BLOOD CULTURE MANUAL
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Introduction

Blood cultures are collected from patients with suspected sepsis or bacteremia. Virtually any organism may cause bacteremia. Thus, the isolation of all organisms from a blood culture must be considered significant and correlated with the clinical picture. At least 2 sets and no more than 3 sets of blood cultures should be collected from a patient with suspected bacteremia prior to the initiation of antimicrobial therapy. Collection of additional blood cultures may be indicated if the patient fails to respond to appropriate antimicrobial therapy or develops a new episode of fever or sepsis following an initial response to therapy. All sets of blood cultures received from a patient will be processed regardless of the number.

Although this section is mainly directed towards the processing of blood cultures, occasionally other specimen types (e.g. Sterile fluids, Bone marrow, abscess material) are received in blood culture bottles and thus their processing and work-up will be described in this manual.

The current blood culture system used in the Microbiology Laboratory is the Virtuo System manufactured by bioMerieux. The Virtuo tracks all bottles automatically loaded and communicating results directly to the LIS. Positive bottles are detected as growth-generated CO_2 causes a colour change in the pH sensitive disc on the bottom of the bottle.

Specimen Collection and Transport

See Specimen Processing Procedure MI_SM_PROC

Reagents/Materials/Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

Procedure

See Specimen Rejection Criteria MI_SM_RJCT to determine suitability of specimen.

A. Processing of Specimens:

See Specimen Processing Procedure MI_SM_PROC

a) Direct Examination:

Gram stain: Positive Blood Cultures Only
Acridine Orange: Positive Blood Cultures NBS, positive graph

b) Culture

Media	**Incubation**
Blood Agar (BA)	CO_2, 35°C x 48 hours
MacConkey Agar (MAC)	CO_2, 35°C x 48 hours
Chocolate Agar (CHOC)	CO_2, 35°C x 48 hours
Fastidious Anaerobe Agar (BRUC)	AnO_2, 35°C x 48 hours

B. Interpretation of Cultures:

Negative Cultures:
Negative Cultures incubating within the Virtuo will automatically result.
Any cultures incubated manually must be manually resulted for each test type.

i) Routine Blood, Bone marrow, sterile fluids, and general fungus/yeast cultures:
Negative bottles are automatically resulted and discarded after 5 days incubation.
"No growth after 5 days incubation." Test comment: **}NG@5**

ii) Bone bank blood cultures:
Negative bottles are automatically resulted and discarded after 7 days incubation.
"No growth after 7 days incubation." Test comment: **}NG@7**

iii) SBE/IE and PUO/FUO, PD Effluent, Brucella:
Negative bottles are automatically resulted and discarded after 21 days incubation.
"No growth after 21 days incubation." Test comment: **}NG21**

Positive Cultures:

Positive cultures are prepared in the specimen processing area.

See Specimen Processing Procedure MI_SM_PROC

Gram stain

i) Read gram stain slide from subcultured positive bottle
ii) Report smear as per Gram stain result reporting.
iii) Phone results as appropriate.
- NOTE: do not mention # of bottles flagged positive or bottle type

The Gram stain may indicate the need for additional media or a change in the incubation conditions. See the table below or the Charge technologist for appropriate additional media.

Additional media for preliminary processing of positive BacT/Alert blood culture bottles:

Gram stain morphology	**Additional Media**	**Incubation**
Mixed gram positive & gram negative bacterial	2 Colistin Nalidixic Agar (CNA)	CO2 35°C x 48 hours AnO_2 35°C x 48 hours
Small gram negative bacilli	Campylobacter Agar (Campy)	Microaerophilic 42°C x 48 hours

Note: If a culture bottle marked as "Brucella" is flagged positive, a small amount of the blood is removed for a Gram smear ONLY. If the Gram smear shows small gram negative bacilli, forward the positive culture bottle to the Public Health Laboratory (PHOL) for identification. If the Gram smear shows organisms other than small gram negative bacilli, notify technician to proceed to subculture the bottle.

