

AEROBES

ORGANISM	SPECIMEN SPECIFIC PHENOTYPE		MEDIA	INOCULUM		SUGGESTED MIC PANEL ²⁾	INCUBATION 35°C		QUALITY CONTROL		COMMENTS ³⁾
				McF equivalent	Suspension medium		Time (h)	Atmosphere	Strain	ATCC®	
Staphylococci	Blood Wounds Sterile sites Indwelling devices	ORSA ORCNS	Mueller Hinton agar (MHA) + 2% NaCl	0.5- 1	Saline	Oxacillin (OX)	24h 48h ORCNS	Ambient	<i>Staphylococcus aureus</i>	43300	- Phenotype interpretation: OX ≥ 4 µg/mL = ORSA OX ≥ 0.5 µg/mL = ORCNS - Heavier inoculum improves detection of low level R
		BORSA	MHA + 2% NaCl	0.5- 1	Saline	Oxacillin (OX) Amoxicillin/ clavulanic acid (XL)	24h	Ambient	<i>S. aureus</i>	29213	- Phenotype interpretation: OX ≥ 4 µg/mL = BORSA - Heavier inoculum improves detection of low level R
		GISA/hGISA ¹⁾	Brain Heart Infusion agar (BHI) ¹⁾	2	Broth	Vancomycin (VA) Teicoplanin (TP)	24h, confirm at 48h	Ambient	<i>S. aureus</i> 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) = GISA/hGISA - Confirm VISA/hVISA by PAP
		GRD	MHA + 5% Blood	0.5	Broth	Vancomycin (VA) Teicoplanin (TP)	18-24h confirm at 48h	Ambient	<i>S. aureus</i> <i>S. aureus</i> <i>S. aureus</i>	29213 700698 700699	- Confirm GISA/hGISA by PAP
		VRSA	MHA	0.5	Saline	Vancomycin (VA)	24h	Ambient	<i>S. aureus</i>	29213	
		ORSA Secondary panel	MHA	0.5	Saline	Clindamycin (CM) Daptomycin (DPC) Linezolid (LZ) Vancomycin (VA) Quinupristin/dalfopristin (QDA) Rifampicin (RI) ¹⁾	16-20h VA full 24h	Ambient	<i>S. aureus</i>	29213	
		Non-ORSA secondary panel	MHA	0.5	Saline	Benzylpenicillin (PG) Erythromycin (EM) Linezolid (LZ) Trimethoprim/sulfamethoxazole (TS) Vancomycin (VA) Ciprofloxacin (CI)	16-20h VA full 24h	Ambient	<i>S. aureus</i>	29213	

ORGANISM	SPECIMEN SPECIFIC PHENOTYPE		MEDIA	INOCULUM		SUGGESTED MIC PANEL ²⁾	INCUBATION 35°C		QUALITY CONTROL		COMMENTS ³⁾
				McF equivalent	Suspension medium		Time (h)	Atmosphere	Strain	ATCC®	
Enterococci	Blood Wounds Sterile sites Urines	VRE ¹⁾	BHI ¹⁾	2	Broth	Vancomycin (VA) Teicoplanin (TP)	24h, confirm at 48h	Ambient	<i>Enterococcus faecalis</i> QC ranges: TP = 0.25-1 µg/mL VA = 2-6 µg/mL	29212	- 0.1 mL inoculum/ 90mm plate - Phenotype interpretation: VA ≥ 32 µg/mL (S) and TP ≥ 6 µg/mL (I, R) = vanA VA ≥ 8-256 µg/mL (I, R) and TP ≤ 4 µg/mL (S) = vanB
			MHA	0.5	Saline	Ampicillin (AM) Daptomycin (DPC) Linezolid (LZ) Quinupristin/dalfopristin (QDA) Vancomycin (VA) Minocycline (MC)	16-20h confirm VA at 24h	Ambient	<i>E. faecalis</i>	29212	
		HLAR	MHA	0.5- 1	Saline	Gentamicin (GM) high range Streptomycin (SM) high range ¹⁾	24h, confirm SM at 48h	Ambient	<i>E. faecalis</i> 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
<i>Enterobacteriaceae</i>	Blood CSF Wounds Respiratory sites Sterile sites Urines (serious infections)		MHA	0.5 (1/mucoid)	Saline	Gentamicin (GM) Piperacillin/ tazobactam (PTc) Cefepime (PM) Ciprofloxacin (CI) Imipenem (IP) Aztreonam (AT)	16-20h	Ambient	<i>Escherichia coli</i> <i>E. coli</i>	25922 35218 (PTc)	
		ESBL	MHA	0.5 (1/mucoid)	Saline	Cefotaxime/cefotaxime + clavulanic acid (CT/CTL) Ceftazidime/ceftazidime + clavulanic acid (TZ/TZL) Cefepime/cefepime + clavulanic acid (PM/PML) ¹⁾	16-20h	Ambient	<i>E. coli</i> (for clav. acid component) <i>Klebsiella pneumoniae</i> (ESBL positive) <i>Pseudomonas aeruginosa</i> (for PM component)	35218 700603 27853	- Investigate ND results by an alternative method

