

Transfusion Reaction Culture

Purpose This procedure provides instruction for Transfusion Reaction Culture for the Microbiology Laboratory.

Policy Statements This procedure applies to microbiologists who perform culture set-up and plate reading.

Principle and Clinical Significance Transfusion of blood products and blood components is usually safe and effective therapy. However, transfusion reactions can occur. A serious complication of transfusion therapy is the transmission of bacterial organisms. Although bacterial contamination of blood and blood products is rare, it remains a significant concern. The use of a closed system for blood collection, the implementation of rigid standards for blood bank operations and stringent attention to skin disinfection has contributed to the reduction of bacterial contamination. If a transfusion reaction should occur, inoculate two sets of blood culture bottles from the suspected blood unit, incubating one set at 35°C and the other set at 25°C.

Test Code TRXN

Reagents	Supplies	Equipment	Media
<ul style="list-style-type: none"> Gram Stain reagents Acridine orange stain 	<ul style="list-style-type: none"> 12 cc syringe 18-gauge needle BD© blood transfer device Glass slide Inoculating loop 	<ul style="list-style-type: none"> BACTEC™ FX Fluorescent series CO₂ incubator Fluorescent microscope Incinerator Microscope Anoxomat 	<p>Refer to the Sunquest specimen label for media information. Note: One “set” consists of an aerobic and an anaerobic blood culture bottle.</p> <ul style="list-style-type: none"> 2 BACTEC™ PEDS PLUS/F aerobic medium (pink cap) 2 BACTEC™ Lytic/10 Anaerobic/F medium (purple cap) Chocolate agar (CHOC) Sheep Blood agar (SB) Anaerobic sheep blood agar (ASB2)

- Sample**
- Acceptable specimens
 - Blood or blood components sterilely transferred to syringe from transfusion bag.
 - If transfusion bag is received, ask Blood Bank to use Syringe Set to remove 12ml of blood or blood components from bag, labeled with patient and unit information.
 - SDES codes/Specimen type
 - DON -; unit number (use free text to add unit number) ; XXXXXXXX
 - If blood, use the code DON - BLD -; unit number XXXXXXXXXXXXXXX
 - If platelets, use free text DON -; PLATELETS unit number XXXXXXXXXXXXXXX
 - If transfusion bag is empty, order culture and result with cancel code QNS. Credit culture in ORM.

Dept	Acc#	Order Code	Test Code	Results	QA Flags
MC	T53888	TRXN	SDES	DON-BLD-W0515110927E	
		TRXN	SREQ	NONE	

Test: SREQ SPECIAL REQUESTS Result:

Composed Text: Preliminary Final

Buttons: Auto Fill, Display Prior Results, Result code lookup, Display Correction, Delete all text, Load default text

Bottom Buttons: Save, Cancel, Help

Special Safety Precautions

Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies:

- [Biohazard Containment](#)
- [Safety in the Microbiology Laboratory](#)
- [Biohazardous Spills](#)

Procedure

A. Specimen processing

1. Order the Transfusion Reaction Culture using the order code **TRXN** and enter the unit type and number.
2. Obtain 2 aerobic 'pink' bottle and 2 anaerobic 'purple' bottles.
3. Cleanse the rubber septum of each blood culture bottle with 70% alcohol. Allow to dry.
4. Inoculate approximately 3 ml into each of 4 blood culture bottles using a blood transfer device, saving a small amount for a Gram stain, and possible subculture. Inoculate bottles with equal amounts if less than 12 ml available.
5. **St. Paul processing:** inoculate bottles and perform Gram Stain. Send bottles, Gram stain slide and any remaining blood in the syringe to Minneapolis. Clearly label RT bottles.
6. **If less than 2 mL is received,** inoculate only the aerobic bottles (2 pink) with equal amounts. Do not perform Gram stain.
7. Incubate one set of blood culture bottles in the BACTEC™ FX Instrument. A set consists of one aerobic bottle (pink) and one anaerobic bottle (purple).
8. Place the other set on the counter for room temperature incubation.
9. If only aerobic bottles inoculated due to low volume, incubate one in the BACTEC™ FX instrument and one at room temperature.
10. Prepare a slide for Gram stain, heat fix, stain and examine immediately.
 - Enter Gram stain result onto the second page of the Transfusion Reaction Report and return to Transfusion Services
 - **Evenings and night staff:** Write Gram Stain results on the Gram Stain Review Log by the microscope.

Transfusion Medicine Service-Laboratory Use Only **TRXR**

Pathologist Report:

Additional testing recommended:

- None
- Hgb (Accession Number: _____)
- Bilirubin (Accession Number: _____)
- Haptoglobin (Accession Number: _____)
- Urine Hgb (Accession Number: _____)
- BNP (Accession Number: _____)
- Bacterial cultures
 - o Date/Time of gram stain and culture: _____
 - o Sample source: bag Segment Other: _____
 - o Culture (TRXN Accession Number: _____)
- Other (Document Accession Number): _____



- **If organisms are observed:**

- Report in the Sunquest MRE as you would a positive BC on Observation line 1.
- Notify the provider and Transfusion Services.
- Directly inoculate a CHOC, SB and ASB2.

