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CRITERIA FOR SUSCEPTIBILITY TESTING

I. Introduction

This section lists the susceptibility testing methods and required antimicrobials for each significant organism appropriate to the site of isolation.

II. Reagents/Materials/Media

Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

III. Method

1. Select significant organisms as per procedure manual of body sites.
2. Identify the selected isolate as per Bacteria and Yeast Workup Manual
3. For identical organisms, as defined in Bacteria and Yeast Workup Manual – Minimal workup, isolated within 1 day from blood and sterile sites OR within 3 days from other sites do not require repeat susceptibility testing EXCEPT oxacillin and vancomycin screens for *Staphylococcus* and *Enterococcus.*
4. Refer susceptibility results back to like sites only and NEVER refer a sterile site to a non-sterile site.
5. Refer the susceptibility result to the previous cultures with the statement "Susceptibility testing not done. Please refer to No. (HIS# if present or LIS#) collected on date ".
6. Follow the table below as a guide for the appropriate method(s)/antimicrobial(s) to be set up.

Organisms	Site	Method	Antimicrobial(s)
Aerobic Gram negative: Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* species	All sites	Vitek ? + KB ?	gns-623 FEP (for all **PMH ONLY**-non-CSF specimens - if CRO is I or R or *P.aeruginosa*)
	If CPD=I or R, *E. coli*, *Klebsiella* species or *Proteus* species	**add** KB ?	AMC, ASM, CRO, TAX, TAZ, CPD, FOX, ROX, TZP, FEP
E. coli O157	Enteric sites	Not tested	
All *Salmonella* species	Blood and Sterile sites	**add** KB ?	NA

Organisms	**Site**	**Method**	**Antimicrobial(s)**
Salmonella species other than *S. typhi*	Enterics from TG and TW Enterics from other hospitals	Vitek ? Not tested	gns-623
Stenotrophomonas maltophilia	All sites	KB ?	SXT, LVX
Burkholderia cepacia	All sites	KB ?	SXT, TAZ, MEM
Pseudomonas aeruginosa, Acinetobacter species *Stenotrophomonas maltophilia* or *Burkholderia cepacia* that is resistant to all routinely tested antimicrobials	All sites	e-test ?	PO
Pseudomonas sp., *Aeromonas* sp., *Plesiomonas* sp. and other afermenters	All sites	Not tested	
Mucoid *P. aeruginosa, Enterobacteriaceae* not growing on Vitek	All sites	KB ?	AMP, KZ, CRO, CIP, SXT, GM, TZP, TOB, CAZ, IMI, CPD
Haemophilus species	All sites Blood and Sterile sites	beta-lactamase beta-lactamase + KB ?	 CRO, CIP, AMP
Moraxella catarrhalis	All sites	Not tested	
Neisseria gonorrhoeae	All sites	Not tested	
Neisseria meningitides	All sites	Not tested	
Other fastidious Gram negatives (e.g. *Pasteurella* species)	All sites	Not tested	
Campylobacter species	All sites	Not tested	
Staphylococcus aureus MRSA – add e-test MU until further notice. (KB-MUP pending validation)	All sites MRSA [redacted] If MRSA from MRSA Screen Test **and** TE **and** SXT=R	Vitek ? + Screen plate ? **a**[redacted] ? **add** e-test ? **add** e-test ?	gps-105 OX, VA [redacted] MU FA

Organisms	Site	Method	Antimicrobial(s)
Coagulase-negative *Staphylococcus*	Urine	Not tested	
	All other sites (test on pooled isolates if more than one morphotype of CNST)	Vitek ? + Screen plate ?	gps-105 VA
Micrococcus species	All sites	Not tested	
Enterococcus species	Urines	Vitek ? + Screen plate ?	gps-105 VA
	Blood & Sterile sites	KB ? + Screen plate ? + beta-lactamase	AMP High level GM and STREP , VA
	All other sites	KB ? + Screen plate ?	AMP VA
	If VA=R, *E. faecalis* or *E. faecium*	**add** VRE MIC panel	
Streptococcus pneumoniae	Blood & Sterile sites	e-test ? + Double disc KB ? + KB ?	P, CRO CC, E LVX, VA
	All other sites	Double disc KB ? + KB ? + If OX=R, then e-test ?	CC, E OX, LVX, VA P, CRO
Group A, B, C, G *Streptococcus*	? Blood and Sterile sites	Double disc KB ? + KB ?	CC, E P, VA
	? Urine, on request ONLY	KB ?	LVX
	? Vaginal GBS screens, on request ONLY	Double disc KB ? + KB ?	CC, E VA
	? Other sites, on request ONLY	Double disc KB ? + KB ?	CC, E LVX, VA

Organisms	Site	Method	Antimicrobial(s)
Streptococcus bovis	Blood & Sterile sites	e-test ?	CRO, P, VA
	Mixed cultures	Not tested	
	Other sites	Not tested	
viridans *Streptococci*	Blood & Sterile sites	e-test for pure cultures?	CRO, P, VA
	Mixed cultures	Not tested	
	Other sites	Not tested	
Streptococcus milleri group	Blood & Sterile sites	e-test ?	CRO, P, VA
	Urine	KB ?	P, LVX
	Other sites	KB ?	CC, E, LVX, P, VA
Listeria species	All sites	Not tested	
Corynebacterium species	All sites	Not tested	
Bacillus species	All sites	Not tested	
Nocardia species	All sites	Not tested	(Sent to PHL on special request)
Anaerobes	All sites	Not tested	(Sent to PHL on special request)

I. Introduction

This section describes the occasions where the drugs tested against isolates showed phenotype that:

1. have never been documented
2. are uncommon, and/or
3. represent results that could easily occur from technical errors and which may have significant clinical consequences.

II. Reagents/Materials/Media

Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

III. Procedure

When any of the listed results in the TABLE 1 below occurs, verify the result as follows:

1. Check purity plate.
2. Check previous reports on the patient.
3. Confirm the identification of the isolate from the original isolation medium.
4. Repeat susceptibility test to confirm result. Use an alternative method if applicable.
5. For isolates that show results other than susceptible for those antimicrobial agents for which only susceptible interpretive criteria are provided by NCCLS guidelines M100-S15 (listed as "not S" above) an for staphylococci with vancomycin – I or R results:
   i. Confirm the organism identification
   ii. Confirm the antimicrobial susceptibility test results
   iii. Freeze the isolate
   iv. Send the isolate to PHL for confirmation.
6. If the result is confirmed, notify the Charge Technologist.
7. The Charge Technologist confirms the result and notifies the Microbiologist.
8. The Microbiologist further confirms the result and notifies the Infection Control Practitioner.

For results marked with *****, LIS reflex rules will automatically report these as R; repeat susceptibility testing is not required if the purity and organism identification is confirmed.

Organism or Group	**Uncommon results**
Any organism	Resistant to all agents routinely tested
Gram-negative organisms	
Enterobacteriaceae	Imipenem, Meropenem – I or R
Citrobacter freundii *Enterobacter* species *Serratia marcescens*	Ampicillin, Cefazolin – S*****
Klebsiella species *Proteous vulgaris* *Providencia* species	Ampicillin – S*****
Escherichia coli, Klebsiella species, *Proteus* species	Cefpodoxime – Vitek=I or R; KB=S
Escherichia coli, Klebsiella species, *Proteus* species	KB-ESBL panel with reduction in zone of inhibition instead of potentiation or no change.
Stenotrophomonas maltophilia	Imipenem, Meropenem – S
Haemophilus influenzae	Aztreonam – not S Imipenem, Meropenem – not S 3rd generation cephalosporin – not S Fluoroqinolone – not S
Neisseria gonorrhoeae	3rd generation cephalosporin – R
Gram-positive organisms	
Enterococcus faecalis *Enterococcus faecium*	Ampicillin or Penicillin – R Daptomycin – not S Quinupristin-Dalfopristin – S Linezolid – I or R Daptomycin – not S Linezolid – I or R
Staphylococcus aureus	Daptomycin – not S Linezolid – not S Quinupristin-Dalfopristin – I or R Vancomycin – I or R
Coagulase-negative *Staphylococcus*	Daptomycin – not S Linezolid – not S Vancomycin – I or R
Streptococcus pneumoniae	3rd generation cephalosporin – R Fluoroquinolone - R Linezolid – not S Vancomycin – not S

Organism or Group	**Uncommon results**
Gram-positive organisms (cont'd)	
beta-haemolytic *Streptococcus*	Ampicillin or Penicillin – not S 3rd generation cephalosporin – not S Daptomycin – not S Linezolid – not S Vancomycin – not S
viridans *Streptococcus*	Daptomycin – not S Linezolid – not S Vancomycin – not S

IV. Reference

Suggestions for Verification of Antimicrobial susceptibility Test Results and Confirmation of Organism identification in Table 8 of Clinical and Laboratory Standards Institute (CLSI) Document - Performance Standards for Antimicrobial Susceptibility Testing M100-S15, Vol. 25 No.1, 2005.

Site: **Urine – Gram Positive Susceptibility Reporting – 1 – *Staphylococcus* species, MRSA**

Antimicrobial Agent	***Staphylococcus aureus***			**MRSA**		
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]
Ampicillin	X[1]	X[1]	X[1]	X[1,5]	X[1, 5]	X[1, 5]
Cefazolin	X[2]	X[2]	X[2]	X[2, 5]	X[2, 5]	X[2, 5]
Cloxacillin	X[2]	X[2]	X[2]	X[2, 5]	X[2, 5]	X[2, 5]
Doxycycline				X[3, 6]	X[3, 6]	X[3, 6]
Fusidic Acid				X[6, 7]	X[6, 7]	X[6, 7]
Mupirocin				X[6]	X[6]	X[6]
Nitrofurantoin	X	X	X	X[5]	X[5]	X[5]
Rifampin				X[6]	X[6]	X[6]
Trimethoprim/Sulfa	X	X	X	X	X	X
Vancomycin	X[4]	X[4]	X[4]	X[5]	X[5]	X[5]

[1] Base on Penicillin or beta-lactamase result
[2] Base on Oxacillin result
[3] Adults only (>13 y); base on Tetracycline result
[4] Report if patient is allergic to Penicillin OR if *Staphylococcus* species is resistant to **All** other antimicrobial agents.
[5] DO NOT report if isolated from Infection Control Screening test
[6] Report ONLY if isolated from Infection Control Screening test. Include Isolate Comment "Susceptibility results are provided to guide decolonization therapy; if decolonization therapy is being considered, please consult your infection control team."
[7] Report only if resistant to Mupirocin.
[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: ***S. saprophyticus*** and **coagulase-negative-*Staphylococcus*** - **DO NOT** report susceptibilities. Report with Isolate Comment - "Susceptibility testing of this organism is not warranted because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated urinary tract infections e.g. nitrofurantoin, trimethoprim/sulfa or fluoroquinolones.

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Urine – Gram Positive Susceptibility Reporting – 2 – *Enterococcus*, *Streptococcus*

Antimicrobial Agent	***Enterococcus* species**[11]			**Group A, B, C, G *Streptococcus***	***Streptococcus milleri***
	MSH[a]	UHN[b]	CHC[c]	All sites Routinely not tested. **See below**[8]**.** For special request:	All sites
Ampicillin	X	X	X		
Ciprofloxacin	X[4,6]	X[5,6]	X[6]		
Clindamycin					
Erythromycin					
Levofloxacin				X[10]	X[6]
Linezolid	X[2]	X[2]	X[2]		
Nitrofurantoin	X	X	X		
Penicillin G					X
Synercid	X[2]	X[2]	X[2]		
Tetracycline	X[1]	X[1]	X[1]		
Vancomycin	X[3, 9]	X[3, 9]	X[3, 9]		

[1] Adults only (>13 y)
[2] If Vancomycin, Ciprofloxacin, Tetracycline, Nitrofurantoin and Ampicillin are R except for *E. gallinarum* and *E. casseliflavus*.
[3] Test but **DO NOT** report unless Vancomycin R or Enterococcus resistant to **All** other antimicrobial agents
[4] TRI only
[5] Test and report on request only
[6] Adults only (>18y)
[7] Always report as sensitive
[8] Report "This organism is intrinsically susceptible to penicillin. If treatment is required and this patient cannot be treated with penicillin, please contact the Microbiology Department within 48 hours to request sensitivity testing."
[9] *E. gallinarum and E. casseliflavus*, report as **R** with the statement "This organism always has intrinsic non-transmissible resistance to vancomycin. The patient does not require isolation."
[10] Report with additional isolate comment "Susceptibility completed as requested" (do not remove original comments). If Levofloxacin is R or patient is <18y, consult the Microbiologist.
[11] DO NOT report susceptibilities if isolated from Infection Control Screening test.

