AEROBES

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6]	INOCULUM		SUGGESTED MIC PANEL [3]	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS [4]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Staphylococci	MRSA	MHA	0.5	Saline	Cefoxitin (FX)	18-24h	35 ± 2°C Ambient air	*Staphylococcus aureus* *S. aureus*	29213™ 43300™	≤ 4 mg/L > 4 mg/L
		Mueller Hinton agar (MHA) + 2% NaCl	0.5-1	Saline	Oxacillin (OX) Amoxicillin/clavulanic acid (XL)	24h	35 ± 2°C Ambient air	*S. aureus*	43300™	- Phenotype interpretation: OX ≥ 4 µg/mL **and** XL ≥ 8 µg/mL - Heavier inoculum improves detection of low level R
	BORSA	MHA + 2% NaCl	0.5-1	Saline	Oxacillin (OX) Amoxicillin/clavulanic acid (XL)	24h	35 ± 2°C Ambient air	*S. aureus*	29213™	- Phenotype interpretation: OX ≥ 4 µg/mL **and** XL ≤ 4 µg/mL
	MRCNS	Mueller Hinton agar (MHA) + 2% NaCl	0.5-1	Saline	Oxacillin (OX)	48h	35 ± 2°C Ambient air	*S. aureus*	43300™	- Phenotype interpretation: OX ≥ 0.5 µg/mL - Heavier inoculum improves detection of low level R
	VISA/hVISA [1] (macromethod)	Brain Heart Infusion agar (BHI) [1]	2	BHI broth	Vancomycin (VA) Teicoplanin (TP) [1]	24h. Confirm at 48h	35 ± 2°C Ambient air	*S. aureus* QC ranges: TP = 0.5-2 µg/mL VA = 1-4 µg/mL	29213™	- 0.1 mL inoculum/ 90mm plate - Phenotype interpretation: VA ≥ 8µg/mL **and** TP ≥ 8µg/mL, **OR** TP ≥12 µg/mL **(alone)** = VISA/hVISA - Confirm VISA/hVISA by PAP
	VISA/hVISA	MHA + 5% blood	0.5	BHI broth	Etest GRD[2] (Vancomycin (VA) / Teicoplanin (TP) [1])	18-24h. Confirm at 48h	35 ± 2°C Ambient air	*S. aureus* *S. aureus* *S. aureus*	29213™ 700698™ 700699™	- Confirm VISA/hVISA by PAP
	VISA/VRSA (standard method)	MHA	0.5	Saline	Vancomycin (VA)	24h	35 ± 2°C Ambient air	*S. aureus*	29213™	- Phenotype interpretation: VA ≥ 4µg/mL = VISA VA ≥ 16µg/mL = VRSA (CLSI)