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

FASTIDIOUS ORGANISMS

ORGANISM	SPECIMEN SPECIFIC PHENOTYPE		MEDIA	INOCULUM		SUGGESTED MIC PANEL ²⁾	INCUBATION 35°C		QUALITY CONTROL		COMMENTS ³⁾
				McF equivalent	Suspension medium		Time (h)	Atmosphere	Strain	ATCC®	
Pneumococci	Blood CSF Respiratory sites		MHA + 5% blood	0.5 (1/mucoid)	Broth	Meropenem (MP) Cefotaxime (CT) Benzylpenicillin (PG) Clindamycin (CM) ¹⁾ Vancomycin (VA) Trimethoprim/sulfamethoxazole (TS)	20-24h	5% CO ₂	<i>Streptococcus pneumoniae</i>	49619	
Streptococci	Blood CSF Wounds Sterile sites Respiratory sites		MHA + 5% blood	0.5 (1/mucoid)	Broth	Benzylpenicillin (PG) Cefotaxime (CT) Chloramphenicol (CL) Ofloxacin (OF) Linezolid (LZ) ¹⁾ Vancomycin (VA) Daptomycin (DPC)	20-24h	5% CO ₂	<i>S. pneumoniae</i>	49619	
<i>Abiotrophia</i> & <i>Granulicatella</i> spp. ¹⁾			MH chocolate agar + 0.001% pyridoxal HCl + 0.01% cysteine	1	Broth		20-24h	5% CO ₂	⁴⁾	⁴⁾	
<i>Haemophilus</i> spp.	Blood CSF Sterile sites Respiratory sites		Haemophilus Test Media (HTM) or MHA + 1% haemoglobin + 1% IsoVitaleX™ (MH chocolate agar)	0.5 (1/mucoid)	Broth	Amoxicillin/ clavulanic acid (XL) Cefotaxime (CT) Meropenem (MP) Trimethoprim/sulfamethoxazole (TS) Chloramphenicol (CL)	20- 24h	5% CO ₂	<i>Haemophilus influenzae</i> <i>H. influenzae</i>	49247 49766 (MP, XM)	
<i>Moraxella catarrhalis</i> ¹⁾			MHA + 5% Blood	0.5	Broth		20-24h	5% CO ₂	⁴⁾	⁴⁾	
Anaerobes	Blood CSF Wounds Respiratory sites Sterile sites		Brucella agar + 5% blood (BBA) + vitamin K (1 µg/mL) + hemin (5 µg/mL)	1	Anaerobic broth	Metronidazole (MZ) Clindamycin (CM) Cefoxitin (FX) Imipenem (IP) Piperacillin/tazobactam (PTc) Benzylpenicillin (PG)	24-72h 48h confirmation of CM results	Anaerobic system	<i>Bacteroides fragilis</i> <i>B. thetaiotaomicron</i> <i>Eubacterium lentum</i>	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	SPECIMEN SPECIFIC PHENOTYPE		MEDIA	INOCULUM		SUGGESTED MIC PANEL ²⁾	INCUBATION 35°C		QUALITY CONTROL		COMMENTS ³⁾
				McF equivalent	Suspension medium		Time (h)	Atmosphere	Strain	ATCC®	
<i>Helicobacter pylori</i> ¹⁾	Stomach biopsy		MHA + 5% blood (≥ 2 weeks old)	3	Broth + 5% serum	Amoxicillin (AC) Clarithromycin (CH) Metronidazole (MZ) Tetracycline (TC)	72h or longer	Micro-aerophilic MZ first 24h anaerobic	<i>Helicobacter pylori</i>	43504	- 1 strip/90mm plate - Strip handle to be placed at edge of plate. - <i>H. pylori</i> colonies are pin-point, translucent and difficult to see. Tilt plate and use oblique light to read endpoint.
Gonococci	Uro-genital tract Oropharynx Conjunctiva Blood CSF Sterile sites		Supplemented GC agar or MH chocolate agar	0.5	Broth	Ciprofloxacin (CI) Benzylpenicillin (PG) Tetracycline (TC) Ceftriaxone (TX) Spectinomycin (SC) ¹⁾	20-24h	5% CO ₂	<i>Neisseria gonorrhoeae</i>	49226	- 3-4 strips/150mm plate
Meningococci ¹⁾	Oro-nasopharynx Blood CSF		MHA + 5% blood or MH chocolate agar	0.5	Broth	Ciprofloxacin (CI) Benzylpenicillin (PG) Trimethoprim/sulfamethoxazole (TS) Meropenem (MP) Ceftriaxone (TX)	24h	5% CO ₂	<i>S. pneumoniae</i> <i>E. coli</i>	49619 25922	- Work in BSC
<i>Campylobacter</i> spp. ¹⁾	Stool Blood Tissue		MHA + 5% blood	1	Broth	Ciprofloxacin (CI) Gentamicin (GM) Erythromycin (EM) Doxycycline (DC)	48-72h	Micro-aerophilic	<i>Campylobacter jejuni</i>	33560	- 3-4 strips/150mm plate - Do not invert plate - <i>Campylobacter</i> colonies may be translucent and difficult to see. Tilt plate and use oblique light to read endpoint.