[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Urine - Gram Negative Susceptibility Reporting

Antimicrobial Agent	**Enterobacteriaceae and *Acinetobacter* spp.**			***Pseudomonas aeruginosa***			***S. maltophilia***	***Burkholderia cepacia***
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]	All Sites	All Sites
Amikacin	X[14]	X[14]	X[14]	X[14]	X[14]	X[14]		
Ampicillin	X[7, 13]	X[7, 13]	X[7, 13]	R[2]				
Cefazolin	X[8, 10, 13]	X[8, 10, 13]	X[8, 10, 13]	R[2]				
Cefepime	X[11, 13]			X[12]				
Ceftazidime				X	X	X		X
Ceftriaxone	X[6, 9, 13]	X[6, 9, 13]	X[6, 9, 13]					
Ciprofloxacin	X[1]	X[1,5]		X[1]	X[1,5]			
Colistin	X[15]	X[15]	X[15]	X[15]	X[15]	X[15]	X[15]	X[15]
Gentamicin	X	X	X	X	X	X		
Imipenem	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]		
Levofloxacin							X[1]	
Meropenem								X
Nitrofurantoin	X[10]	X[10]	X[10]	R[2]				
Ofloxacin		X[1, 5]	X[1, 5]		X[1, 5]	X[1, 5]		
Piperacillin/Tazobactam	X[9, 10]	X[9]		X	X	X		
Trimethoprim/Sulfa	X	X	X	R[2]			X	X
Tobramycin	X	X[3]	X[3]	X	X[3]	X[3]		

[1] Adults only (>18 y) [2] R = Always report as resistant [3] Report if Genta R

[4] Report if R **OR** if R to **All** other Antimicrobial Agents **OR** if only aminoglycoside is S

[5] Report Ofloxacin (base on Ciprofloxacin result) for Enterobacteriaceae on all Bridgepoint, CHC and Ajax/Pickering patients. **DO NOT** report Ciprofloxacin.

[6] Report ceftriaxone only for *Acinetobacter* spp. or if *Enterobacteriaceae* is intermediate or resistant to ceftriaxone

[7] *Klebsiella* spp., *Enterobacter* spp., *Acinetobacter* spp., *H. alvei, Citrobacter* spp., *Pantoea agglonerans, Proteus vulgaris, Proteus penneri,* & *Serratia* species always report Amp as R.

[8] *Enterobacter* spp., *Citrobacter* spp., *Pantoea agglonerans, Proteus vulgaris, Proteus penneri, Acinetobacter* spp. & *Serratia* species always report Cefazolin and Cephalothin as R.

[9] *Cedecea* spp., *Citrobacter* spp., *Enterobacter* spp., *Hafnia* spp., *Morganella morganii, Pantoea agglonerans, Proteus penneri, Proteus vulgaris, Providencia* species, *Seratia* species, if **S**, report with comment "Resistance to extended-spectrum penicillins, beta-lactam/beta-lactamase inhibitor combinations, and cephalosporins may develop during therapy with these agents. For serious infections, these agents should be avoided and consultation with a medical microbiologist or infectious disease physician is strongly recommended."

[10] Do not report for Salmonella species.

[11] Report on PMH patients only if Ceftriaxone is I or R.

[12] PMH only

[13] For *Enterobacteriaceae* if any one of cefotaxime/ceftriaxone or ceftazidime=R, report all as R

[14] Report if both Gentamicin and Tobramycin are R.

[15] For isolates other than Enterobacteriaceae, report if R to all other drugs; base on Polymyxin B.

[a] includes MSH, PMH, Baycrest, TRI, CAMH [b] includes TG, TW, Bridgepoint, Grace [c] includes CHC, Ajax/Pickering

Note: ***Pseudomonas* species (other than *P. aeruginosa*), fastidious gram-negative bacteria** & **non–fermenters** - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Enterics

Antimicrobial Agent	***Shigella* species**			***Salmonella* species**			***Salmonella typhi***			***Vibrio cholerae*[3]**		
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]
Ampicillin	X	X	X		X[2]		X	X	X	X	X	X
Cefepime							X[5]					
Ceftriaxone							X	X	X			
Ciprofloxacin	X[1]	X[1]	X[1]		X[1, 2]		X[1]	X[1]	X[1]	X[1]	X[1]	X[1]
Trimethoprim/Sulfa	X	X	X		X[2]		X	X	X	X	X	X
Tetracycline										X[4]	X[4]	X[4]

[1] Adults only (>18 y)
[2] For TG and TW patients, test but **DO NOT** report.
[3] For all Vibrio species other than *V. cholerae*, **DO NOT** test or report.
[4] Adults only (>13 y)
[5] Report on PMH patients only if Ceftriaxone is I or R, base on Ceftriaxone result.

[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: ***E. coli* O157, *Campylobacter* spp., and *Yersinia* spp.** - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Respiratory and Miscellaneous Non-Sterile Sites - Gram Positive Susceptibility Reporting – 1 – *Staphylococcus*

Antimicrobial Agent	***Staphylococcus* species**			**MRSA**		
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]
Cefazolin	X[2]	X[2]	X[2]	X[2, 6]	X[2, 6]	X[2, 6]
Clindamycin	X[4]	X[4]	X[4]	X[4, 6]	X[4, 6]	X[4, 6]
Cloxacillin	X[2]	X[2]	X[2]	X[2, 6]	X[2, 6]	X[2, 6]
Doxycycline				X[3, 5]	X[3, 5]	X[3, 5]
Erythromycin	X[4]	X[4]	X[4]	X[4, 6]	X[4, 6]	X[4, 6]
Fusidic Acid				X[3, 7]	X[3, 7]	X[3, 7]
Mupirocin				X[3]	X[3]	X[3]
Rifampin				X[3]	X[3]	X[3]
TMP/SMX	X	X	X	X	X	X
Vancomycin	X[1]	X[1]	X[1]	X[6]	X[6]	X[6]

[1] Report if Oxacillin R
[2] Base on Oxacillin result
[3] Report ONLY if isolated from Infection Control Screen. Include Isolate Comment "Susceptibility results are provided to guide decolonization therapy; if decolonization therapy is being considered, please consult your infection control team."
[4] Clinda R = Erythro R
[5] Adults only (>13yrs); base on Tetracycline result
[6] DO NOT report if isolated from Infection Control Screen.
[7] Report only if resistant to Mupirocin.

[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: For organisms isolated from ears and eyes sources and susceptibility result is reported, add comment "These susceptibility testing results are based on guidelines for systemic antimicrobial agents and may not accurately represent activity of topical agents."

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Respiratory and Miscellaneous Non-Sterile Sites - Gram Positive Susceptibility Reporting–2– *Enterococcus, Streptococcus, Corynebacterim* spp., *Bacillus* spp., viridans *Streptococcus, Listeria* spp.

Antimicrobial Agent	***Enterococcus*[1]**			***S. pneumoniae***			**Group A, B, C, G *Streptococcus***	***S. milleri***
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]	All Sites	All Sites
Ampicillin	X	X	X				Routinely not tested. **See below[7].** For special request:	
Cefepime				X[14]				
Ceftriaxone				X[6]	X[6]	X[6]		
Clindamycin				X[4]	X[4]	X[4]	X[4, 12]	X
Erythromycin				X[4, 5]	X[4, 5]	X[4, 5]	X[4, 12]	X
Levofloxacin				X[10, 11]		X[11]	X[2, 11, 12, 15]	X
Linezolid	X[13]	X[13]	X[13]					
Moxifloxacin				X[9, 11]	X[9, 11]			
Penicillin G				X[8]	X[8]	X[8]		X
Synercid	X[13]	X[13]	X[13]					
Vancomycin	X[3]	X[3]	X[3]	X[6]	X[6]	X[6]	X[12,16]	

[1] DO NOT report if isolated from Infection Control Screen.
[2] DO NOT report on GBS Screen or vaginal swab
[3] *E. gallinarum, and E. casseliflavus,* report as **R** with the statement "This organism always has intrinsic non-transmissible resistance to vancomycin. The patient does not require isolation."
[4] Report as R if D-zone is present. Report clindamycin as R if erythromycin is R
[5] Report Erythromycin for respiratory specimens only
[6] Report only if Pen I or R
[7] Report "This organism is intrinsically susceptible to penicillin. If treatment is required and this patient cannot be treated with penicillin, please contact the Microbiology Department within 48 hours to request sensitivity testing."
[8] Base on Oxacillin result if S; base on Penicillin E-test if Oxacillin is R
[9] Base on Levofloxacin result. Report on MSH, PMH, TG and TW patients only.
[10] DO NOT report on MSH and PMH patients.
[11] Adults only (>18 yrs)
[12] Report with additional isolate comment "Susceptibility completed as requested" (do not remove original comments).
[13] If Vancomycin and Ampicillin are R except for *E. gallinarum* and *E. casseliflavus.*
[14] Report on PMH patients only if Cetriaxone is I or R, based on Ceftriaxone result.
[15] If Levofloxacin is R or patient is <18y, consult the Microbiologist.
[16] Report only if either Clindamycin or Erythromycin are I or R.
[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: ***Listeria* species** – DO NOT report susceptibility result. Report with ISOLATE comment –"Routine in vitro susceptibility testing of this organism is unreliable. *Listeria* spp. should be considered resistant to all cephalosporins. The recommended regimen for therapy is ampicillin. If additional advice on antimicrobial therapy is required, please contact the Medical Microbiologist."

Corynebacterim* species, *Bacillus* species, viridans *Streptococcus - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

For organisms isolated from ears and eyes sources and susceptibility result is reported, add comment "These susceptibility testing results are based on guidelines for systemic antimicrobial agents and may not accurately represent activity of topical agents."

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Respiratory and Miscellaneous Non-Sterile Sites - Gram Negative Susceptibility Reporting – 1 – Enterobacteriaceae, *P. aeruginosa*

Antimicrobial Agent	***Enterobacteriaceae*** and ***Acinetobacter* spp.**			***Pseudomonas aeruginosa***		
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]
Amikacin	X[14]	X[14]	X[14]	X[14]	X[14]	X[14]
Ampicillin	X[2, 12, 13]	X[2, 12, 13]	X[2, 12, 13]	R		
Cefazolin	X[3,12,13]	X[3,12,13]	X[3,12,13]	R		
Cefepime	X[9;12]			X[10]		
Ceftazidime	X[4;12]	X[4, 12]	X[4, 12]	X	X	X
Ceftriaxone	X[7, 12]	X[7, 12]	X[7, 12]			
Cefuroxime		X[8, 12]	X[8, 12]			
Ciprofloxacin	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]
Colistin	X[15]	X[15]	X[15]	X[15]	X[15]	X[15]
Gentamicin	X	X	X	X	X	X
Imipenem	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
Piperacillin/Tazobactam	X[7, 11]	X[7]		X	X	X
Trimethoprim/Sulfa	X	X	X	R		
Tobramycin	X	X[6]	X[6]	X	X	X

[1] Adults only (>18 y)

[2] Always report *Klebsiella* spp., *Enterobacter* spp. *Acinetobacter* spp., *H. alvei, Proteus vulgaris, Proteus penneri, Citrobacter* spp. and *Serratia* species as R

[3] Always report *Enterobacter* spp., *Citrobacter* spp., *Proteus vulgaris, Proteus penneri, Acinetobacter* spp. and *Serratia* species as R.

[4] Report only if R. For *Enterobacteriaceae* if cefotaxime/ceftriaxone or ceftazidime R, report both as R

[5] Report if R **OR** if R to **All** other Antimicrobial Agents **OR** if only aminoglycoside is S

[6] Report if Genta is R

[7] *Cedecea* spp., *Citrobacter* spp., *Enterobacter* spp., *Hafnia* spp., *Morganella morganii, Pantoea agglonerans, Proteus penneri, Proteus vulgaris, Providencia* species, *Seratia* species, if S, report with comment "Resistance to extended-spectrum penicillins, beta-lactam/beta-lactamase inhibitor combinations, and cephalosporins may develop during therapy with these agents. For serious infections, these agents should be avoided and consultation with a medical microbiologist or infectious disease physician is strongly recommended."

[8] Report on *Enterobacteriaceae*; Do not report for *Acinetobacter* spp. (Base on KB result) on respiratory specimens only.

[9] Report on PMH patients only if Ceftriaxone is I or R.

[10] PMH only

[11] Do not report for *Salmonella* species.

[12] For *Enterobacteriaceae* if any one of cefotaxime/ceftriaxone or ceftazidime=R, report all as R

[13] Report as I or R if Cefuroxime is I or R.

[14] Report if both Gentamicin and Tobramycin are R.

[15] For Acinetobacter species and *Pseudomonas aeruginosa*, report if R to all other drugs; base on Polymyxin B.

[a] includes MSH, PMH, Baycrest, TRI, CAMH [b] includes TG, TW, Bridgepoint, Grace [c] includes CHC, Ajax/Pickering

Note: ***Pseudomonas* species (other than *P. aeruginosa*), fastidious gram-negative bacteria & non –fermenters** - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

For organisms isolated from **ears and eyes sources** and susceptibility result is reported, add comment "These susceptibility testing results are based on guidelines for systemic antimicrobial agents and may not accurately represent activity of topical agents."

