KCMC Biotechnology Laboratory, Microbiology	STANDARD OPERATING PROCEDURE	Effective Date	SOP-Number MIC.002
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Title: BacT/ALERT AND MycoFLYTIC BLOOD CULTURES FOR BACTERIA AND MYCOBACTERIA

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Annual Review	
By	Date

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Title: BacT/ALERT AND MycoFLYTIC BLOOD CULTURES FOR BACTERIA AND MYCOBACTERIA

PURPOSE

Procedures for processing and handling of BacT/ALERT and MycoFLYTIC blood cultures for bacteria, fungi and mycobacteria.

PRINCIPLE

Blood may be collected in any of the following blood culture bottles: BacT/ALERT SA (adult, bacteria), PF (pediatric, bacteria), MB (mycobacteria) and BD-MycoFLYTIC bottle (mycobacteria). On receipt in the laboratory a BLOOD CULTURE RESULT Teleform is completed on which all pertinent data related to the blood cultures are recorded. The bottles are weighed for volume determination and the original bottle weight (recorded on each bottle) and the filled weight are recorded on the teleform. The BacT/ALERT bottles are loaded into the BacT/ALERT 3D instrument and the date and time recorded. MycoFLYTIC bottles are incubated at 35^{o} C and examined daily under a Wood's (UV) lamp for fluorescent changes in the disk in the bottom of the bottle An aliquot is removed from BacT/ALERT bottles flagged as positive by the instrument or MycoFLYTIC bottles with fluorescent changes, for staining and subculture to appropriate media based on microscopic findings. Data related to the positive blood culture (e.g. date positive, time to positive) are entered on the BLOOD CULTURE RESULT Teleform. A POSITIVE CULTURE FORM is initiated for entering results of microscopic examinations, subsequent culture growth and organism identification test results. When the identity of the organism is known the organism code is entered on the BLOOD CULTURE RESULT Teleform.

CLINICAL APPLICATION

Bacteria may be present in blood in the following situations:

1. Clinical septicemia with invasion of the bloodstream from a focus of infection.
   a. Part of a chronic infection such as disseminated gonococcal disease.
2. "Spillover" from localized infections such as meningitis, pneumonia, UTIs and deep tissue abscesses.
3. Result of localized intravascular infection such as endocarditis and infected thrombi.
4. Multisystem infections such as enteric fever, leptospirosis, or brucellosis whose pathogenesis includes a bacteremic phase.
5. Sudden introduction of bacteria into the bloodstream by trauma to heavily contaminated external sources.
6. An asymptomatic, brief but rapidly clearing introduction of bacteria from minor traumatic events such as dental manipulation.
7. Contamination with skin bacteria acquired during collection.

In many cases blood cultures are the only immediate source of the etiologic age of severe or life threatening infections. Also, they may indicate the severity and extent of dissemination of an infection and therefore are among the most important cultures handled in the laboratory.

SCOPE

This Standard Operating Procedure applies to the processing of blood cultures collected in BacT/ALERT and MycoFLYTIC blood culture media for ISAAC studies 002 A&B and 003 and used by technical staff in the microbiology laboratory who have been trained in the use of the BacT/ALERT instrument, handling of MycoFLYTIC media, completion of data forms, stain and subculture methods.

PRECAUTIONS

Use standard precautions and appropriate protective equipment (gloves) when handling patient samples to protect against bloodborne pathogen exposure. Remove aliquots from positive blood culture bottles in the biohazard safety hood. Refer to SAF.001 BIOHAZARD SAFETY SOP**.**

BLOOD CULTURE COLLECTION/SPECIMEN RECEIPT

Refer to SOPs MIC.012 SPECIMEN COLLECTION ISAAC STUDIES, MIC.011 SPECIMEN RECEIPT AND HANDLING.