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6]	INOCULUM		SUGGESTED MIC PANEL [3]	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS [4]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Staphylococci	MRSA Secondary panel	MHA	0.5	Saline	Clindamycin (CM) Daptomycin (DPC) Linezolid (LZ) Vancomycin (VA) Teicoplanin (TP) [1] Quinupristin/dalfopristin (QDA) Rifampicin (RI) [1]	16-20h. VA and TP full 24h	35 ± 2°C Ambient air	*S. aureus*	29213™	
	Non-MRSA secondary panel	MHA	0.5	Saline	Benzylpenicillin (PG) Erythromycin (EM) Linezolid (LZ) Trimethoprim/sulfamethoxazole (TS) Vancomycin (VA) Teicoplanin (TP) [1] Ciprofloxacin (CI)	16-20h. VA and TP full 24h	35 ± 2°C Ambient air	*S. aureus*	29213™	
Enterococci		MHA	0.5	Saline	Ampicillin (AM) Daptomycin (DPC) Linezolid (LZ) Quinupristin/dalfopristin (QDA) Vancomycin (VA) Teicoplanin (TP) [1] Minocycline (MC)	16-20h. Confirm VA and TP at 24h	35 ± 2°C Ambient air	*E. faecalis*	29212™	
	HLAR	MHA	0.5-1	Saline	Gentamicin (GM) high range Streptomycin (SM) high range [1]	24h. Confirm SM at 48h	35 ± 2°C Ambient air	*E. faecalis* QC ranges: GM = 4-16 µg/mL SM = 64–256 µg/mL	29212™	- Heavier inoculum preferable - SM > 1024 µg/mL or GM > 512 µg/mL = positive HLAR (CLSI) - SM > 512 µg/mL or GM > 128 µg/mL = positive HLAR (EUCAST)
Enterobacteriaceae		MHA	0.5 (1 for mucoid organisms)	Saline	Gentamicin (GM) Piperacillin/ tazobactam (PTc) Cefepime (PM) Ciprofloxacin (CI) Imipenem (IP) Aztreonam (AT)	16-20h	35 ± 2°C Ambient air	*Escherichia coli* *E. coli*	25922™ 35218™ (PTc)	
	ESBL	MHA	0.5 (1 for mucoid organisms)	Saline	Cefotaxime/cefotaxime + clavulanic acid (CT/CTL) Ceftazidime/ceftazidime + clavulanic acid (TZ/TZL) Cefepime/cefepime + clavulanic acid (PM/PML) [1]	16-20h	35 ± 2°C Ambient air	*E. coli* *Klebsiella pneumoniae* (ESBL positive) *Pseudomonas aeruginosa*	35218™ 700603™ 27853™	- Investigate ND results by an alternative method

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6]	INOCULUM		SUGGESTED MIC PANEL [3]	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS [4]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Enterobacteriaceae	MBL MP/MPI [1]	MHA	0.5 (1 for mucoid organisms)	Saline	Meropenem/Meropenem +EDTA (MP/MPI)	16-20h.	35 ± 2°C Ambient air	*K. pneumoniae* *K. pneumoniae*	700603™ BAA-2146™	- Investigate ND results by an alternative method
	AmpC [2]	MHA	0.5	Saline	Cefotetan/Cefotetan + Cloxacillin (CN/CNI)	16-20h	35 ± 2°C Ambient air	*Klebsiella pneumoniae* (negative) *K. pneumoniae* (positive)	700603™ BAA-1144™	- Investigate ND results by an alternative method
Pseudomonas spp.		MHA	0.5 (1 for mucoid organisms)	Saline	Ceftazidime (TZ) Gentamicin (GM) Aztreonam (AT) Ciprofloxacin (CI) Imipenem (IP) Piperacillin/tazobactam (PTc)	16-20h. 48h for slow growers	35 ± 2°C Ambient air	*P. aeruginosa* *E. coli*	27853™ 35218™ (PTc)	
	MBL IP/IPI [1]	MHA	0.5 (1 for mucoid organisms)	Saline	Imipenem/imipenem + EDTA (IP/IPI)	16-20h. 48h for slow growers	35 ± 2°C Ambient air	*P. aeruginosa* (negative) *Stenotrophomonas maltophilia* (positive)	27853™ 13636™	- Investigate ND results by an alternative method
Acinetobacter spp.		MHA	0.5 (1 for mucoid organisms)	Saline	Ceftazidime (TZ) Meropenem (MP) Amikacin (AK) Ampicillin/sulbactam (AB) Levofloxacin (LE) Minocycline (MC)	20-24h. 48h for slow growers	35 ± 2°C Ambient air	*P. aeruginosa* *E. coli*	27853™ 35218™ (AB)	
	MBL IP/IPI [1]	MHA	0.5 (1 for mucoid organisms)	Saline	Imipenem/imipenem + EDTA (IP/IPI)	16-20h. 48h for slow growers	35 ± 2°C Ambient air	*P. aeruginosa* (negative) *Stenotrophomonas maltophilia* (positive)	27853™ 13636™	- Investigate ND results by an alternative method
Burkholderia cepacia		MHA	0.5 (1 for mucoid organisms)	Saline	Trimethoprim/sulfamethoxazole (TS) Ceftazidime (TZ) Levofloxacin (LE) Meropenem (MP) Minocycline (MC)	20-24h. 48h for slow growers	35 ± 2°C Ambient air	*E. coli*	25922™	
Stenotrophomonas maltophilia		MHA	0.5 (1 for mucoid organisms)	Saline	Trimethoprim/sulfamethoxazole (TS) Ceftazidime (TZ) Levofloxacin (LE) Minocycline (MC) Ticarcillin/clavulanic acid (TLc)	20-24h. 48h for slow growers	35 ± 2°C Ambient air	*E. coli* *E. coli*	25922™ 35218™ (TLc)	

