PURPOSE:

To provide susceptibility results on organisms that grow poorly or not at all by MIC.

TEST BY KIRBY-BAUER DISK DIFFUSION:
1. Organisms that fail to grow or grow very poorly in MIC panels. Note: When KB testing is needed on Staphylococci that fail to grow for MICs, send the isolate to Quest.
2. Special request drugs.
3. Haemophilus influenzae or Haemophilus parainfluenzae from sterile sources. Other Haemophilus species will be sent to a reference lab for testing.
4. Stenotrophomonas maltophilia isolates from clinical specimens.

SCOPE: This policy applies to UPMC Hanover

EQUIPMENT NEEDED:

The assay agar medium for most species is Mueller-Hinton agar, pH 7.2-7.4, 60 ml per 15 x 150 mm plate. Appropriate media is indicated below in the instructions for specific susceptibility test panels.
1. Sterile cotton tipped swabs.
2. 5 ml TSB or saline (Haemophilus species)
3. 0.5 McFarland standard with turbidity meter
4. Appropriate media
5. Antibiotic disks stored in refrigerators 12 and 1.
6. Clear ruler or caliper (for reading zone sizes)
7. Materials for disposal of used supplies

SPECIMEN:

Direct Colony Suspension: Several colonies from a fresh 18-24 hour non-selective agar plate to saline or broth.

QUALITY CONTROL:

A. Frequency: For all media, perform upon receipt and each day of patient testing. For all discs, test upon initial use and with each day of patient testing.
B. Record KB QC testing on the worksheets in the "KB" notebook. Ensure all disks and media are within acceptable expiration dates before use. Do not use media/drugs that will expire during the incubation period. Check the dates on all the test drugs before inoculation of media.

PROCEDURE:

1. Touch the tops of at least 3 to 5 colonies of morphologically similar, well-isolated colonies from an 18-24 hour non-inhibitory agar plate using a sterile swab. This avoids testing a mutant colony. Avoid touching the agar. Transfer the inoculum to 5 mL tryptic soy broth (TSB) for aerobic or facultatively anaerobic bacterial species. Inoculum for HTM should be prepared in sterile saline.
2. Using the same swab inoculate a purity plate labeled with the specimen number, date, time set, organism code name and KB. Return the purity plate to bench where order originated. Incubate the purity plate at 35^0 C for 18 to 24 hours in 5-10% CO2, or as otherwise defined for the growth of the organism.
3. Adjust the inoculum of the TSB to match a 0.5 McFarland standard. Vortex thoroughly and proceed within 15 minutes with direct plate inoculation. The correct inoculum at this point contains approximately 1 to 2x 10^8 CFU per ml.
4. A sterile swab is inserted into the inoculated broth and rolled against the tube side as it is withdrawn to remove excess culture. Streak the plate from top to bottom with the swab and side to side across the entire surface of the agar. Turn the plate 60^0 , re-streak, turn 60^0 , and re-streak the entire surface a third time. Finally, run the swab around the periphery of the agar where it meets the side of the dish. Discard the swab into a fresh disinfectant solution.
5. Allow the inoculum to dry for 3-5 minutes but no more than 15 minutes. Do not put the disks on the plates until drying is complete. A disk dispenser evenly distributes the disks, which are then automatically or manually pressed down gently to ensure good contact with the agar surface.
6. Do not report sulfa or trimethoprim drug disks on a blood containing medium, except for S. pneumoniae. Blood contains thymine or thymidine, which reverses the action of these drugs and may lead to false reports of resistance.
7. Invert the plates and incubate them at 35^0 C within 15 minutes after adding the disks. (See directions below for atmospheric conditions for each organism.)

8. After the appropriate period of incubation, examine each plate. If the plate was satisfactorily streaked, and the inoculum concentration was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum concentration was too light and the test must be repeated.
9. Hold the plate a few inches above a black non-reflecting background illuminated with reflected light and with the cover removed.
10. When blood-supplemented media is used, measure the zones of growth inhibition (not the zones of inhibition of hemolysis). Measure the diameters of zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Measure the zones to the nearest whole millimeter, using sliding calipers or a ruler.
11. The endpoint is the widest zone of complete inhibition of growth. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye.

Exceptions:

A. Do not record or report findings from a contaminated assay plate. Different species may stimulate or inhibit the growth of others, leading to false determinations of resistance or sensitivity.

B. SXT: Bacterial species may reproduce for several generations before being inhibited by sulfonamides or trimethoprim sulfa. For trimethoprim-sulfamethoxazole, disregard slight growth (80% or more inhibition) and measure the margin of heavy growth to determine the zone diameter. (Exception = S. pneumoniae)

C. Proteus: The margin of heavy growth of a swarming Proteus is measured and the inner veil of swarming is disregarded.

12. Record growth up to the disk (no zone of inhibition) as 6 mm.
13. Report each drug response as "susceptible", "intermediate", or "resistant".