If gram stain is negative, check the bottle graph. If the graph appears to be positive, recheck and/or repeat the gram stain and/or acridine orange stain. If the graph appears to be negative, enter the gram result "No bacteria seen" under media (GRAM). Do not assign an isolate #. Reload the bottle in controller that bottle originated from.

Sub-cultures

No growth: Read & document no growth on plates from bottles flagged positive but have no bacteria seen in gram. The culture will remain on the "BC Posted – No Iso" worklist. After the 48hours reading of the plates and they are still no growth, press CTRL U before exiting the order to remove the "positive" flag.

No growth, gram stain GPC seen: If patient is indicated with an ESO flag "VDE alert for micro lab", investigate for vancomycin dependent enterococci by subbing from bottle onto BVRE.

Identification

See Blood Culture Workflowfor workflow and duties per shift.

Examine the sub-cultured plates and perform Vitek-MS or full identification as outlined in the BACTERIA AND YEAST WORKUP manual on all isolate types.

For large GNB with unsuccessful MALDI (unusual finding), the aminopeptidase testaminopeptidase test can be used to differentiate GNB from overdecolorized GPB such as *Bacillus sp or Paenibacillus sp*. If the initial gram smear result does not match the aminopeptidase test result, perform gram stain on colony, review for evidence of endospores and consult senior/charge technologist.

If both bottles of one set of blood cultures grow organisms with the same Gram stain result and/or have the same colonial morphology, do Vitek MS only from one bottle of the set. (*The exception to this is gram positive cocci in clusters. In this case, do Vitek MS from growth from both bottles.)*

If multiple sets from the same patient collected within 24 hours of each other are positive and growing **morphologically identical organism**(s), perform and report complete identification from one bottle of each set (*The exception to this is gram positive cocci in clusters. In this case, do Vitek MS from growth from all bottles.)*

If yeast is isolated, do Vitek MS, using formic acid (according to Vitek MS manual). If not identified by Vitek MS, send to PHOL for Identification.

Minimum work-up is performed for identification of isolates from **autopsy blood** specimens.

1. Single isolate culture: Minimum work-up or Vitek MS

2. Mixed culture (≥2): If Vitek-MS is unsuccessful, perform minimum workup, list organisms based on Gram stain morphology, growth requirement and minimum work-up e.g. "Mixed culture including Enterococci, anaerobic gram positive bacilli, aerobic gram negative bacilli…….etc.".

Susceptibility

Refer to Susceptibility Testing Manual.

Note: Set up susceptibilities on CNST from patients with endocarditis or if isolated from a fluid in a blood culture bottle.

If both bottles of one set of blood cultures grow the same organism, perform susceptibility on the organism from one bottle only.

If multiple sets from the same patient collected within **24 hours** of each other are positive and growing **identical organism**(s), susceptibility testing can be referred.

If multiple sets from the same patient collected within **7 days** of each other are positive and growing **identical yeast organisms**, susceptibility testing can be referred.

For isolates of *Staphyloccoccus aureus* or *Enterococcus* one bottle from **each set** of BC bottles must have an oxacillin and/or vancomycin screen performed.

No sensitivity is required for **autopsy blood** or **bone bank** specimens**.**

Blood Culture Isolates to be Frozen and Saved

- Freeze **ALL** isolates from blood culture including all Autopsy isolates
  EXCEPT:
- Enterococcus susceptible to Vancomycin
- *E. coli* susceptible to all tested antimicrobials
- Skin flora (CNST, Micrococcus, *Bacillus* sp., *Corynebacterium* sp. not JK, *Lactobacillus* sp., *Lactococcus* sp., *Proprionibacterium* spp., *Peptostreptococcus* sp.)
- Repeat isolates within 24 hours

- Document freezing in Softstore.

Reporting Results

Negative to Date Bottles and False Positive Bottles:

Preliminary: The LIS will automatically report "Culture received in lab. Results will be reported as soon as they become available" and assign a preliminary status.