FASTIDIOUS ORGANISMS

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6]	INOCULUM		SUGGESTED MIC PANEL [3]	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS [4]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Pneumococci		MHA + 5% blood (CLSI) MHF (EUCAST)	0.5 (1 for mucoid organisms)	MH broth or BHI broth	Meropenem (MP) Cefotaxime (CT) Benzylpenicillin (PG) Clindamycin (CM) [1] Vancomycin (VA) Trimethoprim/sulfamethoxazole (TS)	20-24h	35 ± 2°C 5% CO_2	*Streptococcus pneumoniae*	49619™	
Streptococci		MHA + 5% blood (CLSI) MHF (EUCAST)	0.5 (1 for mucoid organisms)	MH broth or BHI broth	Benzylpenicillin (PG) Cefotaxime (CT) Chloramphenicol (CL) Ofloxacin (OF) Linezolid (LZ) [1] Vancomycin (VA) Daptomycin (DPC)	20-24h	35 ± 2°C 5% CO_2	*S. pneumoniae*	49619™	
Abiotrophia & *Granulicatella* spp. [1]		MH chocolate agar + 0.001% pyridoxal HCl + 0.01% cysteine	1	Broth (MH broth or BHI broth)		20-24h	35 ± 2°C 5% CO_2	[5]	[5]	
Haemophilus influenzae		Haemophilus Test Media (HTM) (CLSI) MHF (EUCAST)	0.5 (1 for mucoid organisms)	MH broth or HTM broth (or BHI broth)	Amoxicillin/ clavulanic acid (XL) Cefotaxime (CT) Meropenem (MP) Trimethoprim/sulfamethoxazole (TS) Chloramphenicol (CL)	20-24h	35 ± 2°C 5% CO_2	*Haemophilus influenzae* *H. influenzae*	49247™ 49766™ (MP, XM)	
Moraxella catarrhalis [1]		MHA + 5% blood (CLSI) MHF (EUCAST)	0.5	MH broth or BHI broth		20-24h	35 ± 2°C 5% CO_2	[5]	[5]	
Anaerobes		Brucella agar + 5% blood + vitamin K (1 µg/mL) + hemin (5 µg/mL) (BBA) (CLSI)	1	Brucella broth or MH broth (or Schaedler Broth + vit K3)	Metronidazole (MZ) Clindamycin (CM) Cefoxitin (FX) Imipenem (IP) Piperacillin/tazobactam (PTc) Benzylpenicillin (PG)	24-72h (48h for CM). Confirm all S results at 48h.	35 ± 2°C Anaerobic system	*Bacteroides fragilis* *B. thetaiotaomicron* *Eubacterium lentum*	25285™ 29741™ 43055™	- For obligate anaerobes, ensure anaerobic conditions are maintained throughout processing - DO NOT VORTEX - Anaerobiosis must be achieved within 1-2 hours for MZ