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Respiratory and Miscellaneous Non-Sterile Sites - Gram Negative Susceptibility Reporting – 2 – *Haemophilus* species, *M. catarrhalis, Neisseria gonorrhoeae, S. maltophilia, B. cepacia, Pseudomonas* species (other than *P. aeruginosa*), fastidious gram-negative bacteria, non-fermenters, *Neisseria meningitidis*

Antimicrobial Agent	***Haemophilus* species**	***S. maltophilia***	***Burkholderia cepacia***
	All sites	All sites	All sites
Beta-lactamase	X[2]		
Ceftazidime			X
Levofloxacin		X[1]	
Meropenem			X
Trimethoprim/Sulfa		X	X

[1] Adults only (>18 y)

[2] If beta-lactamase is negative, add comment "beta-lactamase negative result suggests susceptible to ampicillin."
If beta-lactamase is positive, add comment "beta-lactamase positive result suggests resistance to ampicillin but generally susceptible to amoxicillin-clavulanic acid and cefuroxime."

[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: ***Pseudomonas* species (other than *P. aeruginosa*), fastidious gram-negative bacteria, non-fermenters, *N. gonorrohoeae, N. meningitidis*** - DO NOT report susceptibility result. Report with ISOLATE comment: "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

For ***M. catarrhalis*** - DO NOT report susceptibility result. Report with ISOLATE comment: "The majority of *Moraxella catarrhalis* are resistant to ampicillin. In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

For organisms isolated from **ears and eyes sources** and susceptibility result is reported, add comment "These susceptibility testing results are based on guidelines for systemic antimicrobial agents and may not accurately represent activity of topical agents."

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Spinal Fluids – Gram Positive Susceptibility Reporting

Antimicrobial Agent	*Staphylococcus* species			*Enterococcus* species			*Strep. pneumoniae*	viridans *Strep.* *S. bovis*	*S. milleri* group	Group A,B,C,G *Streptococcus*
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]	All Sites	All Sites	All Sites	All Sites
Ampicillin				X	X	X				
Ceftriaxone							X[8, 4]	X[8, 4]	X[8, 4]	
Cloxacillin	X	X	X							
HLGR[3]				X	X	X				
HLSR[3]				X[2]	X[2]	X[2]				
Linezolid				X[7]	X[7]	X[7]				
Penicillin							X[8]	X[8]	X[8]	X
Piperacillin/Tazo				X[5]						
Synercid				X[7]	X[7]	X[7]				
Trimethoprim/Sulfa	X	X	X							
Tobramycin										
Vancomycin	X[1]	X	X	X[6]	X[6]	X[6]	X[4]	X[4]	X[8, 4]	X[4]

[1] Report if Oxacillin R
[2] Report only if requested.
[3] HLGR = High Level Gentamicin Resistant; HLSR = High Level Streptomycin Resistant. Report base on HLGR using canned message (See Blood and Sterile Fluids HLGR Results Reporting).
[4] Report only if Pen is I or R
[5] Report on PMH patients; base on Ampicillin result
[6] *E. gallinarum and E. casseliflavus* report as **R** with the statement: "This organism always has intrinsic non-transmissible resistance to vancomycin. The patient does not require isolation.
[7] If Vancomycin and Ampicillin are R except *E. gallinarum* and *E. casseliflavus*.
[8] Base on E-test

[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: ***Listeria* species** – DO NOT report susceptibility result. Report with ISOLATE comment –"Routine in vitro susceptibility testing of this organism is unreliable. *Listeria* spp. should be considered resistant to all cephalosporins. The recommended regimen for therapy is ampicillin. If additional advice on antimicrobial therapy is required, please contact the Medical Microbiologist."

***Corynebacterim* species, *Bacillus* species** - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

If all antimicrobial agents are resistant, inform the Microbiologist on-call

Spinal Fluids – Gram Negative Susceptibility Reporting – 1 – Enterobacteriaceae and *Acinetobacter* spp., *Salmonella* species including *S. typhi*

Antimicrobial Agent	**Enterobacteriaceae** and ***Acinetobacter* spp.**			***Salmonella* species** including ***S. typhi***		
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]
Amikacin	X[7]	X[7]	X[7]			
Ampicillin	X[4, 6]	X[4, 6]	X[4, 6]	X	X	X
Ceftazidime	X[2, 6]	X[2, 6]	X[2, 6]			
Ceftriaxone	X[5, 6]	X[5, 6]	X[5, 6]	X	X	X
Colistin	X[8]	X[8]	X[8]			
Gentamicin	X	X	X			
Imipenem	X[3]	X[3]	X[3]			
Piperacillin/Tazobactam	X[5]	X[5]				
Trimethoprim/Sulfa	X	X	X	X	X	X
Tobramycin	X	X[1]	X[1]			

[1] Report if Genta R
[2] Report only if R.
[3] Report if R **OR** if R to **All** other Antimicrobial Agents **OR** if only aminoglycoside is S
[4] Always report *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Proteus vulgaris, Proteus penneri, Acinetobacter* spp., *H. alvei* and *Serratia* species as Ampicillin R
[5] *Cedecea* spp., *Citrobacter* spp., *Enterobacter* spp., *Hafnia* spp., *Morganella morganii, Pantoea agglonerans, Proteus penneri, Proteus vulgaris, Providencia* species, *Seratia* species, if **S**, report with comment "Resistance to extended-spectrum penicillins, beta-lactam/beta-lactamase inhibitor combinations, and cephalosporins may develop during therapy with these agents. For serious infections, these agents should be avoided and consultation with a medical microbiologist or infectious disease physician is strongly recommended."
[6] For *Enterobacteriaceae* if any one of cefotaxime/ceftriaxone or ceftazidime=R, report all as R
[7] Report if both Gentamicin and Tobramycin are R.
[8] For Acinetobacter species, report if R to all other drugs; base on Polymyxin B.

[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: If all antimicrobial agents are resistant, inform the Microbiologist on-call

Spinal Fluids – Gram Negative Susceptibility Reporting – 2 – *Pseudomonas aeruginosa, Pseudomonas* spp. (other than P. aeruginosa), fastidious gram-negative bacteria, non-fermenters, *M. catarrhalis, N. gonorrhoeae , N. meningitidis, Stenotrophomonas maltophilia, Burkholderia cepacia, Haemophilus* species.

Antimicrobial Agent	***Ps. aeruginosa***			***S. maltophilia***	***B. cepacia***	***Haemophilus species.***
	MSH[a]	UHN[b]	CHC[c]	All sites	All sites	All sites
Amikacin	X[3]	X[3]	X[3]			
Ampicillin	R					X[1]
Ceftazidime	X	X	X		X	
Ceftriaxone						X
Colistin	X[4]	X[4]	X[4]	X[4]	X[4]	
Gentamicin	X	X	X			
Imipenem	X[2]	X[2]	X[2]			
Meropenem					X	
Piperacillin/Tazobactam	X	X	X			
TMP/SMX	R			X	X	
Tobramycin	X	X	X			

[1] Base on beta-Lactamase result and KB Ampicillin
[2] Report if R **OR** if R to **All** other Antimicrobial Agents **OR** if only aminoglycoside is S
[3]Report if both Gentamicin and Tobramycin are R.
[4]Report if R to all other drugs; base on Polymyxin B.
[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: ***Pseudomonas* species (other than *P. aeruginosa*), fastidious gram-negative bacteria, non-fermenters, *N. gonorrhoeae*** and ***N. meningitidis*** - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

For ***M. catarrhalis*** - DO NOT report susceptibility result. Report with ISOLATE comment: "The majority of *Moraxella catarrhalis* are resistant to ampicillin. In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Blood and other Sterile Sites - Gram Positive Susceptibility Reporting – 1 – *Staphylococcus, Enterococcus*

Antimicrobial Agent	*Staphylococcus* species			*Enterococcus* species		
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]
Ampicillin				X	X	X
Cefazolin	X[2]	X[2]	X[2]			
Clindamycin	X[5]	X[5]	X[5]			
Cloxacillin	X[2]	X[2]	X[2]			
Erythromycin	X[5]	X[5]	X[5]			
HLGR[3]				X	X	X
HLSR[3]				X[4]	X[4]	X[4]
Linezolid				X[7]	X[7]	X[7]
Pipercillin/Tazobactam				X[1]	X[1]	
Synercid				X[7]	X[7]	X[7]
TMP/SMX	X	X	X			
Vancomycin	X[8]	X	X	X[6]	X[6]	X[6]

[1] Base on ampicillin result (PMH, TGH, TWH)
[2] Base on Oxacillin result
[3] HLGR = High Level Gentamicin Resistant; HLSR = High Level Streptomycin Resistant
Report base on HLGR using canned message (See Blood and Sterile Fluids HLGR Results Reporting).
[4] Report only if requested.
[5] Clinda R = Erythro R
[6] *E. gallinarum and E. casseliflavus* report as **R** with the statement "This organism always has intrinsic non-transmissible resistance to vancomycin. The patient does not require isolation."
[7] If Vancomycin and Ampicillin are R except *E. gallinarum* and *E. casseliflavus*.
[8] Only if Oxacillin=R.

[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Blood and other Sterile Sites - Gram Positive Susceptibility Reporting – 2 - *S. pneumoniae*, viridans *Streptococcus, Streptococcus bovis,*, *S. milleri* group, Group A, B, C, G *Streptococcus, Listeria* species, *Corynebacterim* species, *Bacillus* species

Antimicrobial Agent	*S. pneumoniae*			viridans *Strep. Streptococcus bovis*			*S. milleri* group	Group A, B, C,G *Strep.*
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]	All Sites	All Sites
Ceftriaxone	X[2, 5]	X[2, 5]	X[2, 5]	X[2, 5]	X[2, 5]	X[2, 5]	X[2, 5]	
Cefepime	X[6]			X[6]				
Clindamycin	X	X	X					X[3]
Erythromycin	X	X	X					X[3]
Levofloxacin	X[1, 8]		X[1]					
Moxifloxacin	X[1,7]	X[1,7]						
Penicillin	X[2]	X[2]	X[2]	X[2, 4]	X[2, 4]	X[2, 4]	X[2, 4]	X
Vancomycin	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[2, 5]	X[5]

[1] Adults only (>18 y)
[2] Base on E-test
[3] Report as R if D-zone is present. Report clindamycin as R if erythromycin is R
[5] Report only if Pen I or R
[4] For viridans S*treptococcus* and *S. milleri*, also report MIC value as Isolate Comment
[6] Report on PMH patients only if Ceftriaxone is I or R, base on Ceftriaxone result.
[7] Base on Levofloxacin result; Report on MSH, PMH, TG and TW patients only.
[8] DO NOT report on MSH and PMH patients.

[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: ***Listeria* species** – DO NOT report susceptibility result. Report with ISOLATE comment –"Routine in vitro susceptibility testing of this organism is unreliable. *Listeria* spp. should be considered resistant to all cephalosporins. The recommended regimen for therapy is ampicillin. If additional advice on antimicrobial therapy is required, please contact the Medical Microbiologist."

***Corynebacterim* species, *Bacillus* species**. - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

If all antimicrobial agents are resistant, inform the Microbiologist on-call.

Blood and other Sterile Sites - Gram Negative Susceptibility Reporting -1 - Enterobacteriaceae and *Acinetobacter* spp., *Salmonella* species including *S. typhi*

Antimicrobial Agent	Enterobacteriaceae and *Acinetobacter* spp.			*Salmonella* spp. including *S. typhi*		
	MSH[a]	UHN[b]	CHC[c]	MSH[a]	UHN[b]	CHC[c]
Amikacin	X[13]	X[13]	X[13]			
Ampicillin	X[1]	X[1]	X[1]	X	X	X
Cefazolin	X[2, 12]	X[2, 12]	X[2]			
Cefepime	X[5, 12]					
Ceftazidime	X[6, 9, 12]	X[6, 12]	X[6, 12]			
Ceftriaxone	X[10, 12]	X[10, 12]	X[10, 12]	X	X	X
Ciprofloxacin	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]
Colistin	X[14]	X[14]	X[14]			
Gentamicin	X	X	X			
Imipenem	X[8]	X[8]	X[8]			
Nalidixic Acid				X[11]	X[11]	X[11]
Piperacillin/Tazobactam	X[10]	X[10]				
TMP/SMX	X	X	X	X	X	X
Tobramycin	X	X[3, 7]	X[7]			

[1] Always report *Klebsiella* spp, *Enterobacter* spp, *Acinetobacter* spp., *H. alvei, Citrobacter* spp., *Proteus vulgaris, Proteus penneri*,and *Serratia* species as R

[2] Always report *Enterobacter* spp, *Citrobacter* spp., *Proteus vulgaris, Proteus penneri, Acinetobacter* spp, and *Serratia* species as R.

[3] TG and TW – report Tobramycin on all PD effluents

[4] Adults only (>18 y)

[5] Report on PMH patients only if Cetriaxone is I or R.

