STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: Kirby Bauer Testing	**Revision Date:** 20-April-2018	**Issue Date:** 20-April-2016
Document Number: MIC51000	**Status: Approved**	
Distribution: Microbiology Test Manual	**Page:** 1 of 6	
Approved by: S. Asmussen, Manager of Diagnostic Services	**Signed by:** 	

PURPOSE:

Anti-microbial disk diffusion testing is a technique used to determine the *in vitro* susceptibility of bacteria that grow aerobically to certain antimicrobial agents. Agar disk diffusion is used to test many common, rapidly growing, non-fastidious and certain fastidious organisms. Disk diffusion tests use the principle of standardized methodology and zone diameter measurements correlated with minimal inhibitory concentrations (MICs) with strains known to be susceptible or resistant to various antimicrobial agents

SAMPLE INFORMATION:

Type	Well isolated colonies, 18-24hours old

REAGENTS and/or MEDIA:

- Mueller-Hinton Agar: Oxoid – store at 8°C
- Mueller-Hinton Agar with Blood – store at 8°C
- Haemophilus Test Media – store at 8°C
- Antimicrobial disks – store at -20°C

SUPPLIES:

- Wooden applicator sticks
- DensichekPlus
- Cotton-tipped swabs
- Sterile 0.45% saline

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

QUALITY CONTROL:

- Weekly KB testing is performed – see Procedure **MIC60300 - Weekly KB and ETEST QC**

PROCEDURE INSTRUCTIONS:

Step	Action
Performing Kirby Bauer Disk Diffusion Testing	
1	Consult Dynalife AST Manual under the organism section for the appropriate antibiotics, media, and McFarland standard required **NOTE:** Most organisms are set up at a 0.5 McFarland standard with the exception of mucoid strains – consult the Dynalife AST Manual
2	Dispense sterile saline into a plastic test tube
3	Ensure that the daily/monthly maintenance has been completed on the DensichekPlus – See Procedure **MIC70100 DenischekPlus Use and Maintenance**

4	Using a wooden applicator stick, select 2-3 isolated colonies from an 18-24 hours old culture plate
5	Add a test tube cap and vortex for 2-3 seconds
6	Insert saline suspension into the DensichekPlus and spin 360°
7	The desired saline suspension should read at a turbidity between 0.50-0.62 McFarland Adjust the saline suspension accordingly
8	Once the desired turbidity is obtained, the saline suspension should be used within 15minutes
9	With a cotton tipped applicator, dip into the suspension.
10	Rotate the swab several times and press it firmly on the inside wall of the tube above the fluid level to remove excess fluid
11	Inoculate the surface of the agar plate by streaking the swab over the entire surface
12	Turn the plate 60° and repeat a second time
13	Turn the plate 60° and repeat a third and final time ensuring that the entire surface of the plate is covered
14	Rim the edge of the plate with the swab and discard the swab in the AccelTB waste container
15	Allow the surface of the agar plate to dry for 3-5 minutes but no more than 15minutes
16	Dispense the appropriate disks onto the surface of the agar. No more than 5 disks are to be placed on the 100-mm plate*****. Ensure that the disks are not placed too close to the edge of the plate, as this can result in an unreadable zone.
17	Once the disk is placed on the agar it should not be moved, as the drug diffuses out almost immediately
18	All plates should be incubated within 15mins after the disks are applied *****Incubate Mueller Hinton Agar at 35°C in ambient air for 16-18 hours *****Incubate Mueller Hinton with Blood at 35°C in CO2 for 16-18 hours *****Incubate Haemophilus Test Medium at 35°C in CO2 for 16-18 hours

*** See Special Considerations**

SPECIAL CONSIDERATIONS:

Streptococcus pneumoniae	• Susceptibility testing is performed on Mueller Hinton with Blood agar • Use colonies taken from a 20-24 hour overnight subculture • Place no more than 4 disks on a 100-mm agar plate • Incubate the plates at 35°C in 5% CO_2 for 20-24 hours • See CLSI guidelines for interpretations
Beta streptococci	• Susceptibility testing is performed on Mueller Hinton with Blood agar • Incubate the plates at 35°C in 5% CO_2 for 16-18 hours • Inducible clindamycin resistance (ICR) is performed by placing the erythromycin and clindamycin disks 12mm away from each other. This test is also called the D-test • A Positive ICR is viewed by the flattening of the clindamycin zone adjacent to the erythromycin(called the D-zone)  See • Clinical and Laboratory Standards Institute (CLSI) guidelines for interpretations
Haemophilus spp	• Susceptibility testing is performed on Haemophilus Test Media • Use a colonies taken from a 20-24 hour subculture • Place no more than 4 disks on a 100-mm plate • Incubate the plates at 35°C in 5%CO_2 for 16-18 hours
Staphylococcus aureus Staphylococcus lugdenensis	• Susceptibility testing is performed on Mueller Hinton Agar • If indicated, perform Cefoxitin screening • See Procedure **MIC50600DiscDiffusion-**

	CefoxitinPRO.doc • See CLSI guidelines for interpretations

READING PLATES:

- Measure the diameters of the zone of complete inhibition judged by the unaided eye
- Measure the zones to the nearest millimeter
- Measure the zones with a ruler held to the back of the inverted Petri Plate. **Exception:** Mueller Hinton with Blood plates require reading the zones from the upper surface of the agar plate illuminated and the cover removed
- The zone margin is the area showing no obvious, visible growth detectable by the **unaided** eye
- Zone of growth should be measured for beta hemolytic Streptococci on Mueller Hinton with Blood plates NOT zone of hemolysis
- For trimethoprim and sulfonamides some slight growth may occur; measure the more obvious margin to determine the zone diameter
- See CLSI guidelines for specific zone diameter interpretive criteria

INTERPRETATION OF RESULTS:

IF	THEN
Confluent lawn of growth, distinct circular zone of inhibition	Plate was streaked correctly, density of inoculum was correct
Individual colonies visible	Density of inoculum was too light - Repeat
Discrete colonies growing in the zone of inhibition	Repeat testing with pure culture. If the repeat is the same, measure the colony-free inner zone

RELATED DOCUMENTS:

- MIC50600DiskDiffusion-CefoxitinPRO.doc
- MIC70100DenischekPlusPRO.doc
- MIC60300WeeklyKB/ETEST QCPRO.doc

REFERENCES:

- Clinical and Laboratory Standards Institute. (n.d.). Performance Standards for Antimicrobial Disk Susceptibility Tests Vol.32 No.1. pp. 1-27.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Dec13	Initial Release	Darrach (A)
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell