Table 1 Main pathogen-specific biomarkers used in routine practice in critically ill patients

Biomarker	Methods	Infection diagnosis	Sample (Cut-off)	Diagnostic accuracy		Comments
				Sensitivity (95%CI)	Specificity (95%CI)	
Influenza A/B Ag test	EIA ICT FIA	Influenza pneumonia	nasal swab other respiratory samples	0.69 (0.64-0.74)	0.97 (0.96-0.98)	Sensitivity varies according to test method (higher sensibility with FIA > ICT - EIA) and population. Rapid results with ICT.
SARS-CoV-2 Ag test	ICT FIA	SARS-CoV-2 pneumonia	nasal swab other respiratory samples	0.70 (0.69-0.71)	0.98 (0.98-0.98)	Higher sensitivity with nasal swab (*versus* other respiratory samples), among symptomatic patients (*versus* asymptomatic) and with higher viral load (RT-PCR cycle threshold ≤25). Rapid results.
***Streptococcus pneumoniae* urinary Ag test**	ICT FIA	Pneumococcal pneumonia	urine	0.72 (0.62-0.80)	0.83 (0.65-0.93)	Sensitivity varies depending on the pneumococcal serotype. Higher sensitivity with FIA > ICT, and in pneumonia with positive blood or pleural fluid cultures. No impact of antibiotic exposure on sensitivity. False positives: *Streptococcus pneumoniae* colonisation in children, vaccination (48h), prior infection (several months). Rapid results (15 minutes). Can also be used on CSF in suspected pneumococcal meningitis.
***Legionella* urinary Ag test**	EIA ICT FIA	Legionellosis caused by *Legionella* spp.	urine	0.79 (0.71-0.85)	1.00 (0.99-1.00)	Mainly detect *Legionella pneumophila* serogroup 1 (LP1), resulting in higher sensitivity for legionellosis cause by LP1 0.84 (0.75-0.90). Higher sensitivity with FIA > ICT > EIA, and in severe legionellosis. No impact of antibiotic exposure on sensitivity. Rapid results (ICT/FIA 15 minutes, EIA 90 minutes).
Glutamate dehydrogenase (GDH)	EIA	*Clostridium difficile* infection	unformed stool	0.94 (0.89-0.97)	0.90 (0.88-0.92)	At low CDI prevalence (5%), PPV 34-38% and NPV 100%. Rapid results.
***Clostridium difficile* toxins A/B**	EIA	*Clostridium difficile* infection	unformed stool	0.83 (0.76-0.88)	0.99 (0.98-0.99)	At low CDI prevalence (5%), PPV 69-81% and NPV 99%. Rapid results (30 minutes). Several tests include both detection of GDH and toxins A/B. A positive GDH result but negative toxins A/B detection may indicate a false positive GDH, a false negative toxins A/B result, CDI with toxin levels below the threshold of detection, or toxigenic *Clostridium difficile* carriage.
(1,3)-β-D-glucan (BDG) (Fungitell® assay)	protease zymogen-based colorimetric assay	Invasive *Candida* infection	Serum (>80 pg/mL)	0.81 (0.74-0.86)	0.60 (0.49-0.71)	Early positivity (24-72h before blood culture), slow decreasing kinetics (up to 7 weeks persistence after positive blood culture). Sensitivity depends on fungal species (lower sensibility for *C. parapsilosis*) At low prevalence of invasive *Candida* infection (<5%), PPV 10-15% and NPV >95%. Specificity and PPV can be increased by two consecutive positive samples, increased cut-off value, or combination with other specific biomarker for *Candida* such as mannan or *Candida albicans* germ tube-specific antibody. BDG test requires glucan-free laboratory equipment. Numerous causes of false-positive results, but less frequent in current clinical practice than in theory: fungal colonization, severe mucositis, disruption of gastrointestinal tract integrity, blood transfusions, albumin, immunoglobulin, hemodialysis/hemofiltration, surgical gauze, β-lactam antibiotics, enteral nutrition, Gram-positive bacteremia, sample contamination.
		Pneumocystis jirovecii pneumonia	Serum (>80 pg/mL)	0.91 (0.87-0.94)	0.79 (0.72-0.84)	Increased sensitivity in HIV patients 0.94 (0.91-0.96) *versus* non HIV patients 0.86 (0.78-0.91). At low/intermediate pre-test probability (≤20% in non-HIV and ≤50% in HIV), NPV ≥95%.

						A negative BDG cannot rule out the diagnosis among patients with a higher likelihood of *Pneumocystis jirovecii* pneumonia.
Galactomannan (GM)	EIA	Invasive pulmonary aspergillosis	Serum (ODI ≥0.5)	0.74 (0.64-0.82)	0.85 (0.77-0.90)	Increasing cut-off (ODI ≥1) increased both sensitivity and specificity. False negatives are frequent in non-neutropenic critically ill patients, except for Influenza-associated pulmonary aspergillosis. Causes of false-positive results: intestinal mucositis, β-lactams antibiotics.
			Serum (ODI ≥1)	0.79 (0.60-0.91)	0.88 (0.78-0.94)	
			BAL (ODI ≥0.5)	0.79 (0.65-0.88)	0.84 (0.74-0.91)	Increasing cut-off (ODI ≥1) increased both sensitivity and specificity. More useful for the diagnosis of invasive pulmonary aspergillosis in non-neutropenic critically ill patients than that serum GM.
			BAL (ODI ≥1.0)	0.90 (0.77-0.96)	0.94 (0.88-0.97)	
Cryptococcal Ag test	EIA ICT	Cryptococcal meningitis	Serum Cerebrospinal fluid	0.99 (0.88-100) 0.99 (0.96-100)	0.95 (0.88-0.98) 0.99 (0.97-100)	Detects all cryptococcal serotypes. In HIV adults with cryptococcal meningitis symptoms, a negative serum cryptococcal Ag test may rule out cryptococcal meningitis. Rapid results (10 minutes) with point of care lateral flow ICT.

Ag antigen; *BAL* bronchoalveolar lavage; *BDG* (1,3)-β-D-glucan*; CDI Clostridium difficile* infection; *CI* confidence interval; EIA enzyme immunoassay; *FIA* fluorescence immunoassay; *GM* galactomannan; *GDH* glutamate dehydrogenase; *HIV* human immunodeficiency virus; *ICT* immunochromatographic test; *NPV* negative predictive value; *ODI* optical density index; *PPV* positive predictive value.

For Influenza A/B Ag test, pooled sensitivity and specificity are presented for ICT only. For legionellosis diagnostic, reference test = positive culture and/or PCR and/or serology. For CDI diagnosis, reference test = cell cytotoxicity neutralization assay. BDG diagnostic accuracy for invasive *Candida* infection was assessed in an ICU population at risk for ICI, reference standard = European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) criteria for proven invasive candidiasis. For *Pneumocystis jirovecii* pneumonia diagnostic, reference test = cytological sputum staining, except 2 studies with PCR. GM diagnostic accuracy for invasive pulmonary aspergillosis was assessed in patients with impaired immunity suspected of having invasive aspergillosis, reference standard = EORTC/MSG criteria for proven/probable aspergillosis. Cryptococcal antigen diagnostic accuracy for cryptococcal meningitis was assessed in HIV-positive patients with central nervous system symptoms, reference test = cerebrospinal fluid fungal culture.