[6] Report only if R

[7] Report if Genta is R

[8] Report if R **OR** if R to **All** other Antimicrobial Agents **OR** if only aminoglycoside is S

[9] Always report for PMH

[10] *Cedecea* spp., *Citrobacter* spp., *Enterobacter* spp., *Hafnia* spp., *Morganella morganii, Pantoea agglonerans, Proteus penneri, Proteus vulgaris, Providencia* species, *Seratia* species, if **S**, report with comment "Resistance to extended-spectrum penicillins, beta-lactam/beta-lactamase inhibitor combinations, and cephalosporins may develop during therapy with these agents. For serious infections, these agents should be avoided and consultation with a medical microbiologist or infectious disease physician is strongly recommended."

[11] DO NOT Report. If Ciprofloxacin is S and Nalidixic Acid is R, report isolate comment "This isolate is tested Fluoroquinolone-susceptible and nalidixic acid-resistant; may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with extraintesinal salmonellosis."

[12] For *Enterobacteriaceae* if any one of cefotaxime/ceftriaxone or ceftazidime=R, report all as R

[13] Report if both Gentamicin and Tobramycin are R.

[14] For Acinetobacter species, report if R to all other drugs; base on Polymyxin B.

[a] includes MSH, PMH, Baycrest, TRI, CAMH

[b] includes TG, TW, Bridgepoint, Grace

[c] includes CHC, Ajax/Pickering

Note: If all antimicrobial agents are resistant, inform the Microbiologist on-call

Blood and other Sterile Sites - Gram Negative Susceptibility Reporting -2 - *Pseudomonas aeruginosa, Pseudomonas* spp. (other than P. aeruginosa), fastidious gram-negative bacteria, non-fermenters, *M. catarrhalis, N. gonorrhoeae , N. meningitidis, Stenotrophomonas maltophilia, Burkholderia cepacia, Haemophilus* species.

Antimicrobial Agent	***P. aeruginosa***			***S. maltophilia***	***B. cepacia***	***Haemophilus species***
	MSH[a]	UHN[b]	CHC[c]	All sites	All sites	All sites
Amikacin	X[5]	X[5]	X[5]			
Ampicillin	R					X[1]
Cefazolin	R					
Cefepime	X[3]					
Ceftazidime	X	X	X		X	
Ceftriaxone						X
Ciprofloxacin	X[2]	X[2]	X[2]			X[2]
Colistin	X[6]	X[6]	X[6]	X[6]	X[6]	
Gentamicin	X	X	X			
Imipenem	X[4]	X[4]	X[4]			
Levofloxacin				X[2]		
Meropenem					X	
Piperacillin/Tazobactam	X	X	X			
TMP/SMX	R			X	X	
Tobramycin	X	X	X			

[1] Base on beta-lactamase result and KB Ampicillin
[2] Adults only (>18 y)
[3] For PMH only
[4] Report if R **OR** if R to **All** other Antimicrobial Agents **OR** if only aminoglycoside is S
[5] Report if both Gentamicin and Tobramycin are R.
[6] Report if R to all other drugs; base on Polymyxin B.
[a] includes MSH, PMH, Baycrest, TRI, CAMH
[b] includes TG, TW, Bridgepoint, Grace
[c] includes CHC, Ajax/Pickering

Note: ***Pseudomonas* species (other than *P. aeruginosa*), fastidious gram-negative bacteria, non–fermenters, *N. gonorrhoeae*** and ***N. meningitidis*** - DO NOT report susceptibility result. Report with ISOLATE comment "In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

For ***M. catarrhalis*** - DO NOT report susceptibility result. Report with ISOLATE comment: "The majority of *Moraxella catarrhalis* are resistant to ampicillin. In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

If all antimicrobial agents are resistant, inform the Microbiologist on-call

ANTIMICROBIAL RELATED LIS CANNED MESSAGES

I. Introduction

Antimicrobial related canned messages are built into the Laboratory Information System to provide uniform reporting phrases to be used when certain pre-described conditions are met.

II. Procedure

A. Automatic Canned Messages:

The lists below are automatic canned messages that will appear when set conditions are met. The message will appear in a warning box before exiting an order.

1. When the message code appears, press F12 to save.
2. Continue with another F12 to save the order.
3. View the report.
4. If the same message had been save previously (i.e. appeared more than once), go to the Isolate Comment window, press CTLR and L at the line to remove the duplicate line.
5. Re-status as required.
6. Press F12 to save the order.

Ear and Eye specimens with susceptibility results
LIS Isolate Canned Message Code: **&eye**; attached to Organism classes A and B with sources EYE, EYEN, EAR and drugs am, betalac, cc, peng, sxt.
"These susceptibility testing results are based on guidelines for systemic antimicrobial agents and may not accurately represent activity of topical agents."

For **MSH MRO's**
LIS Isolate Canned Message Code: **\MRES**, attached to drug – tax **"MULTIPLY ANTIBIOTIC RESISTANT ORGANISM. THIS PATIENT MUST BE ON "CONTACT PRECAUTIONS" UNTIL FURTHER NOTICE FROM INFECTION CONTROL."**

For **MSH MRSA's**
LIS Isolate Canned Message Code: **\MRSI**, attached to organism staamr **"THIS PATIENT MUST BE ON "MRSA PRECAUTION" UNTIL FURTHER NOTICE."**

MRSA isolated from MRSA Screen Susceptibility Result Comment
LIS Isolate Canned Message Code: **\MRSS**; linked to drug code - oxa
"Susceptibility results are provided to guide decolonization therapy; if decolonization therapy is being considered, please consult your infection control team."

***Staphylococcus saprophyticus* and CNST from urine**
LIS Isolate Canned Message Code: **\ssap**; attached to organism stasap.
"Susceptibility testing of this organism is not warranted because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated urinary tract infections e.g. nitrofurantoin, trimethoprim/sulfa or fluoroquinolones."

***ß-haemolytic Streptococcus* Groups A, B, C and G**
LIS Isolate Canned Message Code: **\GBS**; attached to organism straga, strpyo, strgrc, strgrg.
"This organism is intrinsically susceptible to penicillin. If treatment is required AND this patient cannot be treated with penicillin, please contact the Microbiology Department within 48 hours to request sensitivity testing."

For **MSH VRE's**
LIS Isolate Canned Message Code: **\VREI**, attached to organisms entfac and entfae
"THIS PATIENT MUST BE ON "VRE PRECAUTION" UNTIL FURTHER NOTICE."

Vancomycin for ***E. gallinarum, and E. casseliflavus*** report as **R** with the statement
LIS Isolate Canned Message Code: **\EntV**; attached to organisms - entgal and entcas.
"This organism always has intrinsic non-transmissible resistance to vancomycin. The patient does not require isolation."

For ***Listeria* species**:
LIS Isolate Canned Message Code: **\LIST**; attached to organisms lismoc and lismon.
"Routine in vitro susceptibility testing of this organism is unreliable. *Listeria* spp. should be considered resistant to all cephalosporins. The recommended regimen for therapy is ampicillin. If additional advice on antimicrobial therapy is required, please contact the Medical Microbiologist."

For isolates that for which **susceptibility testing is not routinely performed and/or is unreliable**:
LIS Isolate Canned Message Code: **\NSEN**; attached to organisms and Isolate Comment keypad.
"In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

For ***Cedecea* spp., *Citrobacter* spp., *Enterobacter* spp., *Hafnia* spp., *Morganella morganii, Pantoea agglonerans, Proteus penneri, Proteus vulgaris, Providencia* species, *Serratia* species** that are ceftriaxone or pipercillin/tazobactam = **S**:
LIS Isolate Canned Message Code: **&cit** or **&ent** or **&ser**; attached to Organisms ceddav, cedlap, cedspp, Classes d, e, H, L, S, and T.

"Resistance to extended-spectrum penicillins, beta-lactam/beta-lactamase inhibitor combinations (e.g. pipercillin/tazobactam), and cephalosporins may develop during therapy with these agents. For serious infections, these agents should be avoided and consultation with a medical microbiologist or infectious disease physician is strongly recommended."

For **MSH *E. coli, Klebsiella* species, *Proteus*** Class A ESBL, Infection Control message:
Isolate canned message code **&taz** linked to organisms *E. coli*, Class J and Class T:
"MULTIPLY ANTIBIOTIC RESISTANT ORGANISM. THIS PATIENT MUST BE ON "CONTACT PRECAUTIONS" UNTIL FURTHER NOTICE FROM INFECTION CONTROL."

ESBL Comments
Attached to organisms esccol, Class J and Class T
Desbinh=Y Dfox=S **~&cla** "The susceptibility pattern suggests that this organism contains a class A extended spectrum beta-lactamase (ESBL)."
Dtaz=R Desbinh=N Dfox=R or I **~&claC** "The susceptibility pattern suggests that this organism contains a class C extended spectrum beta-lactamase (ESBL)."
Desbinh=Y Dfox=R or I **~&clAC** "The susceptibility pattern suggests that this organism contains class A and C extended spectrum beta-lactamases (ESBL)."
Dtaz=R Desbinh=N Dfox=S **~&esbl** "The susceptibility pattern suggests that this organism contains an extended spectrum beta-lactamase (ESBL) other than class A or C."

For ***Haemophilus* species** from Respiratory and Miscellaneous Sites – LIS Isolate Canned Message Code, attached to organism Class X:
If beta-lactamase is negative, **\BLa-** "beta-lactamase negative result suggests susceptible to ampicillin."
If beta-lactamase is positive, **\BLa+** "beta-lactamase positive result suggests resistance to ampicillin but generally susceptible to amoxicillin-clavulanic acid and cefuroxime."

For ***Salmonella* species**:
Nalidixic acid confirming Fluoroquinolone Susceptibility in *Salmonella* species Preliminary Report Comment
"Susceptibility testing suggests that this organism may not be fully susceptible to fluoroquinolones. Further testing is being completed and results will be available tomorrow. If you are interested in preliminary susceptibility results, please contact the microbiology laboratory."

Comment after KB – Nalidixic Acid has been completed:
LIS Isolate Canned Message Code: **&sal**; attached to organisms Class q and saltyp: Dcip=S Dna=R RI RR R1 Ra Rb Re Rl Rk ~&sal
If Ciprofloxacin is S and Nalidixic Acid is R, report isolate comment "This isolate is tested Fluoroquinolone-susceptible and nalidixic acid-resistant; may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with extraintesinal salmonellosis."

For ***M. catarrhalis*** - LIS Isolate Canned Message Code: **\mcat**; attached to Organism: "The majority of *Moraxella catarrhalis* are resistant to ampicillin. In vitro susceptibility testing for this organism is not routinely performed and/or is unreliable. If advice on antimicrobial therapy is required, please contact the Medical Microbiologist".

B. Canned Messages to be selected from the Isolate Comment keypad:
The listed below are canned messages to be selected from the Isolate Comment keypad when needed.

1. At the LIS Isolate Comment Window, type the appropriate number on the keypad.
2. Press F12 to save.
3. Continue with another F12 to save the order.
4. View the report.
5. Status the report as required.

For **INH** reporting if 0.1 mg/L=R and 0.4mg/L=S:
LIS Isolate Canned Message Code: **\INHr**; select from keypad
"This isolate has low-level resistance to isoniazid (INH). Patients infected with strains exhibiting this level of INH resistance may benefit from continuing therapy with INH. Consultation with a specialist experienced in the treatment of tuberculosis is recommended."

BORSA (DENKA *mec*A-negative *S. aureus* with oxacillin MIC>=4mg/L)
LIS Isolate Canned Message Code: **\BORS**; select from Isolate Keypad
"This organism is resistant to cloxacillin by a mechanism different from that in typical MRSA. Consultation with a Microbiologist or Infectious Disease physician is advised."

If susceptibility is done on request for ***ß-haemolytic Streptococcus*** **Groups A, B, C & G**
Do not remove original canned message. Add message from Isolate Comment keypad code "Susceptibility\done" – "Susceptibility completed as requested."

Enterococcus from Blood and Sterile Sites:
If high level gentamicin is **susceptible** (regardless of streptomycin result), select from Isolate Comment Keypad **\EGMS**: "Serious enterococcal infections may require an aminoglycoside for synergy. Please contact the Medical Microbiologist for treatment advice".
If high level gentamicin is **resistant** (regardless of streptomycin result), select from Isolate Comment Keypad **\EGMR**: "This organism is high level aminoglycoside resistant. Please contact the Medical Microbiologist for treatment advice".

E. coli, Klebsiella **species,** ***Proteus*** **cefpodoxime=I or R**, set up KB ESBL, Preliminary Report Comment; select from ISOLATE keypad - **\podR**
"Susceptibility testing suggests that this organism may contain an extended spectrum beta-lactamase. Further testing is being completed and results will be available tomorrow. If you are interested in preliminary susceptibility results, please contact the microbiology laboratory."