NOTES:

1. Nitrofurantoin results should be obtained or reported only for urinary tract infections.
2. Common sources of error:
   A. clerical error in transcribing zone diameters or interpretations.
   B. reader error in measuring zone diameters.
   C. contamination of the inoculum.
   D. an inoculum which is too concentrated or too dilute.
   E. failure to thoroughly mix the 0.5 McFarland standard prior to use.
   F. loss of antibiotic disk potency.

3. A (+) beta-lactamase result indicates resistance to penicillins (ampicillin and penicillin), regardless of zone size around a penicillin or ampicillin disk.

KB drugs included in susceptibility test panels:

1. Gram (+) KB Enterococci:

Preferred test method is MIC. For isolates that fail to grow in the MIC panel test by KB; order panel as KB Pos.

QC- SA 25923

Media- plain MH agar Atmosphere- 35°± 2 in ambient air for 24 hours

Report antibiotics marked with X, per source (U=urine source, S=systemic)

Drug	Code	Ent. species (S)	VA-R Ent. species (S,U)	Ent. species (U)
Ampicillin	AM	X	X	X
Daptomycin^*	DAPTO	X	X	X
Nitrofurantoin	FD		X**	X
Tetracycline	TE		X**	X
Vancomycin	V	X	X	X
Linezolid	LZD		X	

^KB by E-test *No respiratory specimens **Urine only

Note: No CLSI guidelines exist for susceptibilities of Enterococcus sp. tested by Blood KB. Do not use the Blood KB method for poorly growing Enterococcus sp.

CLSI Zone Size Interpretations-Enterococcus species

Drug	Code	S	SDD	I	R
Ampicillin	AM	≥17	-	-	≤16

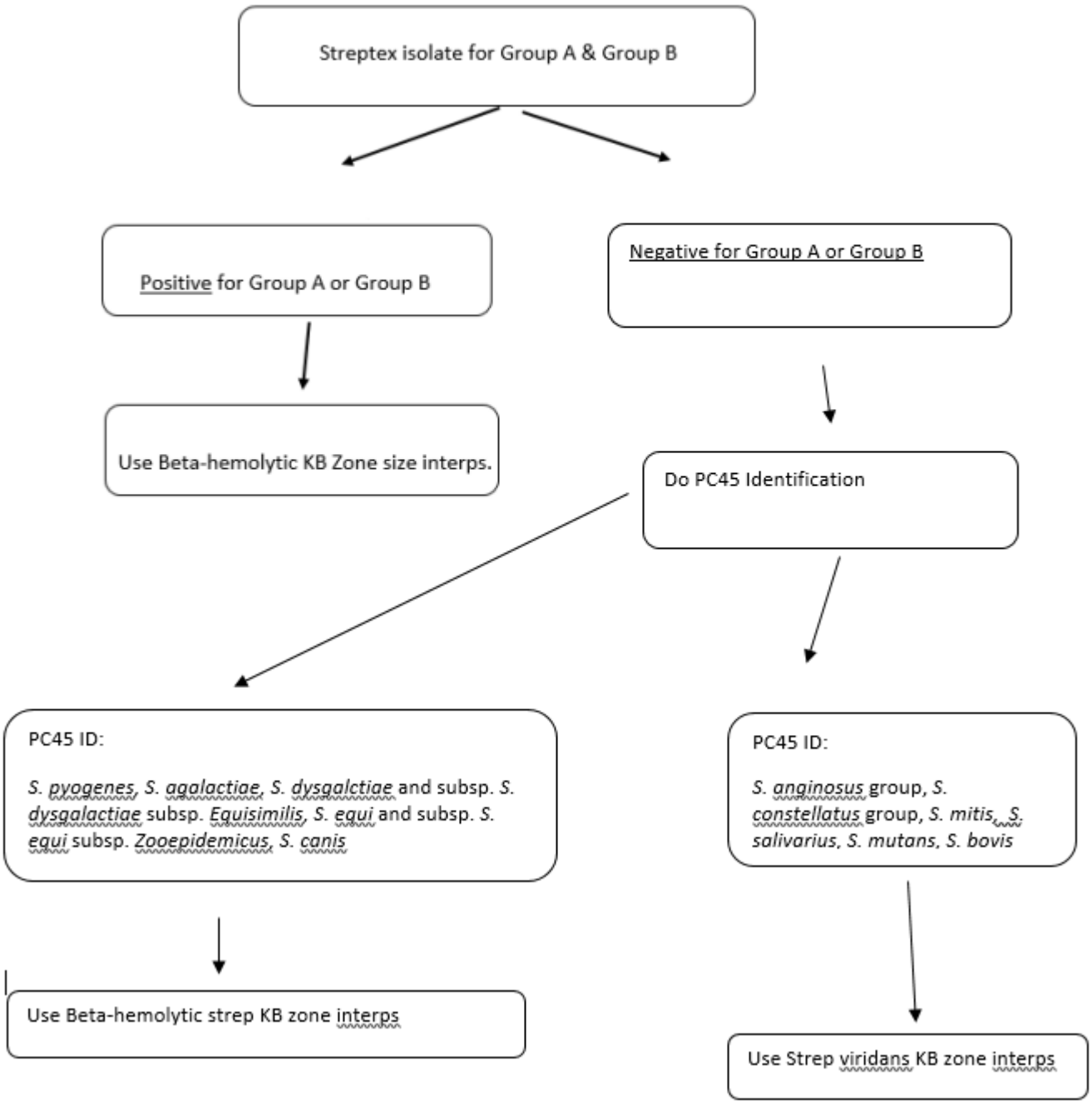
Daptomycin *E. faecalis* *E. faecium*	DAPTO	 ≤2 -	 - ≤4	 4 -	 ≥8 ≥8
Nitrofurantoin	FD	>17	-	15-16	≤14
Tetracycline	TE	≥19	-	15-18	≤14
Vancomycin	V	>17	-	15-16	≤14
Linezolid	LZD	≥23	-	21-22	≤20