FASTIDIOUS GRAM-POSITIVE ORGANISMS ¹⁾

ORGANISM	SPECIMEN SPECIFIC PHENOTYPE	MEDIA	INOCULUM		SUGGESTED MIC PANEL ²⁾	INCUBATION 35°C		QUALITY CONTROL		COMMENTS ³⁾
			McF equivalent	Suspension medium		Time (h)	Atmosphere	Strain	ATCC®	
<i>Arcanobacterium</i> spp., <i>Listeria monocytogenes</i> , <i>Erysipelothrix</i> , <i>Lactobacillus</i> , <i>Corynebacterium</i> , <i>Bacillus</i> spp., <i>Rothia</i> , <i>Pediococcus</i> , <i>Leuconostoc</i> , and <i>Gemella</i> spp.		MHA + 5% blood	1	Broth		20-24h (48h if required)	5% CO ₂	⁴⁾	⁴⁾	

FASTIDIOUS GRAM-NEGATIVE ORGANISMS ¹⁾

ORGANISM	SPECIMEN SPECIFIC PHENOTYPE	MEDIA	INOCULUM		SUGGESTED MIC PANEL ²⁾	INCUBATION 35°C		QUALITY CONTROL		COMMENTS ³⁾
			McF equivalent	Suspension medium		Time (h)	Atmosphere	Strain	ATCC®	
<i>Bartonella</i> spp.	Periodontal Blood Respiratory Bite wounds	MH chocolate agar	1	Broth		3-5 days	5% CO ₂	⁴⁾	⁴⁾	
<i>Bordetella</i> spp.		Regan-Lowe Bordet–Genjou or MHA + 5% blood	3	Broth		3-5 days	Ambient in bags, moist	⁴⁾	⁴⁾	
<i>Capnocytophaga</i> spp.		BBA	1	Broth		48h	5% CO ₂	⁴⁾	⁴⁾	
<i>Legionella</i> spp.		Buffered Charcoal Yeast Extract (BCYE)	1	Broth		3-5 days	5% CO ₂	⁴⁾	⁴⁾	
<i>Pasteurella</i> spp.		MHA + 5% blood	1	Broth		48h	5% CO ₂	⁴⁾	⁴⁾	
<i>Francisella tularensis</i>		Cysteine Heart Agar + 10% blood ¹⁾ (or + 2% haemoglobin ²⁾) alternatively, Glucose Cysteine Blood Agar	1 McF	Suspend 48h colonies from chocolate agar in broth	Clindamycin (CM) Tetracycline (TC) Ciprofloxacin (CI) Gentamicin (GM)	48-72h	37 °C 5% CO ₂	⁴⁾	⁴⁾	- Testing should only be performed in appropriate reference laboratories. - Perform all work within BSC Class IIA and minimum BSL 2 environment.