APPENDIX I - DISK DIFFUSION

I. Introduction

The disk diffusion method of susceptibility testing (also known as the Kirby-Bauer (KB) method) has been standardized primarily for testing of rapidly growing bacteria. To perform the test, filter paper disks impregnated with a specific amount of antimicrobial agent are applied to the surface of an agar medium that has been inoculated with a known amount of the test organism. The drug in the disk diffuses through the agar. As the distance from the disk increases, the concentration of the antimicrobial agent decreases creating a gradient of drug concentrations in the agar medium. Concomitant with diffusion of the drug, the bacteria that were inoculated and that are not inhibited by the concentration of the antimicrobial agent continue to multiply until a lawn of growth is visible. In areas where the concentration of drug is inhibitory, no growth occurs, forming a zone of inhibition around each disk. Criteria currently recommended for interpreting zone diameters and MIC results for commonly used antimicrobial agents are published by NCCLS. Results are reported categorically as Susceptible (S), Intermediate (I), or Resistant (R).

II. Materials

Antimicrobial disks (store frozen with a desiccant)
Mueller Hinton Agar (MH)
Mueller Hinton Blood Agar (MHB)
Haemophilus Test Media (HTM)
Trypticase Soy Broth (TSB) (3 mL)
VITEK colorimeter
Sterile saline
Sterile swabs

III. Procedure

1. Allow disks to come to room temperature before opening the container.

2. Using the Vitek colorimeter, prepare a suspension of the test organism in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies. If there is not enough growth, inoculate the organism into TSB, and incubate at 35^{o}C for 2-4 hours or until it reaches the turbidity of a 0.5 McFarland standard.

3. Using a sterile cotton swab, inoculate the organism onto an appropriate agar plate, streaking in 3 directions over the entire agar surface. For organisms that grow rapidly use MH agar. For *Haemophilus* species use HTM and for *S. pneumoniae* use MHB. For other organisms that do not grow on MH, use MHB.

4. Using forceps or a disk dispenser, apply the appropriate Antimicrobial disks onto the agar. Place the disks with an equal distance apart from each other and put no more than 6 disks on a 100mm diameter plate.

5. Incubate plates as follows:
Campylobacter species - microaerophilically at 35°C x 18 hours
Haemophilus species - CO_2, 35°C x 18 hours
S. pneumoniae - CO_2, 35°C x 20 to 24 hours
S. aureus and *Enterococcus* species for Methicillin and Vancomycin - O_2, 35°C x 24 hours
Others - O_2, 35°C x 18 hours

IV. Interpretation

After incubation, measure the diameters of the zone of complete inhibition with callipers using transmitted light. For MH agar, measure from the back of the plate.

Refer to NCCLS Document M100-S15 (M2) for the zone size interpretations. Report susceptible, resistant and intermediate as appropriate.

V. Quality Control

Test the following organisms each time a new batch of MH agar is prepared and once weekly. Subculture the organisms from the TSB slant (in fridge) to BA the day before setting up the QC.
S. aureus ATCC 25923
E. coli ATCC 25922
P. aeruginosa ATCC 27853
In addition, test *S. faecalis* ATCC 29212 each time a new batch of MH is prepared.

Perform weekly Quality Control on HTM agar with *Haemophilus influenzae* ATCC 49247. Test for growth of *Haemophilus influenzae* ATCC 10211 on each new batch HTM.

See NCCLS Document M100-S15 (M2) Table 3 for acceptable QC results.

VI. Reference

Clinical and Laboratory Standards Institute (CLSI) Document - Performance Standards for Antimicrobial Disk Susceptibility Testing M2-A8, 2003.

APPENDIX II – DOUBLE DISK DIFFUSION for ERYTHROMYCIN and CLINDAMYCIN on ß-haemolytic *Streptococci* Groups A, B, C, G and *Streptococcus pneumoniae*

I. Introduction

Macrolide (erythromycin) resistant ß-haemolytic *Streptococci* and *Streptococcus pneumoniae* isolates may have constitutive or inducible resistance to lincosamides (clindamycin). The mechanisms of resistance include:

- Ribosomal modification encoded by an *erm* gene; also refer to as MLS_B (macrolide, lincosamide and type B streptogramin) resistance.
- Efflux of the antibiotic encoded by a *mef* gene; resistant only to macrolide
- Drug inactivation

Inducible clindamycin resistance can be detected using a disk approximation test with a clindamycin disk placed beside an erythromycin disk as part of the normal disk diffusion test.

II. Materials

Antimicrobial disks – clindamycin (DA, 2 µg) and erythromycin (E, 15 µg)
Mueller Hinton Blood Agar (MHB)
Trypticase Soy Broth (TSB) (3 mL)
VITEK colorimeter
Sterile saline
Sterile swabs

III. Procedure

1. Allow disks to come to room temperature before opening the container.

2. Using the Vitek colorimeter, prepare a suspension of the test organism in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies.

3. Using a sterile cotton swab, inoculate the standardized organism onto an MHB agar plate, streak in three directions over the entire agar surface.

4. Place plate on disk template (Figure 1.)

5. Using forceps or a disk dispenser, apply the clindamycin and erythromycin disks onto the agar 12 mm apart from each other edge to edge using template below (Figure 1). Other antimicrobial disks can be placed on the same agar plate if needed.

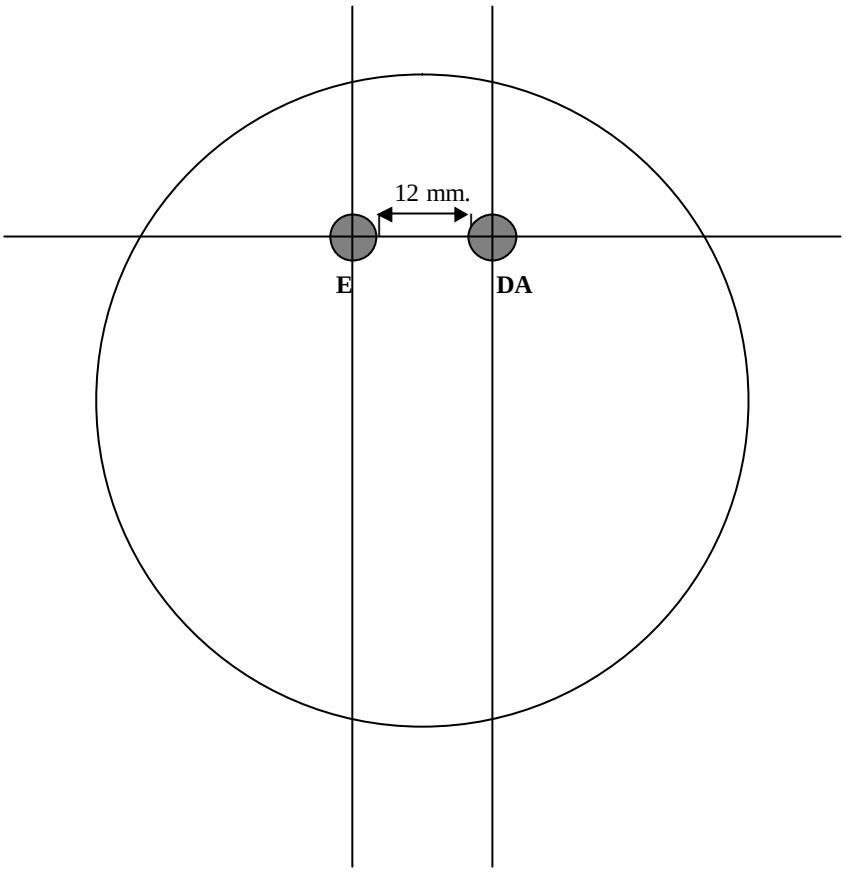


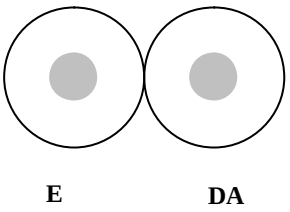
Figure1. Template for Clindamycin and Erythromycin disks placement

6. Incubate plates in CO_2 at 35°C for 20 to 24 hours

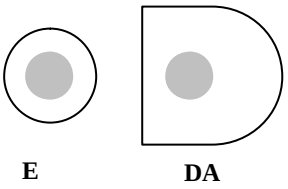
IV. Interpretation

1. After incubation, measure the diameters of the zone of complete inhibition with callipers/ruler. Refer to Clinical and Laboratory Standards Institute (CLSI) Document - M100-S15 (M2) for the zone size interpretations.
2. Enter zone size measurements into the LIS.
3. Organisms that show flattening of the clindamycin zone adjacent to the erythromycin disk in the shape of the letter D (referred to as a "D" zone) have inducible clindamycin resistance. Enter into the LIS the presence or absence of "D" zone as "Y" or "N" under LIS drug "D zone". Isolates that show the presence of D zone will be automatically reflexed in the LIS to report as "clindamycin resistant".

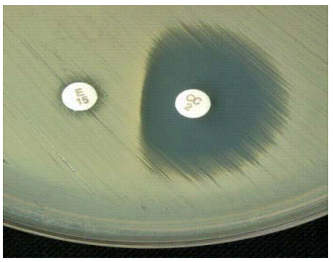
Examples of Zone of Inhibition Patterns and their Interpretation:

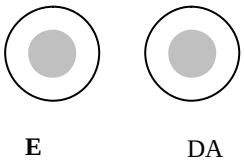


Both E and DA are Susceptible.
Report both E and DA as S

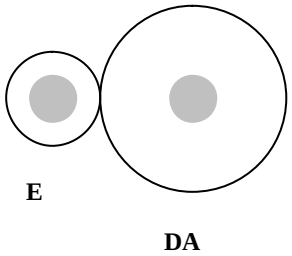


Both E and DA (measured at the narrowest side) are I or R; "D" zone is positive – Inducible MLS_B; presumed genotype: *erm*
Report both E and DA as R





Both E and DA I or R – Inducible or constitutive MLS_B; presumed genotype: *erm*
Report both E and DA as R



E is I or R and DA is S – M phenotype; presumed genotype: *mef*.
Report E as I or R and DA as S.

V. Quality Control

See Clinical and Laboratory Standards Institute (CLSI) Document - M100-S15 (M2) Table 3 for acceptable QC results.

VI. References

Clinical and Laboratory Standards Institute (CLSI) Document - Performance Standards for Antimicrobial Disk Susceptibility Testing M2-A8, 2003.

Clinical and Laboratory Standards Institute (CLSI) Document - Performance Standards for Antimicrobial Disk Susceptibility Testing Information Supplement Table 2H M2-Disk Diffusion M100-S15 2005.

Quality Management Program-Laboratory Services (QMP-LS) Committee Comments BACT-020, Vol. 3, 2.2:721-724.

Streptococci and *Staphylococcus* (overview of macrolides and lincosamide resistance) Leclercq. CID 2002;34:482-92

Streptococcus pneumoniae
Descheemaeker et al. JAC 2000 45:167-173

Beta-haemolytic streptococcus (Groups A,B,C,G)
GAS Descheemaeker et al. JAC 2000 45:167-173
GBS de Azavedo et al. AAC 1001;45:3504-3508
GCS & GGS Kataja et al. AAC 1998;42:1493-1494

APPENDIX III - DOUBLE DISK TEST FOR ESBL

I. Introduction

Class A or Bush Group 1 extended spectrum beta-lactamases (ESBLs) are inhibited by clavulanic acid. This may be detected by testing the suspected organism to a 3rd generation cephalosporin alone and in combination with clavulanic acid. If the combination results in an expanded zone of inhibition compared to that of the 3rd generation cephalosporin alone, it is indicative of the presence of an ESBL.

II. Materials

Mueller-Hinton (MH) agar (150) mm
20/10 mg amoxicillin-clavulanate disc
30 mg ceftazidime disc
30 mg ceftriaxone or cefotaxime disc
30 mg aztreonam disc
10 mg cefpodoxime disc (optional)
30 mg cefoxitin disc
Quality control strain: *E. coli* ATCC 51446

III. Procedure

1. Prepare a bacterial suspension of the organism to be tested that has a turbidity equivalent to that of a 0.5 McFarland standard.

2. Inoculate a Mueller-Hinton agar plate with this suspension in accordance with NCCLS M100-S10 (M2) guidelines for disc diffusion testing.