2. Gram (+) KB Beta and Viridans strep:

Preferred test method is MIC. For isolates that fail to grow in the MIC panel test by KB. Order panel as KB Strep. Use Beta Strep zone sizes and interpretations for Strep pyogenes (Group A), Strep agalactiae (Group B), Strep dysgalactiae, S. equi, and S. canis (including their sub-species). For all minute colony beta streps, perform PC45 for ID if clinically important source, use zone sizes and interpretations for viridans strep. Refer to algorithm below.

Identification and KB guidelines for Beta-hemolytic Streptococcus species from

Clinically Significant Sources



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INTERPRETATION:

QC-STRPNE 49619
MEDIA- Blood MH agar Atmosphere-35°± 2 in 5%CO2 for 20-24 hours
Report antibiotics marked with X: U=urine source, S=systemic source

Drug	Code	BES-U	BES-S	BES-Prenatal*	STRVIR
Ampicillin	AM	X			
Cefotaxime	CTX		X		
Ceftriaxone	CAX	X	X		X
Clindamycin	CD			X	
Erythromycin	E			D-test only	
Penicillin	P		X	X	E-test only
Vancomycin	V	X	X	X	X

*Only used for prenatal prophylaxis on Group B Strep screens and urines of pregnant women when Penicillin-allergic.

CLSI Zone Size Interpretations-Viridans Strep

Drug	Code	S	I	R
Ceftriaxone	CAX	≥27	25-26	≤24
Penicillin*	P			
Vancomycin	V	≥17		

*No CLSI guidelines for P on this organism group by KB method. Do E-test.

CLSI Zone Size Interpretations-Beta Strep

Drug	Code	S	I	R
Ampicillin	AM	≥24		
Ceftriaxone	CAX	≥24		
Clindamycin	CD	≥19	16-18	≤15
Erythromycin	E	≥21	16-20	≤15

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Penicillin	P	≥24		
Vancomycin	V	≥17		

Providing weekly QC is within range, interpret the diameters of the zones of inhibition according to the current CLSI Performance Standards for Antimicrobial Susceptibility Testing. Results will be recorded as "S, I, or R" according to the measuements.

METHOD LIMITATIONS:

- Place no more than 12 disks on a 150-mm plate.
- Place no more than 5 disks on a 100-mm plate.
- Some organisms may not be reported from Kirby-Bauer sensitivities, as they only have values for MICs. See current Performance Standards for those organisms acceptable for KB reporting.
- Except for colonies of MRSA or VRE, always disregard minute colonies visible only by transmitted light or by examination only under a magnifying device.
- Disregard swarming *Proteus* species, and measure edge of the obvious inhibition under the veil of swarming.
- When measuring zone sizes of sulfonamides, trimethoprim or trimethoprim-sulfamethoxazole, disregard light growth (read for 80% or more inhibition), and measure the edges of heavy growth.
- Large colonies growing within the inhibition zone may represent a mixed culture or resistant variants. Subculture these, identify them, and retest for

susceptibility. If repeat testing gives the same results (and culture is confirmed to be pure), or a zone indicating resistance, report as resistant.

- Blood components (5% sheep blood in BAP plates), contain thymidine. Thymidine acts as an antagonist and results in decreased activities of sulfonamides, trimethoprim, and trimethoprim-sulfamethoxazole, try not to test these drugs on Mueller-Hinton agar supplemented with 5% Sheep blood.
- Sometimes unsupplemented MH agar contains enough thymidine to allow the organism to grow several generations before inhibition of sulfonamides and.or trimethoprim. Disregard the resulting light haze, and read for 80% or more inhibition.

REFERENCES:

Isenberg, Henry D., Clinical Microbiology Procedures Handbook, American Society for Microbiology, 1325 Massachusetts Ave., N.W., Washington, D.C. 20005, Section 5.1.5 thru 5.1.11.1992.

Performance Standards for Antimicrobial Susceptibility Testing, M100, 29th Edition. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA 19087. USA. www.clsi.org.

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