- Incubate subculture plates as follows:

- CHOC/SB - 35°C in CO₂ incubator for 2 days
- ASB2 - 35°C in anaerobic jar/bag for 2 days

11. The provider will order a BC on the patient. If orders are not received with the bottles, call the nurse to order the BC.

B. 5 day standard incubation

- a. Incubate one set of blood cultures at 35°C in the BACTEC™ FX following the 5-day Bactec™ protocol.
- b. Incubate the other set of blood cultures at 25°C for 5 days.
- c. Incubate blind subculture plates as follows:
 - CHOC/SB - 35°C in CO₂ incubator for 2 days
 - ASB2 - 35°C in anaerobic jar for 2 days
- d. **Day shift, Desk 2-**Confirm bottles are in Bactec before updating culture.

C. Culture Procedure. Macroscopically examine Room Temperature bottle(s) and update negative TRXN manually in Sunquest MRE for 5 days.

Day #1:

1. Macroscopically examine the **room temperature** bottle(s) **twice a day for 2 days**. Record in workups. Refer to [MC 1.03 Bactec FX Blood Brucella procedure](#) for further instructions.
2. Perform 24 h blind subcultures and AO stain on the RT 25°C set of blood culture bottles.
 - a. Cleanse the bottle septum with 70% alcohol. Allow to dry.
 - b. Remove 0.5 ml of blood and inoculate a CHOC from the aerobic bottle and a CHOC & ASB2 from the anaerobic bottle.
 - c. Prepare a slide for acridine orange stain (AO).
 - d. Read AO with fluorescent microscope. If positive, perform Gram stain.
 - e. If positive, report in the Sunquest MRE and notify the provider and Transfusion Services as for a positive BC.
 - f. Blot the oil from the slide and save for one week.

3. Initial Blind subcultures

- a. If initially set because of a positive initial gram, examine blind subculture plates at 24 h and 48 h before discarding as negative.

- b. If growth is present, Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, etc.
- c. Set up definitive biochemical or identification procedures on isolated colonies.
- d. Perform antimicrobial susceptibility testing on isolated colonies.
- e. Subculture organisms that are not well isolated to appropriate media for further work-up.
- f. Re-incubate plates and subcultures for an additional day.
- g. Report preliminary results.
- h. Notify Transfusion Services and the provider of any positive findings.

Day # 2:

1. Macroscopically examine the **room temperature** bottle(s) **twice a day for 2 days**. Record in workups.
2. Examine blind subculture plates at 24 h and 48 h before discarding as negative.
3. Perform 48 h blind subcultures and AO stain on the RT 25°C set of blood culture bottles. Follow instructions list above in section C.2a-f.
4. Examine plates from the previous day.
5. Read and record identification tests and susceptibilities if any, from the previous day.
6. Set up additional tests as needed.
7. Send updated report.
8. Save a representative primary plate, whether a complete work-up was performed or not, at room temperature for 7 days in case a physician calls for further studies.

4. Additional Days:

- a. Examine blind subculture plates at 24 and 48 h before discarding as negative.
- b. Macroscopically examine bottle(s) for 5 days.
- c. As needed, complete identification and susceptibility testing procedures until all significant isolates are finished.
- d. Send updated report and finalize.

5. Terminal AO and subculture:

- a. Perform terminal subcultures (TSUB) and AO stain on the RT 25°C set of blood culture bottles at 5 days.
- b. Cleanse the bottle septum with 70% alcohol. Allow to dry.
- c. Remove 0.5 ml of blood and inoculate a CHOC from the aerobic bottle and a CHOC & ASB2 from the anaerobic bottle.
- d. Prepare a slide for acridine orange stain (AO).
- e. Read AO with fluorescent microscope. If positive, perform Gram stain.
- f. If positive, report in the Sunquest MRE and notify the provider and Transfusion Services as for a positive BC.
- g. Blot the oil from the slide and save for one week.
- h. Examine plates from the previous day.
- i. Hold blind subculture plates for 48 hours before discarding as negative.

Hold negative RT 25°C bottles for 5-7 days until all plates have been read, before issuing a final report.