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6]	INOCULUM		SUGGESTED MIC PANEL [3]	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS [4]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Helicobacter pylori [1]		MHA + 5% blood (≥ 2 weeks old) (CLSI)	3	MH broth + 5% serum or BHI broth + 5% serum	Amoxicillin (AC) Clarithromycin (CH) Metronidazole (MZ) Tetracycline (TC)	72h or longer	35 ± 2°C Micro-aerophilic, except MZ - first 24h anaerobic	*Helicobacter pylori*	43504™	- 1 strip/90mm plate - Strip handle to be placed at edge of plate. - *H. pylori* colonies are pin-point, translucent and difficult to see. Tilt plate and use oblique light to read endpoint.
Gonococci		Supplemented GC agar (CLSI) MH chocolate agar	0.5	MH broth (or BHI broth)	Ciprofloxacin (CI) Benzylpenicillin (PG) Tetracycline (TC) Ceftriaxone (TX) Spectinomycin (SC) [1]	20-24h	35 ± 2°C 5% CO_2	*Neisseria gonorrhoeae*	49226™	- 3-4 strips max./150mm plate to facilitate reading
Meningococci [1]		MHA + 5% blood (CLSI) MH chocolate agar	0.5	Broth (BHI broth)	Ciprofloxacin (CI) Benzylpenicillin (PG) Trimethoprim/sulfamethoxazole (TS) Meropenem (MP) Ceftriaxone (TX)	24h	35 ± 2°C 5% CO_2	*S. pneumoniae* *E. coli*	49619™ 25922™	- Work in BSC
Campylobacter spp. [1]		MHA + 5% blood (CLSI) MHF (EUCAST)	1	Broth (BHI broth)	Ciprofloxacin (CI) Gentamicin (GM) Erythromycin (EM) Doxycycline (DC)	48-72h	35 ± 2°C Micro-aerophilic	*Campylobacter jejuni*	33560™	- 3-4 strips max./150mm plate to facilitate reading - Do not invert plate - *Campylobacter* colonies may be translucent and difficult to see. Tilt plate and use oblique light to read endpoint.

FASTIDIOUS GRAM-POSITIVE ORGANISMS [1)]

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6)]	INOCULUM		SUGGESTED MIC PANEL [3)]	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS [4)]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Arcanobacterium spp., *Listeria monocytogenes**, *Erysipelothrix*, *Lactobacillus*, *Corynebacterium*, *Bacillus* spp., *Rothia*, *Pediococcus**, *Leuconostoc**, and *Gemella* spp.*		MHA + 5% blood (CLSI) * MHF (EUCAST)	1	Broth (BHI broth)		20-24h (48h if required)	35 ± 2°C 5% CO_2	[5)]	[5)]	

FASTIDIOUS GRAM-NEGATIVE ORGANISMS [1]

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6]	INOCULUM		SUGGESTED MIC PANEL [3]	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS [4]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Bartonella spp.		MH chocolate agar	1	Broth (BHI broth)		3-5 days	35 ± 2°C 5% CO_2	[5]	[5]	
Bordetella pertussis		Regan-Lowe Bordet-Jengou or MHA + 5% blood	3	Broth (BHI broth)		3-5 days	35 ± 2°C Ambient air in bags, moist	[5]	[5]	
Capnocytophaga spp.		BBA	1	Broth (BHI broth)		48h	35 ± 2°C 5% CO_2	[5]	[5]	
Legionella spp.		Buffered Charcoal Yeast Extract (BCYE)	1	Broth (BHI broth)		3-5 days	35 ± 2°C 5% CO_2	[5]	[5]	
Pasteurella spp.		MHA + 5% blood (CLSI) MHF (EUCAST)	1	Broth (BHI broth)		48h	35 ± 2°C 5% CO_2	[5]	[5]	
Francisella tularensis		Cysteine Heart Agar + 10% blood [1] (or + 2% haemoglobin [3]) alternatively, Glucose Cysteine Blood Agar	1	Suspend 48h colonies from chocolate agar in broth (BHI broth)	Clindamycin (CM) Tetracycline (TC) Ciprofloxacin (CI) Gentamicin (GM)	48-72h	37 ± 2°C 5% CO_2	[5]	[5]	- Testing should only be performed in appropriate reference laboratories. - Perform all work within BSC Class IIA and minimum BSL 2 environment.
HACEK group		MHA + 1% haemoglobin + 1% IsoVitalex or HTM or BBA	1	Broth (BHI broth)	Levofloxacin (LE) Imipenem (IP) Trimethoprim/sulfamethoxazole (TS) Ceftriaxone (TX)	24-72h	35 ± 2°C 5% CO_2	*H. influenzae* *H. influenzae*	49247™ 49766™ (IP)	