3. Place the amoxicillin-clavulanic acid disc towards the centre of the plate.

4. Carefully measure 15 mm out from the edge of that disc at 90^{o} angles marking the plate.

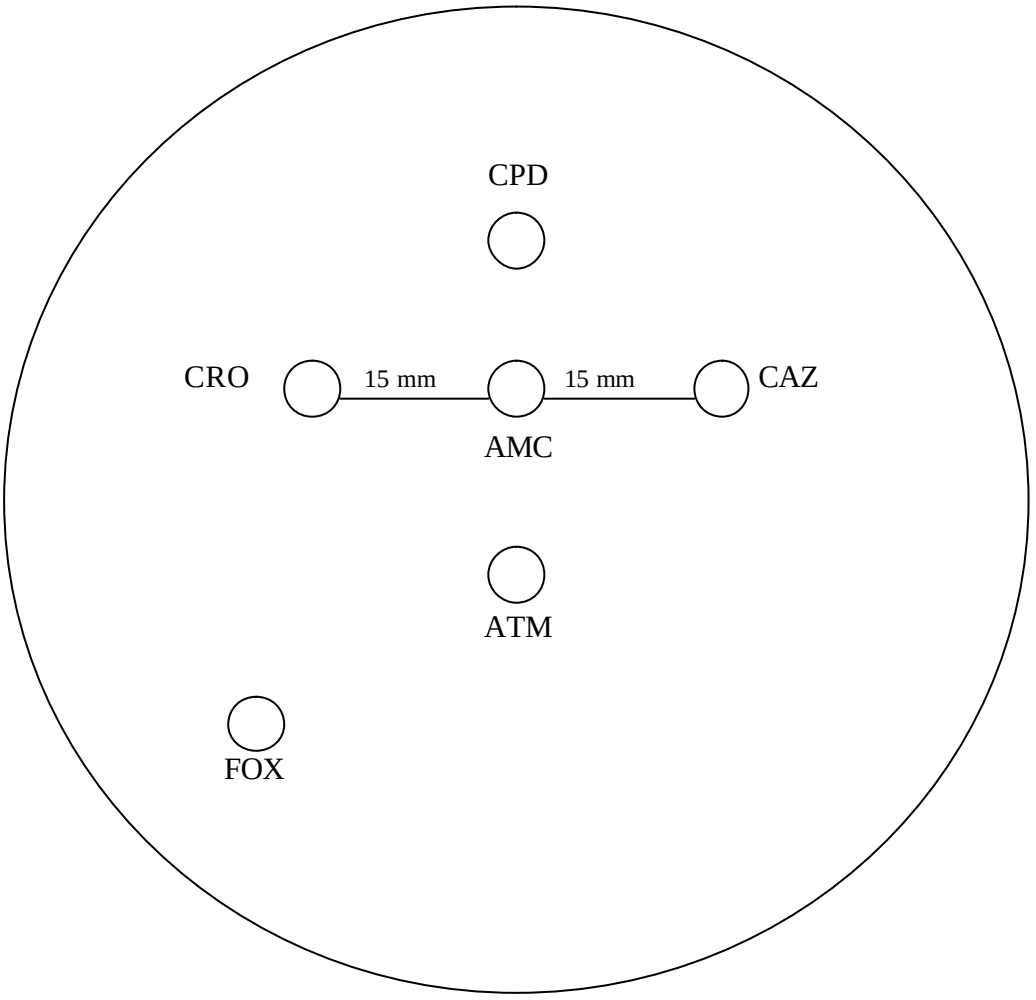
5. Place a ceftazidime disk on the plate so that its inner edge is 15 mm (the mark) from the amoxicillin-clavulanic acid disc (See Figure 1 KB-ESBL Template).

6. Do the same with cefotaxime (or ceftriaxone), aztreonam and cefpodoxime discs so that they are spaced 90^{o} apart and 15mm from the centre disc.

7. Place a cefoxitin disc in any available space remaining on the plate.

8. Incubate 35°C, in O_2 x 18-24 hours and record the zone diameters for the all cephalosporins as per NCCLS guidelines.

Figure 1. KB-ESBL Template



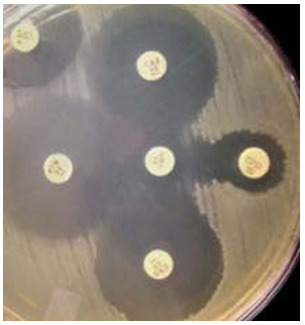
IV. Interpretation

Note: The following applies to cefpodoxime-nonsusceptible *E. coli*, *Klebsiella* species and *Proteus* species only.

1. Document zone size for all antibiotics.
2. Observe for **potentiation** of the inhibition zone (i.e. **increase** in the inhibition zone) of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid (enter **Y**es or **N**o to the "drug" named ESBL Inhibitor in the LIS).
3. If a reduction of zone of inhibition of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid is observed, recheck the identification of the isolate and repeat testing. Notify the charge technologist if result remains unchanged.

Class A ESBL present:

i) Potentiation of the inhibition zone of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid (see below for examples of different patterns of potentiation that can be seen with organisms that contain Class A ESBLs)
ii) Susceptibility to cefoxitin
iii) Susceptibility or resistance to any one of ceftazidime, ceftriaxone or aztreonam


Class A and Class C ESBL present:

i) Potentiation of the inhibition zone of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid
ii) Resistant or Intermediate to cefoxitin.
iii) Susceptibility or resistance to any one of ceftazidime, ceftriaxone or aztreonam

Class C-ESBL present:

i) No potentiation with clavulanic acid
ii) Resistance or Intermediate to cefoxitin
iii) Resistance to any one of ceftazidime, ceftriaxone or aztreonam.

ESBL not Class A or Class C present:

i) No potentiation with clavulanic acid
ii) Susceptibility to cefoxitin
iii) Resistance to any one of ceftazidime, ceftriaxone or aztreonam

ESBL absent:

i) No potentiation with clavulanic acid
ii) Susceptibility or resistance to cefoxitin
iii) Susceptibilty to all of ceftazidime, ceftriaxone or aztreonam

V. Reporting

Reporting Comment	**Potentiation** of the inhibition zone of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid (enter **Y** or **N** to the "drug" named ESBL Inhibitor in the LIS)	Cefoxitin	Ceftazidime, ceftriaxone or aztreonam
The susceptibility pattern suggests that this organism contains a class A extended spectrum beta-lactamase (ESBL).	**Y**es	S	S/R
The susceptibility pattern suggests that this organism contains class A and C extended spectrum beta-lactamases (ESBL).	**Y**es	I/R	S/R
The susceptibility pattern suggests that this organism contains a class C extended spectrum beta-lactamase (ESBL).	**N**o	I/R	R
The susceptibility pattern suggests that this organism contains an extended spectrum beta-lactamase (ESBL) other than class A or C.	**N**o	S	R
Not ESBL – no reporting comment	**N**o	S/R	S

VII. References

1. Cormican MG, Marshall SA, Jones RN. 1996. Detection of extended-spectrum beta-lactamase (ESBL)-producing strains by the Etest ESBL screen. J. Clin. Microbiol. 8:1880-1884.

2. Jacoby GA, Han P. 1996. Detection of extended-spectrum beta-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. J. Clin. Microbiol. 34:908-911.

3. Jacoby GA. 1994. Genetics of extended-spectrum beta-lactamases. Eur. J. Clin. Microbiol. Infect. Dis. Suppl 1,13:2-11.

4. Livermore DM. 1995. Beta-lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev.8:557-584.

5. Moland ES, Sanders CC, Thomson KS. 1998. Can results obtained with commercially available MicroScan microdilution panels serve as an indictor of beta-lactamase (ESBL)-production among *Escherichia coli* and *Klebsiella* isolates with hidden resistance to expanded spectrum cephalosporins and aztreonam? J. Clin. Microbiol. 36:2575-2579.

6. NCCLS. Performance standards for antimicrobial susceptibility testing; Eighth informational supplement. NCCLS document M100-SB [ISBN 1-26238-337-X]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 1998)

7. Sanders CC, Barry AL, Washington JA, Shubert C, Moland ES, Traczewski MM, Knapp C, Mulder R. 1996. Detection of extended-spectrum beta-lactamase-producing members of the family Enterobacteriaceae with the Vitek ESBL test. J. Clin. Microbiol. 34:2997-3001.

8. Swenson JM, Ferraro MJ, Jorgensen JH, Tenover FC. 1996. Can different breakpoints be used to detect extended-spectrum beta-lactamase (ESBL)-producing organisms? Abstr D-22, ICAAC meeting, New Orleans, Louisianna, Sept 15 -18, 1996.

9. Vercauteren E, Descheemaeker P, Ieven M, Sanders CC, Goossens H. 1997. Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella* spp. in a Belgian teaching hospital. J. Clin. Microbiol. 35:2191-2197.

APPENDIX IV - BETA-LACTAMASE TESTING

Introduction

This method can be used to detect beta-lactamase production in *Haemophilus* spp., *Neisseria gonorrhoeae*, *M. catarrhalis*, enterococci and staphylococci. Refer to CRITERIA FOR SUSCEPTIBILITY TESTING for when testing for beta-lactamase is required. The current method used in the laboratory is the chromogenic cephalosporin [cefinase (nitrocefin) disk] test. The test organism is inoculated directly onto the filter paper disk impregnated with nitrocefin. If the organism produces beta-lactamase, it will hydrolyze the chromogenic cephalosporin causing an electron shift that result in a coloured product.

II. Materials

Cefinase disks (BBL) (store refrigerated)
Sterile distilled water
Microscope slides
Sterile Pasteur pipettes

III. Procedure

1. Using forceps remove the required number of disks from the dispenser and place on a microscope slide. Use 1 disk per organism.

2. Using a sterile Pasteur pipette, moisten each disk with a drop of sterile water.

3. With a sterile loop or applicator stick, pick several similar colonies from the agar plate and smear onto the surface of the disk.

4. Observe the disk for up to 5 minutes for a colour change. For staphylococci, observe the disks for up to 60 minutes.

IV. Interpretation

Positive:	the appearance of a pink colour
Negative:	no colour change

V. Quality Control

Set up positive and negative controls whenever a test is performed.

Positive: *H. influenzae* ATCC 35056, ß-lactamase positive.
Negative: *H. influenzae* ATCC 10211, ß-lactamase negative.

VI. Reference

APPENDIX V - OXACILLIN SCREEN PLATE

I. Introduction

This is an agar dilution method using a single concentration of oxacillin incorporated into Mueller Hinton (MH) agar to screen for resistant strains of *S. aureus*.

II. Materials

Control plate (MH with 4% NaCl)
Screen plate (MH with 4% NaCl and 6 µg/mL OX)
VITEK colorimeter
Sterile saline
Sterile swabs

III. Procedure

1. Using the VITEK colorimeter, prepare a suspension of the test organism (from solid medium after overnight culture) in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies (inoculum prepared for VITEK can be used).

2. Using a sterile swab, spot inoculate the suspension onto the screen and control plates. Numerous organisms can be tested on one plate.

3. After the inocula have dried, incubate the plate at 35°C, O_2 for 24 hours.

4. All resistant isolates on the screen plate must be checked for purity (e.g. Gram stain, *S. aureus* tube coagulase or slide agglutination and sub-culture). The resistance must be confirmed by seting up a Denka MRSA Screen for confirmation of MRSA (Refer to DENKA MRSA SCREEN). Send a preliminary report as ISOLATE: Methicillin-resistant *S. aureus* and report to infection control.

IV. Interpretation

Growth on the screen plate indicates that the organism is methicillin resistant and therefore is considered resistant to all beta-lactam Antimicrobials (eg. penicillin, oxacillin, cephalosporins). Note: There must be growth on the control plate.

V. Quality Control

Controls must be tested each day. The organisms are to be sub-cultured from the TSB slant (in fridge) to Blood agar each day.

Sensitive: *S. aureus* ATCC 29213

Resistant: *S. aureus* LPTP 8610-1
S. aureus ATCC 43300

VI. Reference

Clinical and Laboratory Standards Institute (CLSI) Document – Methods for Dilution Antimicrobial Susceptibility Testing M7-A6, 2003.

APPENDIX VI - DENKA MRSA SCREEN

Principle

To be used as a screening test for the detection of Methicillin Resistant *S. aureus* (MRSA) from isolated colonies.

Reagents

MRSA screening kit
Microcentrifuge tubes
Boiling water bath
Wooden sticks
Loops
Micropipettes

Method

1. Add 4 drops of extraction reagent #1 into a microcentrifuge tube.

2. Take one heavy loopful of the *Staphylococcus aureus* colonies from a blood plate and suspend the cells in the microcentrifuge tube.

3. Place in a boiling water bath for 3 minutes.

4. Remove microcentrifuge tube and let cool to room temperature.

5. Add one drop of extraction reagent #2 to the tube and mix well.

6. Centrifuge at high speed for 5 minutes.

7. For each specimen to be tested, allot and label one circle of the test card for testing with sensitized latex and one with control latex.

8. Place 50 microliters of the specimen onto 2 of the test circles and add one drop of the sensitized cells to one circle and one drop of the latex control to the other.

9. Mix the sample and latex together.

10. Rotate the card by hand for 3 minutes and observe for agglutination.

11. If negative rotate for another 3 minutes.

Interpretation

Sensitized latex	Control latex	Result
+	-	MRSA
-	-	Not MRSA
+ or -	+	Indeterminant

For DENKA – negative isolates:

a. Perform Oxacillin-induced DENKA test:
   i Sub-culture isolate onto a BA and add a Oxacillin disc. Incubate in O_2 at 35°C overnight.
   ii Repeat DENKA using growth around the disc.
b. If Oxacillin-induced DENKA is positive and it is a confirmed *S. aureus* report as MRSA.

If Oxacillin-induced DENKA is negative, check the Vitek Oxcillin MIC:

a. Oxacillin =>4mcg/L, isolate is a BORSA; report as *S. aureus* with Isolate comment "This organism is resistant to cloxacillin by a mechanism different from that in typical MRSA. Consultation with a Microbiologist or Infectious Disease physician is advised."
b. Oxacillin <4mcg/L, isolate is NOT a MRSA; report as *S. aureus* oxacillin-S.