EQUIPMENT AND SUPPLIES

Equipment:

BacT/ALERT 3D Microbial Detection instrument
UV lamp
Slide warmer

Supplies:

BacT/ALERT Mycobacterial Enrichment Fluid
Alcohol wipes
Glass slide
Syringe with needle

Staining Reagents:

Gram stain
Kinyoun AFB stain
Acridine Orange stain

Media for subculturing positive vials (organism dependent):

Blood agar

Chocolate agar
MacConkey agar
Sabouraud Dextrose agar

Forms:

BLOOD CULTURE RESULT Teleform
POSITIVE CULTURE form
MycoFLYTIC EXAMINATION RECORD form

BLOOD CULTURE DATA RECORDING

1. Record patient and specimen collection information on a BLOOD CULTURE RESULT Teleform for each set of blood cultures received.
2. Weigh each bottle and record uninoculated weight (written on the bottle) and inoculated weight on the BLOOD CULTURE RESULT Teleform.

ADDITION OF ENRICHMENT FLUID TO MB BOTTLES

1. Add 1 ml Mycobacteria Enrichment Fluid to BacT/ALERT MB bottles as follows:
   a. Take blood culture bottle to biohazard hood.

      Caution: Perform this procedure in the biohazard safety hood. Wear appropriate personal protection for working with mycobacterial cultures.

   b. Wipe bottle septum with 70% alcohol and let dry.
   c. Using a syringe remove 1 ml of MB Enrichment fluid and inject into MB bottle.
   d. Discard syringe in sharps disposal container. **DO NOT** reuse syringe to add enrichment fluid to another bottle.

LOADING BOTTLES IN BacT/ALERT INSTRUMENT

1. Load bottles into BacT/ALERT instrument. Refer to SOP MIC.001OPERATION of BacT/ALERT MICROBIAL DETECTION SYSTEM.
2. Record Load Date and Time on BLOOD CULTURE RESULT Teleform.
3. File form in “Blood Culture Pending” folder.

INCUBATION TIME

BacT/ALERT bottles:

Routine Incubation:
Bacterial blood cultures (BacT/ALERT SA, PF) - 5 days.

Mycobacterial cultures (BacT/ALERT MB) – 42 days

MycoFLYTIC bottles:

Routine incubation – 42 days

EXAMINATION OF MycoFLYTIC BOTTLES

1. Examine bottles daily.
2. Remove bottles from incubator and take to Dark Room for examination under UV light.
3. Turn on the UV light and let it warm up for 2-3 minutes.
4. Invert the bottle and place the disk at the bottom under the light.
5. If any fluorescence is observed, consider it a positive bottle.
6. Record findings on MYCOFLYTIC EXAMINATION RECORD FORM.

POSITIVE BLOOD CULTURES

Follow instructions for unloading positive bottles in MIC.001 OPERATION OF BacT/ALERT 3D MICROBIAL DETECTION SYSTEM.

1. Record Date Positive and Time to Positive on BLOOD CULTURE RESULT Teleform.
2. Initiate a POSITIVE CULTURE FORM for recording results of microscopic examinations and culture data. Use separate form for each organism isolated.

EXAMINATION OF POSITIVE VIALS

NOTE: Wear gloves and work under the biosafety hood when removing aliquots from positive blood culture vials.

1. Wipe the top of the septum with 70% alcohol and let dry.
2. Withdraw 0.5 ml of blood with a syringe and place in a sterile test tube. Discard the syringe immediately into sharps box. DO NOT replace needle cap.
3. Transfer a small loopful of the aliquot to a clean glass slide.
4. Dry slide on slide warmer for 2 hours..
5. Stain with Gram's (SA/PF positive bottles) or Kinyoun AFB stains (MB bottles). Refer to MIC.021 GRAM STAIN PROCEDURE and MIC.004 KINYOUN STAIN PROCEDURE.
6. If Kinyoun stain on aliquot from MB bottle is negative, prepare a new slide and stain with Gram stain.

POSITIVE STAINS

1. Record smear results on the. POSITIVE CULTURE FORM. Save slides until workup is complete.
2. Place the vial in the rack labeled POSITIVE on the counter in the microbiology laboratory until growth on solid media is observed. Vials may be discarded once culture workup is completed.

