	0	0	27

I. Ampicillin, *Enterococcus* spp.

		VITEK®2					
		Susceptible		I	Resistant		
		<=2	4	8	16	>16	Total
SpecifAST®	<=2	1	0	0	0	0	1
	4	2	0	0	0	0	2
	8	1	0	0	0	0	1
	16	0	0	0	0	0	0
	>16	0	0	0	0	5	5
	Total	4	0	0	0	5	9

J. Vancomycin, *Enterococcus* spp.

		VITEK®2							
		Susceptible				Resistant			
		<=0.5	1	2	4	8	16	>16	Total
SpecifAST®	<=0.5	5	0	0	0	0	0	0	5
	1	1	3	0	0	0	0	0	4
	2	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	1	1
	>32	0	0	0	0	0	0	0	0
	Total	6	3	0	0	0	0	1	10

Interpretation of susceptibility test results according to EUCAST 2021 clinical breakpoints. Categorical agreement (green) Minor errors (orange) Major and very major errors (red). ATU: Area of technical uncertainty; I: Susceptible, increased exposure.

Table S4. Sensitivity and specificity of SpecifAST® method compared to VITEK®2.

Gram negatives	Sensitivity (95%-CI)	Specificity (95%-CI)	Resistance prevalence
Ciprofloxacin	100%	100%	9/45 (20%)
Cefotaxim	100%	100%	7/46 (19%)
Meropenem	100%	100%	0/46 (0%)
Gram positives			
Ampicillin	100%	75% (32.6 - 117.4)	8/12 (66.7%)
Oxacillin	92.9% (78.9 – 106.9)	85.7% (71.7 – 99.7)	14/42 (33.3%)
Cefoxitin	75% (53.8 - 96.2)	100%	16/41 (39.0%)
Vancomycin	100%	96.2% (91.0 - 101.4)	1/53 (1.9%)
Mean	91% (83.1 - 98.9)	97% (94.8 – 99.2)	55/285 (19.3%)

Sensitivity and specificity with corresponding 95% confidence intervals, and resistance prevalence. CI: Confidence interval.