Interpretation

1. If the culture is negative, it is unlikely that the blood or blood product was contaminated with bacteria at the time of the transfusion reaction.
 2. A positive culture does not positively identify infection as the cause of the transfusion reaction. A positive blood culture or blood product does not establish the source of contamination unless both the blood bank and patient's samples yield the same organism. PFGE may be needed to determine if the strains are similar.
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**Procedure
Notes**

1. *Yersinia enterocolitica*, *Ps. fluorescens*, *Ps. putida*, and *Enterobacter* sp. can proliferate at 0-6°C, the temperature at which blood is stored. These organisms have been implicated in transfusion reactions. Gram-negative bacilli can produce endotoxins that will cause rapid and sometimes irreversible shock when present in the blood.
2. Platelets are the most common cause of transfusion-related sepsis caused by organisms such as *Corynebacterium* sp., coagulase-negative staphylococci, *S. aureus*, *Pseudomonas* sp., and *Enterobacter cloacae*. Platelets are stored at 22°C and provide an excellent medium for bacterial growth.
3. Contamination of blood products can be from skin flora, environmental organisms introduced at the time of collection or processing, or an asymptomatic bacteremia in the blood of a healthy donor. Water baths in which frozen blood products are thawed have also been a source of contamination.
4. If it is clinically necessary to culture a blood unit, the recipient's blood should be cultured simultaneously.

**Result
Reporting**

1. **Culture Results:** Record culture results and culture work-ups in Sunquest MRE *Culture Entry* tab in Observations or Workups by using customized keyboards or by entering a code in the result box.
2. **No growth cultures:** Update culture status in the Observation result box (*Culture Entry* tab), by using the "No Growth" update key ('). Report as "No growth "x" days".
3. **Final culture on day 5 or when testing is complete as No Growth 5 days.**
4. **Positive culture:**

Observations: 1. 4+ COAGULASE NEGATIVE STAPHYLOCOCCI (2 COLONY MORPHOLOGIES)

Workups:	Wkup # 1	Workup Components
	Med: SB	GMS : STPH
	Desc: WH	SC : SB
	Id: CNS	SLC : POS
		VMIC : 1

5. **Notify provider and Transfusion Services of positive results. Document in workup.**
6. Review **Culture Summary** for accuracy before filing report.
7. If a culture requires a correction, the code **CORR** (corrected report) must be reported on an observation line in the *Direct Exam* or *Culture Entry* tab. Refer to policy MCVI 5.1 Mislabeled / Unlabeled Specimens and Correcting Patient Data for Sunquest report entry information.
8. If growth should occur or additional testing should be requested after the culture has been finalized, remove the final status and send out a supplementary report. The code SRPT (supplementary report) must be used in SREQ or *Culture Observations* as follows:
 - Updated or new culture information: In the *Culture Entry* tab, enter SRPT on an observation line followed by new results.
 - Requests for additional testing: In the *Misc. Updates* tab, enter **SRPT** in SREQ followed by the request.
 - Re-final the culture when identifications and/or testing are complete.

Appendices

WORKLABEL MEDIA DEFINITION, BATTERY : TRXN
SPEC MEDIA
0 BPNK,BPRL,BP25,BPR25

References

1. Leber, Amy Section 13.3.5, Epidemiologic and Infection Control Microbiology, Culture of blood bank products, *Clinical Microbiology Procedures Handbook*, 2016, American Society for Microbiology, Washington, D.C.
2. Leber, Amy Section 3.4.1, Aerobic Bacteriology, Blood Cultures, *Clinical Microbiology Procedures Handbook*, 2016, American Society for Microbiology, Washington, D.C.

**Training Plan/
 Competency
 Assessment**

Training Plan	Initial Competency Assessment
1. Employee must read the procedure 2. Employee will observe trainer performing the procedure. 3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.	1. Direct observation

**Historical
 Record**

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.0	Pat Ackerman	04/11/1994	Initial Version
1.1	Pat Ackerman	08/12/2003	
1.2	Pat Ackerman	01/14/2008	Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements; added notification of blood bank Technologist; added Hyper-link to labeling policy. Updated blood culture bottle information.
1.3	Pat Ackerman	10/12/2009	Updated protocol length to 5 days from 7 days.
1.4	Tina Gronquist	06/16/2014	Updated into online format.
2	Becky Carlson	4/14/2015	Re-numbered from MC 432
3	Susan DeMeyere	12/7/2018	Add instructions for empty or low volume. Removed anaerobic culturing from aerobic bottles.
4	Susan DeMeyere	10/15/2020	Removed use of Fenwal sampling site coupler. Blood Bank will provide syringes. Added macroscopic examination of manual bottles.
5	Susan DeMeyere	2/17/2022	Added instructions for gram stain result on Transfusion Reaction form and return to Transfusion Services
6	Susan DeMeyere	7/6/2022	Added instructions for processing in St Paul.
7	Susan DeMeyere	7/22/2022	Added additional instructions for St Paul processing, hold plates 5 days when QNS for bottles, Desk 2 tech check for bottles in Bactec.
8	Susan DeMeyere	10/1/2024	Revised instructions <2 ml of sample