NEGATIVE STAINS ON POSITIVE BOTTLES

1. Prepare another smear and stain with Acridine Orange (AO). Refer to MIC.024 ACRIDINE ORANGE STAIN PROCEDURE.
2. If AO is negative, subculture SA and PF bottles to Blood and Chocolate agars and MB bottles to Middlebrook agar and reload bottles in the BacT/ALERT instrument.
3. If bottle continues to be flagged positive without microscopic evidence or growth on subculture, consult supervisor for handling advice.
4. If bacteria are seen on Acridine Orange stain yet still cannot be found on GS consult supervisor.

NOTE: Organism that may be difficult to see on microscopic examinations include *Legionella, Campylobacter, Bartonella, Leptospira, Mycoplasma*..

SUBCULTURES OF POSITIVE VIALS

1. Subculture blood culture aliquot to appropriate media based on microscopic findings as directed in the table below.

BOTTLE	MORPHOLOGY	MEDIA (35° C)
BacT/ALERT SA/PF	Gram pos cocci- prs or chains	BAP[a] + optochin and bacitracin disks
	Gram pos cocci- clusters	BAP
	Gram pos bacilli	BAP
	Gram neg cocci	BAP, CHOC
	Gram neg bac - large	BAP, MAC
	Gram neg bac - small	BAP, CHOC, MAC
	Gram neg bac -curved	CHOC
	Gram neg bacilli, coccobacillary	BAP, CHOC, MAC
	Fungi (yeasts or molds)	SAB (Incubate at RT)
	No organisms observed	CHOC
BacT/ALERT MB, MycoFLYTIC	Acid-fast bacilli	Middlebrook 7H10 Agar, LJ
	Non-AFB	Perform Gram stain and proceed as above based on morphology.

[a] Abbreviations: BAP-Blood agar Plate, CHOC–Chocolate Agar, MAC- MacConkey Agar,
SAB – Sabouraud Dextrose Agar, RT – Room Temperature, LJ-Lowenstein Jensen Medium..

IDENTIFICATION AND SUSCEPTIBILITY TESTING

Isolates will be identified and susceptibility testing performed to the extent that the KCMC/ISAAC Microbiology laboratory is capable. All isolates will be archived (frozen at -80° C) and sent to Duke University Medical Center Clinical Microbiology Laboratory for

preservation and testing that exceeds capabilities of the KCMC Biotechnology Microbiology Laboratory.

1. All organisms isolated from blood must be identified to species level and serogroup or serotype determined, if applicable, to the extent possible in the laboratory.
2. Susceptibility tests will be performed on clinically significant isolates. The following are considered skin contaminants and testing will not be performed unless clinically indicated:

   Single isolations of:
   Coagulase negative staphylococci
   Streptococcus, viridans group
   Corynebacterium spp.
3. Susceptibility tests will be performed on subsequent isolation of organisms encountered in previously positive cultures ≥3 days ago.

REPORTING POSITIVE RESULTS

1. Telephone appropriate Study Coordinator with results of all positive blood culture smears and cultures.
2. Document date and time of call on BLOOD CULTURE RESULT Teleform.
3. Issue an ISAAC Laboratory Report Form with all positive smear results (preliminary report) and final results of culture. Send to ISAAC Study Coordinator to be placed in patient's medical record.

NEGATIVE BLOOD CULTURES

Blood cultures that have not turned positive after 5 days of incubation for bacteria or 42 days for mycobacteria will be flagged as Negative by the BacT/ALERT instrument.

1. Negative bottles should be removed from the instrument and reports issued as soon as convenient. Positive cultures take precedence.
2. Unload negative bottles (Refer to OPERATION of BacT/ALERT INSTRUMENT SOP MIC.001.).
3. Record negative result on the BLOOD CULTURE RESULT Teleform
4. Report negative result on Isaac Laboratory Report form. Send to ISAAC Study Coordinator.

REFERENCES

Dunne, WM, Nolte, FS, Wilson, ML. Cumitech 1B: Blood Cultures III. J. Hindler, Coord.Ed. American Society for Microbiology, Washington, DC.

Weinstein, MP. 1996. Current Blood Culture Methods and Systems, Clinical concepts, Technology and Interpretation of Results. CID 23:40.

APPENDIX – MycoFLYTIC EXAMINATION RECORD FORM