Quality Control

Positive and negative controls must be set up once per week.

Positive: *S. aureus* (ATCC 43330)
Negative: *S. aureus* (ATCC 29213)

Reference

1. Denka Seiken Co., Ltd., Tokyo, Japan, Denka MRSA Screen package insert June 1998.

APPENDIX VII - SERUM BACTERIOSTATIC & BACTERICIDAL TITRES

I. Introduction

In the treatment of a patient with bacterial endocarditis or osteomyelitis, it is often important to know whether the prescribed dosages of Antimicrobials are achieving blood levels sufficiently high enough to kill the causative organism.

The bacteriostatic level is the dilution of serum that inhibits visible bacterial growth; the bactericidal level is the serum dilution that kills 99.9% of the initial inoculum.

NOTE: This test is to be performed only with the approval of a microbiologist.

II. Specimen Collection

The dose, the time the dose was given, and the time of collection must be recorded on the requisition. Pre- and post-dose blood specimens are obtained in serum separator tubes. The pre-dose blood specimen is drawn immediately before administering the next dose of Antimicrobial in order to evaluate the pre (trough) level. Blood for the post-dose (peak) level should be drawn 1 hour after an intravenous infusion has been started, 1 hour after an intramuscular dose and 1 to 2 hours after an oral dose.

III. Reagents/Materials/Media

Mueller Hinton Broth (MHB) (100 mL)
Blood Agar (BA)
Sterile 13 x 100 mm glass tubes
Sterile 10 mL pipettes
Sterile yellow pipette tips
Test tube racks
Pipetter
Precision pipette to deliver 20 ?L

IV. Procedure

A. Processing of Specimens
Upon arrival in the laboratory, centrifuge the blood and aseptically transfer the serum into a sterile vial.

B. Preparation of bacterial suspension:

Inoculate several colonies of a pure culture of the patient's organism (overnight sub-culture) into 5 mL MHB. Incubate on a shaker at 36°C for a minimum of 3 hours or until it achieves turbidity greater than the 0.5 McFarland standard (approximately 1.3 x 10^8 CFU/mL).

C. Serum dilution:

a. Place 12 sterile test tubes in a rack for each serum sample to be diluted.
b. Number the tubes 1 to 12.
c. Aseptically pipette 1.0 mL of patient's serum into tubes 1 and 2.
d. Aseptically pipette 1.0 mL of MHB into tubes 2 - 12.
e. With a new 1.0 mL sterile pipette transfer 1.0 mL of serum from tube 2 to tube 3. Mix well.
f. Serially dilute the serum by sequentially transferring 1.0 mL of the mixture through to tube 10. Discard 1.0 mL of the mixture from tube 10. No serum is to be added to tube 11 (positive inoculum control) or to tube 12 (broth sterility control). The final dilution of serum in tube 10 is 1:512 and final volume in all tubes should be 1.0 mL.

D. Inoculating Broth

a. Using the Vitek colourimeter, dilute the bacterial suspension to the turbidity of the 0.5 McFarland standard using MHB.
b. Prepare a 1:4 dilution of the standardized inoculum by adding 1.0 mL of inoculum to 3.0 mL MHB. Mix well.
c. Using a precision pipette, dispense 20 µL (0.02 mL) of diluted inoculum into tubes 1 through 11. To inoculate, insert the pipette tip well under the surface of the Antimicrobial containing serum broth mixture. AVOID ANY CONTACT BETWEEN THE TIP AND THE WALLS OF THE TUBE - to prevent transfer of organisms to the inside of tube above the meniscus. Mix by flushing 2 or 3 times without creating air bubbles or splashing. Use a new tip for each tube.
d. Incubate all tubes at 37°C for 20 hours in a CO_2-free incubator.
e. From the 1:4 dilution of the standardized inoculum, dilute 1:250 in MHB (0.1 mL in 24.9 mL MHB) to achieve an inoculum of 10^5 CFU/mL.
f. Perform a colony count to confirm the bacterial count in the final inoculum. Transfer 0.001 mL of diluted inoculum to BA by using a urine loop and distribute evenly on the surface of a BA plate.
g. Incubate the BA plate overnight at 35°C.

Determination of serum bacteriostatic titres

1. After incubation, tube 12 (broth sterility control) should be clear while tube 11 (positive inoculum control) should be turbid.

2. Record the colony count. The colony count plate should have 75-150 colonies. If the colony count is <75 or >150 consult the charge technologist before reading the tubes.

3. The highest dilution of serum that completely inhibits visible growth represents the bacteriostatic titre.

Determination of serum bactericidal titre

1. Vortex all tubes without visible growth for 15 seconds.

2. Use a urine loop to subculture all of the clear tubes onto 1/4 BA. Incubate at 37°C for 18 hours.

3. After incubation, read the plates and record the colony count.

4. The first dilution showing 99.9% killing activity (ie. no growth on sub-culture) is reported as the serum bactericidal titre.

V. Reporting Results

Telephone all results when available. Report as follows and give a copy of the report to the microbiologist:

Pre-dose serum bacteriostatic titre -
Pre-dose serum bactericidal titre -

Post-dose serum bacteriostatic titre -
Post-dose serum bactericidal titre -

VI. Reference

National Committee for Clinical Laboratory Standards. Methodology for the Serum Bactericidal Test, NCCLS Document M21-P,Vol. 7, No. 1, 1987.

APPENDIX VIII - BROTH MACRODILUTION AND AGAR DILUTION

I. Introduction

These tests are not routinely done and will only be performed following consultation with a microbiologist. Refer to the NCCLS standard M7-A6 (January 2003) for methodology.

APPEDIX IX - BROTH MICRODILUTION MIC

I. Introduction

Dilution susceptibility testing methods are used to determine the minimal concentration of an antimicrobial agent required to inhibit or kill a microorganism. Antimicrobial agents are usually tested at $\log_2$ (twofold) serial dilutions, and the lowest concentration that inhibits visible growth of an organism is regarded as the MIC. The concentration range used may vary with the drug, the organism tested, and the site of infection. The method and principles of the microdilution method is essentially the same as the macrodilution method except that the antimicrobial dilutions are in 0.1 ml volumes contained in wells of a microdilution tray (usually 96 well trays). Results obtained may be reported as the actual MIC or categorically as Susceptible (S), Intermediate (I), or Resistant (R). Interpretive categories are published and up-dated regularly by NCCLS.

II. Materials

Sterile saline
Transfer pipettes
Sterile distilled water
Vitek colorimeter
MIC microtitre panel
Inoculator (tray and lid)

III. Procedure

1. Prepare a suspension of the test organism in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies.

2. Transfer l.5 mL of the suspension to the bottom of the inoculating tray and add approximately 40 mL of sterile distilled water.

3. Aseptically replace the transfer lid into the inoculating tray making sure no bubbles are under the prongs.

4. Lift the transfer lid and center it over the previously thawed MIC panel.

5. Lower the transfer lid into the panel so the prongs enter all wells except the control well.

6. Remove transfer lid and cover the panel with a dummy MIC panel.

7. Incubate for 18-24 hours at 35°C, depending on drugs/bugs tested.

IV. Interpretation

The highest dilution of the Antimicrobial that completely inhibits visible growth represents the minimum inhibitory concentration (MIC).

V. Quality Control

Panels are Quality Controlled with the appropriate ATCC control organisms.

VI. Reference

Clinical and Laboratory Standards Institute (CLSI) Document – Methods for Dilution Antimicrobial Susceptibility Testing M7-A6, 2003.

APPENDIX X - E-TEST

I. Introduction

The E test (also known as the Gradinet Diffusion Method) is based on the same principle as the disk diffusion method. It is an in vitro method for quantitative antimicrobial susceptibility testing whereby a preformed antimicrobial gradient from a plastic-coated strip diffuses into an agar medium inoculated with the test organism. The MIC is read directly from a scale on the top of the strip at a point where the ellipse of organism growth inhibition intercepts the strip.

II. Materials

E-test strips (store frozen)
Mueller Hinton Agar (MH)
Mueller Hinton Blood Agar (MHBA)
Haemophilus Test Media (HTM)
Trypticase Soy Broth (TSB) (3 mL)
VITEK colorimeter
Sterile saline
Sterile swabs

III. Procedures

1. Allow E-test strips to come to room temperature before opening the container.

2. Using the Vitek colorimeter, prepare a suspension of the test organism in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies. If there is not enough growth, inoculate the organism into TSB, and incubate at 35°C for 2-4 hours or until it reaches the turbidity of a 0.5 McFarland standard.
   For mucoid organisms, adjust suspension to 1 McFarland standard.

3. Using a sterile cotton swab, inoculate the organism onto an appropriate agar plate, streaking in 3 directions over the entire agar surface. For organisms that grow rapidly use MH agar. For *Haemophilus* species use HTM and for *S. pneumoniae* and viridans streptococci use MHBA. For other organisms that do not grow on MH, use MHBA.

4. Using forceps, apply the appropriate Antimicrobial strips onto the agar. Use one plate per Antimicrobial strip.

5. Incubate plates as follows:

   Haemophilus species - CO_2, 35°C x 18 hours
   Streptococcus pneumoniae - CO_2, 35°C x 20 to 24 hours
   Staphylococcus aureus and *Enterococcus* species for Methicillin and Vancomycin - O_2, 35°C x 24 hours
   Others - O_2, 35°C x 18 hours

IV. Interpretation

After incubation read the MIC value at the point of intersection between the zone edge and the E-test strip. Since E-test comprises a continuous gradient, MIC values in between two-fold dilutions can be obtained. Always round up these values to the next two-fold dilution before interpretation.

See e-test Reading Guide

For polymyxin B see etest Polymyxin B Reading

V. Quality Control

The 3 E-test strips are tested once weekly with *S. aureus* ATCC 29213. The organism is sub-cultured from the TSB slant (in fridge) to BA the day before setting up the QC.

Expected Results*:

	MIC
Penicillin	0.25-2.0 µg/mL
Ceftazidime	4.0-16.0 µg/mL
Ceftriaxone	1.0-8.0 µg/mL
Cefotaxime	1.0-4.0 ?g/mL

? As per NCCLS document M100-S15 (M7), Table 3, January 2005.

VI. Reference

APPENDIX XI - VANCOMYCIN & HIGH LEVEL AMINOGLYCOSIDE TESTING

I. Introduction

Synergy between ampicillin, pencillin or vancomycin and an aminoglycoside for *Enterococcus* species can be predicted by high level aminoglycoside (HLAR - gentamicin and streptomycin) screening test. Vancomycin resistance of *Enterococcus* species can be detected by BHI vancomycin agar screen plate containing 6mg/L of vancomycin.

II. Materials

Control plate (Brain Heart Infusion Agar)
Entero HLAR and Vancomycin Screen plates
VITEK colorimeter
Sterile saline
Sterile swabs

III. Procedure

1. Using the VITEK colorimeter, prepare a 0.5 McFarland suspension in sterile saline (inoculum from VITEK can be used).

2. Using a sterile swab, spot inoculate the suspension onto each of the test and control plates.

3. After the inocula have dried, incubate the plate at 35^{o}C for up to 48 hours.

IV. Interpretation

Check the control plate for adequate growth. Then check the drug plates for absence or presence of growth; any growth is considered significant. Read plates at 24 hours and record results. If there is no growth on the drug plates, re-incubate plate for an additional 24 hours.

V. Quality Control

Control strains are tested with each plate.

	Expected results of each quadrant			
	C	**G**	**S**	**V**
E. faecalis (ATCC 19966)	+	+	-	-
E. faecalis (ATCC 49533)	+	-	+	-
E. gallinarum (ATCC 35038)	+	-	-	+
E. casseliflavus (ATCC 12755)	+	-	+	+
S. aureus (ATCC 29213)	+			-

C = Growth Control; G = Gentamicin; S = Streptomycin; V = Vancomycin

VI. Reporting Results

Blood cultures and sterile sites:

? If high level gentamicin is **susceptible** (regardless of streptomycin result) report as:
  - ✍ "**Serious enterococcal infections may require an aminoglycoside for synergy. Please contact the Medical Microbiologist for treatment advice".**

? If high level gentamicin is **resistant** (regardless of streptomycin result) report as:
  - ✍ **"This organism is high level aminoglycoside resistant. Please contact the Medical Microbiologist for treatment advice".**

? Record the streptomycin result in the LIS. Report result only upon request.

Urines and other sites:

? Do not report HLAR.
? Report Vancomycin result as per specimen type specific reporting tables.

VII. Reference

PML Technical Manual data sheet No. 323, Nov. 1993.

APPENDIX XII - VANCOMYCIN SCREEN PLATE FOR STAPHYLOCOCCUS

I. Introduction

This is an agar dilution method using a single concentration of Vancomycin (6 µg/mL) incorporated into Brain Heart Infusion (BHI) agar to screen for resistant strains of *Staphylococcus aureus*.