MYCOBACTERIA AND AEROBIC ACTINOMYCETES ¹⁾

ORGANISM	SPECIMEN SPECIFIC PHENOTYPE		MEDIA	INOCULUM		SUGGESTED MIC PANEL ²⁾	INCUBATION 35°C		QUALITY CONTROL		COMMENTS ³⁾
				McF equivalent	Suspension medium		Time (h)	Atmosphere	Strain	ATCC®	
<i>Mycobacterium tuberculosis</i>	Respiratory sites Wounds Sterile sites		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 at 37 ± 1 °C	5-10% CO ₂	<i>Mycobacterium tuberculosis</i> <i>M. tuberculosis</i>	27294 AW388 (not ATCC)	- Pre-incubation 24h - 1 strip/90mm plate, seal plates - Perform all work within BSC class IIA
Nontuberculous mycobacteria	Sputum Blood CSF Skin lesions		MHA + 10% OADC + 5% blood (<i>M. kansasii</i> use same agar as for MTB)	1 (3/ <i>M. kansasii</i>)	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 (<i>M. marinum</i> at 30 °C)	5% CO ₂	<i>M. avium</i> <i>M. avium</i> spp. <i>avium</i> <i>M. marinum</i> <i>M. kansasii</i>	700898 35713 927 12478	- 1 strip/90mm plate, seal plates - Antibiogram is species specific
Rapid growing mycobacteria	Wound Respiratory sites Sterile sites Middle ear Eye		MHA + 5% blood	1	Saline	Cefoxitin (FX) Imipenem (IP) Ciprofloxacin (CI) Clarithromycin (CH)	48-72h at 30-35 °C	Ambient, moist	<i>M. fortuitum</i> <i>M. peregrinum</i>	6841 700686	- Subculture twice before preparing inoculum - 3-4 strips/150mm plate
<i>Nocardia</i> spp.	Respiratory sites Sterile sites Blood CSF Pus		MHA + 5% blood	1	Broth	Amikacin (AK) Trimethoprim/sulfamethoxazole (TS) Ciprofloxacin (CI) Clarithromycin (CH) Imipenem (IP)	48-72h (dependent on spp.)	Ambient	<i>S. aureus</i>	29213	

FUNGI

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

1) In the USA, For Research Use Only (RUO).

2) Example based on CLSI®, Performance Standards for Antimicrobial Susceptibility Testing supplement M100-S. Please use your own selection of MIC panels.

3) Additional Etest information is available at www.biomerieux.com/techlib.

4) 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.

KEY:

BORSA: Borderline ORSA (non mec A resistance) due to type A macro-inducible β -lactamase inhibited by clavulanic acid

BSC: Biological Safety Cabinet

ESBL: Extended Spectrum β -Lactamase

GISA/hGISA: Glycopeptide Intermediate/ hetero-Intermediate *Staphylococcus aureus*

HLAR: High Level Aminoglycoside Resistance

MBL: Metallo β -Lactamase

ND: Non Determinable

OADC: Oleic acid, Albumin, Dextrose Complex.

ORCNS: Oxacillin-Resistant Coagulase-Negative Staphylococci (mec A+)

ORSA: Oxacillin-Resistant *Staphylococcus aureus* (mec A+)

PAP: Population Analysis Profile

VRE: Vancomycin-Resistant Enterococci

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