MYCOBACTERIA AND AEROBIC ACTINOMYCETES [1)]

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6)]	INOCULUM		SUGGESTED MIC PANEL [3)]	INCUBATION		RECOMMENED QUALITY CONTROL		COMMENTS [4)]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Mycobacterium tuberculosis		Middlebrook 7H11 + 10% OADC agar	3-4	M7H9 broth + 0.5% tween + 2% glycerol Vortex with sterile glass beads 3-5 mins, settle 20 mins & adjust supernatant to correct turbidity.	Ethambutol (EB) Ethionamide (ET) Isoniazide (IZ) Rifampicin (RI)	5-10 days	37 ± 2°C 5-10% CO_2	*Mycobacterium tuberculosis* *M. tuberculosis*	27294™ AW388 (not ATCC)	- Pre-incubation 24h - 1 strip/90mm plate, seal plates - Perform all work within BSC class IIA
Nontuberculous mycobacteria		MHA + 10% OADC + 5% blood (*M. kansasii* use same agar as for MTB)	1 (3 for *M. kansasii*)	M7H9 broth Vortex with sterile glass beads 3-5 mins, settle 20 mins & adjust supernatant to correct turbidity.	Amikacin (AK) Ciprofloxacin (CI) Clarithromycin (CH) Rifampicin (RI)	5-10 days	35 ± 2°C (*M. marinum* at 30°C) 5% CO_2	*M. avium* *M. avium* spp. *avium* *M. marinum* *M. kansasii*	700898™ 35713™ 927™ 12478™	- 1 strip/90mm plate, seal plates - Antibiogram is species specific
Rapid growing mycobacteria		MHA + 5% blood	1	Saline	Cefoxitin (FX) Imipenem (IP) Ciprofloxacin (CI) Clarithromycin (CH)	48-72h	30-35°C Ambient air, moist	*M. fortuitum* *M. peregrinum*	6841™ 700686™	- Subculture twice before preparing inoculum - 3-4 strips max./150mm plate to facilitate reading
Nocardia spp.		MHA + 5% blood	1	Broth (BHI broth)	Amikacin (AK) Trimethoprim/sulfamethoxazole (TS) Ciprofloxacin (CI) Clarithromycin (CH) Imipenem (IP)	48-72h (dependent on spp.)	35 ± 2°C Ambient air	*S. aureus*	29213™	

FUNGI

ORGANISM	SPECIFIC PHENOTYPE	MEDIA [6]	INOCULUM		SUGGESTED MIC PANEL [3]	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS [4]
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
Yeast		RPMI 1640 + 2% glucose + MOPS + 1.5% Bacto agar	0.5 (1 for *Cryptococcus neoformans*)	Saline	Fluconazole (FL) Itraconazole (IT) Amphotericin B (AP) [1] Flucytosine (FC) Voriconazole (VO) Caspofungin (CS) [1]	24-48h. 48-72h for *C. neoformans*	35 ± 2°C Ambient air in bags, moist	*Candida albicans* *C. krusei* *C. parapsilosis*	90028™ 6258™ 22019™	- Once plate inoculated, re-dip swab and streak again.
Mould [1]		RPMI 1640 + 2% glucose + MOPS + 1.5% Bacto agar	0.5 *Aspergillus* spp. (1 for *Fusarium*, *Rhizopus* spp.)	Saline + Tween 20	Amphotericin B (AP) [1] Itraconazole (IT) Voriconazole (VO) [1] Posaconazole (POS) [1] Caspofungin (CS) [1]	16-72h (Dependent on genus)	35 ± 2°C Ambient air in bags, moist	*C. parapsilosis* *Aspergillus flavus* *A. fumigatus*	22019™ 204304™ 204305™	