II. Materials

Control plate (Brain Heart Infusion Agar)
Screen plate (Brain Heart Infusion Agar with 6 µg/mL Vancomycin)
VITEK colorimeter
Sterile saline
Sterile swabs

III. Procedure

1. Using the VITEK colorimeter, prepare a 0.5 McFarland suspension in sterile saline (inoculum from VITEK can be used).

2. Using a sterile swab, spot inoculate the suspension onto each of the test and control plates.

3. After the inocula have dried, incubate the plate at 35°C in O_2 for 24 hours.

4. All resistant isolates on the screen plate must be checked for purity (e.g. Gram stain, tube coagulase or slide agglutination and sub-culture). The resistance must be confirmed by repeating the screen plate and setting up e-test (Refer to APPENDIX X - E-TEST). Report preliminary results to infection control.

IV. Interpretation

Check the control plate for adequate growth. Then check the drug plates for absence or presence of growth; any growth is considered significant. Read plates at 24 hours and record results. Note: There must be growth on the control plate.

V. Quality Control

Control strains are tested on every plate.

	C	V
E. faecalis (ATCC 19966)	+	-
E. faecalis (ATCC 49533)	+	-
E. gallinarum (ATCC 35038)	+	+
E. casseliflavus (ATCC 12755)	+	+
S. aureus (ATCC 29213)	+	-

C = Growth Control; V = Vancomycin

VI. Reference

bioMerieux procedure update May 28, 2004

APPENDIX XIII – Antimicrobial Abbreviations

Abbrevaitions - Antimicrobial Disks

ANTIMICROBIAL	DISK (Manufacturer)	Concentration (?g)
Amikacin	AK (Oxoid)	30
Amoxacillin/Clavulanic Acid	AMC	30
Ampicillin	AMP (Oxoid)	10
Aztreonam	ATM	30
Cefazolin	KZ (Oxoid)	30
Cefepime	FEP	30
Cefixime	CFM	5
Cefotaxime	CTX	30
Cefotetan	CTT (Gen. Diag.)	30
Cefoxitin	FOX (Oxoid)	30
Ceftazidime	CAZ (Oxoid)	30
Ceftriaxone	CRO (Oxoid)	30
Cefuroxime	CXM	30
Cephalothin	KF	30
Cefpodoxime	CPD	10
Chloramphenicol	C	30
Ciprofloxacin	CIP (Oxoid)	5
Clarithromycin	CLR	15
Clindamycin	DA (Oxoid)	2
Colistin	CT	10
Cotrimoxazole	SXT (Oxoid)	
Erythromycin	E (Oxoid)	15
Fusidic Acid	FD	10
Gentamicin	CN (Oxoid)	10
Imipenem	IPM (Difco)	10
Levofloxacin	LVX	5
Meropenem	MEM	10
Metronidazole	MTZ (Oxoid)	
Minocycline	MH	30
Mupirocin	MUP	5
Nalidixic Acid	NA	30
Nitrofurantoin	F (Oxoid)	300
Norfloxacin	NOR (BBL or Difco)	10
Novobiocin	NV	5

ANTIMICROBIAL	**DISK (Manufacturer)**	**Concentration (?g)**
Oxacillin	OX (Oxoid)	1
Penicillin	P (Oxoid)	10
Piperacillin	PRL (Oxoid)	100
Pipercillin/Tazobactam	TZP	110
Rifampin	RA	5
Teicoplanin	TE	30
Tetracycline	TE (Oxoid)	30
Timentin	TIM	85
Tobramycin	TOB (Oxoid)	10
Trimethoprim/Sulfamethoxazole	SXT	
Vancomycin	VA (Oxoid)	30

Abbreviations – E-Test Strips

ANTIMICROBIAL	ABBREVIATION	CONCENTRATION (?g)
Amikacin	AK	30
Ampicillin	AM	30
Azithromycin	AZ	30
Cefotaxime	CT	100
Cefotetan	CN	100
Cefoxitin	FX	100
Ceftazidime	TZ	30
Ceftriaxone (For CHC Pneumo)	TX	30
Cefuroxime	XM	30
Cephalotin	CE	30
Chloremphenicol	CL	30
Ciprofloxacin	CI	30
Clarithromycin	CH	30
Clindamycin	CM	100
Colistin	CO	
Doxycycline	DC	30
Erythromycin	EM	30
Fusidic Acid		30
Gentamycin (Low Level)	GM	30
High Level Gentamicin	GM	30
Imipenem	IP	30
Metronidazole	MZ	100
Mupirocin	MU	100
Oxacillin	OX	30
Penicillin	PG	100
Pipercillin	PP	100
Pipercillin/Tazobactam	PTC	30
Polymyxin B	PO	
Streptomycin (High Level)	SM	30
Teicoplanin		30
Tetracycline		30
Vancomycin	VA	100

Abbreviations – LIS (Soft Computer Corporation)

ANTIMICROBIAL	ABBREVIATION
Amikacin	an
Amoxicillin	amx
Amoxicillin / Clavalanic Acid	amc
Ampicillin	am
Ampicillin / Sulbactam	ama
Azithromicin	azi
Aztreonam	azm
?eta-lactamase	blac
?-lactamase	beta
Carbenicillin	cb
Cefaclor	ccl
Cefamandole	cm
Cefazolin	cz
Cefipime	cpo
Cefixime	cfm
Cefotaxime	tax
Cefotetan	cte
Cefoxitin	fox
Cefpodoxime	cpd
Cefpodoxime	cpod
Cefpodoxime / Clavulanic Acid	cpodc
Ceftazidime	taz
Ceftizoxime	zox
Ceftriaxone	ctr
Cefuroxime	roxh
Cefuroxime – Axetil	roxa
Cefuroxime-sodium	rox
Cephalothin	cf
Chloramphenicol	C
Ciprofloxacin	cip
Clarifloxacin	clar
Clarithromycin	cla
Clinafloxacin	cflox
Clindamycin	cc
Cloxacillin	clx
Colistin	ct
Dalfopristin	dalfo
Doxycycline	dx
D-zone	dzone

ANTIMICROBIAL	ABBREVIATION
Erythromycin	e
ESBL Potentiation	esbinh
Everninomycin	ever
Fusidic Acid	fa
Gatifloxacin	gat
Gentamicin	gm
Gentamicin 2000	gm2000
Gentamicin 500	gm500
Imipenem	imi
Kanamycin	k
Levofloxacin	lev
Linezonomycin	linezo
Meropenem	mem
Metromidazole	mtz
Mezlocillin	mz
Minocycline	mn
Mupirocin	mup
Nalidixic Acid	na
Netilmicin	net
Nitrofurantoin	fd
Norfloxacin	nor
Ofloxacin	ofx
Oxacillin	ox
Penicillin	peng
Piperacillin	pip
Pipercillin / Tazobactam	tzp
Polymyxin B	pb
Pristinamycin	pris
Ramoplanin	ramo
Rifampin	rif
Streptomycin	strep
Streptomycin 2000	st2000
Sulfisoxazole	sox
Synercid	suncd
Teicoplanin	tei
Tetracycline	tet
Ticarcillin	tic
Ticarcillin/Clavulanic Acid	tcc

ANTIMICROBIAL	ABBREVIATION
Tobramycin	tob
Trimethoprim	tmp
Trimothoprim/sulfamethoxazole	sxt
Vancomycin	va

OXACILLIN AND VANCOMYCIN SCREEN RECORDING SHEET FOR *S. aureus*

Policy # MI\ANTI\04\03a\v03

Date: _____ Set-up by: _____ Lot# (OXA_____ (VANC)_____ Read by: 18h_____ 24h_____

#	ATCC Control Or Lab No.	OXA 18 h 24	**VANC** 18 h 24		#	Lab No.	OXA 18 h 24		**VANC** 18 h 24		#	Lab No.	OXA 18 h 24		**VANC** 18 h 24	
1	OXA: 8610 VANC: 49532	R	S		14						27					
2	OXA: 43300 VANC: 49573	R	R		15						28					
3	OXA: 43387 VANC: 49533	S	S		16						29					
4	OXA: 29213 VANC: 29213	S	S		17						30					
5					18						31					
6					19						32					
7					20						33					
8					21						34					
9					22						35					
10					23						36					
11					24						37					
12					25											
13					26											

QUAD SCREEN RECORDING SHEET FOR ENTEROCOCCUS and CNST

Policy# MI\ANTI\04\08a\v03

Date: _____ Set-up by: _____ Lot# (vanc) : _____ (HLA) _____ Read by: 18h_____ 24h_____ 48h_____

#	Lab No.	VA 18 h 24		GM 24h	**STR** 24 h 48		#	Lab No.	VA 18 h 24		GM 24h	**STR** 24 h 48	#	Lab No.	VA 18 h 24		GM 24h	**STR** 24 h 48	
1	*E. faecalis* ATCC 49532	S		R	S		14						27							
2	*E. gallinarum* ATCC 49573	R		S	S		15						28							
3	*E. faecalis* ATCC 49533	S		S	R		16						29							
4	*S .aureus* ATCC 29213	S		--	–		17						30							
5							18						31							
6							19						32							
7							20						33							
8							21						34							
9							22						35							
10							23						36							
11							24						37							
12							25						38							
13							26						39							

APPENDIX XIV - VITEK

I. Introduction

The VITEK System is an automated, short-incubation micro broth dilution system capable of performing susceptibility testing of most rapidly growing gram-positive and gram-negative aerobic bacteria. It can yield a result in a period of 4 to 10 hours. Results are automatically transferred from the VITEK System to the Laboratory Information System (LIS) via a computer interface. Results are reported qualitatively as either Susceptible (S), Intermediate (I), or Resistant (R).

II. Materials

Cards (stored refrigerated):

GNI --- Gram negative identification card
GPI --- Gram positive identification card
GNS – 606 --- Gram negative susceptibility card
GPS –105 --- Gram positive susceptibility card

1.8 mL 0.45% sterile saline
VITEK colorimeter
MLA pipette
Pipette tips
Transfer tubes
Filling Stand

III. Procedure

A. Preparation of Inoculum:

Use fresh 24 hours culture.

If insufficient growth for setting up inoculum, sub-culture isolate onto a Blood Agar (BA) plate, incubate for a few hours and set up from a sweep of the growth.

Sub-culture frozen or freeze dried isolates twice before setting up any Vitek card.

Gram Negative (GNI / GNS)
Organism ? 1.8 mL saline (1 McFarland) – 50 ?L ? 1.8 mL saline
GNI **GNS**

Gram Positive (GPI / GPS)
Organism ? 1.8 mL saline (0.5 McFarland) – 200 ?L ? 1.8 mL saline
GPI **GPS**

Vortex suspensions for ID card inoculations
Inoculate cards within 15 minutes.

B. Card Inoculation:

1. Allow card to reach room temperature.
2. Remove protective package liner, visually inspect for holes or cracks in the foil material.
3. Label card with **Last 6 digits of Lab # + isolate #**. Isolate # should be identical in corresponding ID and susceptibility cards.
4. Mark **external tests marks** (positives) on appropriate cards:
   oxidase/catalase
   beta hemolysis/coagulase
   Corresponding ID and susceptibility cards should have identical external tests marked.
5. Insert transfer tube into inlet port.
6. Place inoculated test tube in filling stand.
7. Place assembled card and transfer tube on filling stand with the long portion of the transfer tube into the test tube.
8. Place filling stand with the assembled test kit into filling module.
9. Press '**FILL**' button **once**. 'READY' light will go out after a few moments.
10. When the 'READY' light returns, remove filling stand from filling module.
11. Cut transfer tube and seal card in heater/sealer module.
12. Check card for bubbles.
13. For ID Cards, remove bubbles by shaking.
14. For sensitivity cards, DO NOT SHAKE. Small bubbles will not cause problems. If very large bubbles are present, discard the card and repeat.
15. Insert card into tray.
16. **Put filled card into reader/incubator within 15 minutes.**
15. Check time and location of the next tray to be read by:
    a) click on "**VITEK**" in main menu
    b) click on "**READER**"
    c) click on "**STATUS**"
    Open reader/incubator.

Put new card into tray following 'next tray to be read'. Example: Next tray to be read: tray a put new card into tray b. (If b is full, trays c or d can also be used).

To Exit "STATUS" WINDOW:
a) click on '**FILE**"
b) click on '**QUIT**"

NHI, ANI CARDS:

After inoculation, incubate in non-CO_2 incubator for 4 hours.

At amscommand, type **'nhi'** (not capitals)
Enter ID #
Catalase
MTM
Indole
Results of card per NIH insert

C. Quality Control:

QC all new lots of **identification cards** once when received in the lab. No further QC is required if all results are within limits. Perform QCs for 5 consecutive days if out-of-control results are observed (see QA technologist).

QC all new lots of **susceptibility cards** once when received and once weekly when lot is in use. Perform daily QCs when any out-of-control results are observed (see QA technologist).

See VITEK manual for QC organisms to be set up and set up procedure.