1) **In the USA, For Research Use Only (RUO). The contents of this document do not in any way indicate or imply new *in vitro* diagnostic uses of Etest, outside those which are FDA-cleared for certain antibiotics and organism groups.**
2) Worldwide, For Research Use Only (RUO).
3) Example based on CLSI®, Performance Standards for Antimicrobial Susceptibility Testing supplement M100-S. Please use your own selection of MIC panels.
4) Additional Etest information is available at www.biomerieux.com/techlib.
5) For QC of fastidious organisms, please refer to local recommendations and/or CLSI M45-A Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.
6) For media available in the bioMérieux range, see page 10 (consult www.biomerieux.com/techlib for availability of certificates of compatibility).

KEY:

BORSA	Borderline ORSA (non-mec A resistance) due to type A macro-inducible ß-lactamase inhibited by clavulanic acid
BSC	Biological Safety Cabinet
ESBL	Extended Spectrum ß-Lactamase
HLAR	High-Level Aminoglycoside Resistance
MBL	Metallo ß-Lactamase
MRCNS	Methicillin-Resistant Coagulase-Negative Staphylococci (mec A+)
MRSA	Methicillin-Resistant *Staphylococcus aureus* (mec A+)
ND	Non-Determinable
OADC	Oleic acid, Albumin, Dextrose Complex
PAP	Population Analysis Profile
VISA/hVISA	Vancomycin Intermediate/ hetero-Intermediate *Staphylococcus aureus*

MEDIA AVAILABLE IN THE BIOMERIEUX RANGE

Consult the bioMerieux Technical Library (www.biomerieux.com\techlib) for availability of certificates of compatibility

For non-fastidious bacteria: • Mueller Hinton 2 agar bioMérieux Ref. 43301/43309 - 20 / 100 plates 90 mm bioMérieux Ref. 43302 - 20 plates 145 mm bioMérieux Ref. 43511 - 20 plates 120 x 120 mm bioMérieux Ref. 41864 - 6 x 200 ml bioMérieux Ref. 51075 - 500 g	**For fastidious bacteria:** EUCAST recommendation: • Mueller Hinton 2 horse blood NAD agar bioMérieux Ref. 43901/43919 - 20 / 100 plates 90 mm bioMérieux Ref. 43904 - 20 plates 120 x 120 mm CLSI recommendation: • Mueller Hinton 2 sheep blood agar bioMérieux Ref. 43321/43329 - 20 / 100 plates 90 mm bioMérieux Ref. 43324 - 20 plates 120 x 120 mm
For anaerobes: • Brucella blood agar (with Vitamin K1 + Hemin) bioMérieux Ref. 411 968 - 20 plates 90 mm	**For fungi and yeasts:** • RPMI agar bioMérieux Ref. AEB 122 180 - 10 plates 90 mm bioMérieux Ref. AEB 122 182 - 10 plates 140 mm

SUSPENSION MEDIA AVAILABLE IN THE BIOMERIEUX RANGE

• Brain-Heart Infusion broth bioMérieux Ref. 42081 - 20 x 9 ml	• API® NaCl 0.85 % Medium (saline) bioMérieux Ref. 20070 – 100 x 2ml bioMérieux Ref. 20040 – 100 x 3ml bioMérieux Ref. 20230 – 100 x 5ml	• Schaedler Broth + vit. K3 bioMérieux Ref. 42106 - 20 x 13 ml

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