Negative report:

Final:	i) Routine Blood, Bone marrow Sterile Fluids, Blood products, Fungus, Yeast	"No growth after 5 days incubation".
	ii) Bone Bank bloods	"No growth after 7 days incubation".
	iii) Brucella, PD Effluent, SBE / IE, PUO / FUO	"No growth after 21 days incubation".
	iv) Dimorphic fungi	"No fungus isolated". (See Mycology Manual)

Positive report:

For Gram stain resulting:

In LIS "MEDIA" Window, under GRAM media, pick from keypad:

1. The bottle type the organism was from e.g. from FO2.
2. Then pick the organism seen e.g. gram positive cocci in clusters
3. Then the isolate code to be transferred to the "ISOLATE" Window (if this is the first time this organism is seen in this order; omit this keypad pick if this is the second time this organism is seen in this order.)

Go to the "TEST" Window:

1. For Blood Culture test, **REMOVE** preliminary statement "Culture received in lab…." Add "UPDATED REPORT".
2. For fluids or aspirates in blood culture bottles report Gram results under the "ISOLATE" window of LIS as Isolates 1 "Gram positive cocci" etc. **REMOVE**

preliminary statement "No growth to date……" and add "UPDATED REPORT" and Status the test (C&S or FLDM) as preliminary (^P).
No work has to be documented under test code '?BTLE'

3. Go to the "ISOLATE" Window.
4. Go to the ISOLATE COMMENT field.
5. From isolate keypad, select "BLDC" to go to Blood Culture specific keypad.
6. Select >SMEAR, then select the appropriate comment for the organism morphology and "seen" e.g. "~in clusters seen".
7. Press "Verify all"
8. Save the isolate and return to "TEST" window.
9. Status the Test as preliminary (^P).

For all sites, telephone **the ward/ordering physician as soon as the Gram stain result is available**.

NOTE: do not mention # of bottles flagged positive or bottle type

If another bottle of the same set becomes positive with the same organism, no further report is required.

Notify Infectious Disease Team (ID) as appropriate.

For Culture results:

Remove "~seen" comment from ISOLATE COMMENT field and add "isolated"

Report organism with the corresponding antibiotic susceptibilities results and comments as appropriate, refer to Susceptibility Manual and Vitek MS Species List.

Notify MOH with appropriate isolates from reportable diseases listreportable diseases list

For identification and susceptibility results, call the results to the **wards** as soon as they become available as follows:

Hospital	**Monday - Friday**	**Weekend / Holidays**
TGH	No call*	No call*
TWH	No call*	No call*
TRI	No call*	No call*
PMH	No call*	**Call**
MSH all wards and patients admitted from Emergency ward	No call*	No call*
MSH Emergency Ward not admitted or discharged	No call*	No call*
Bridgepoint,	No call*	No call*
Baycrest	No call*	No call*

CAMH	**Call**	**Call**
Grace	**Call**	**Call**

***Unless** a new organism is isolated that was not seen on the initial Gram stain, or the organism has been identified as *Streptococcus pneumoniae, Listeria monocytogenes, Staphylococcus aureus, Streptococcus pyogenes, Neisseria meningitides, Salmonella* species or *Cryptococcus neoformans.*

S. aureus reported on Blood culture will have a comment automatically appended:

"Treatment for S. aureus bacteremia requires a MINIMUM of two weeks of INTRAVENOUS antimicrobials. Infectious Diseases consultation is advised. (Ref: CMAJ 2019 September 3;191:E967)"

When both bottles in the set are completed, assign "Interim" status (^L). Senior staff will review and finalize the report.

Infection Control Team Reporting:

Notify as per QPCMI16003

Infectious Diseases Team Reporting:

If *S. aureus*, Yeast or Fungus is isolated (presumptive or confirmed) from an inpatient at the TGH, TWH, PMH or MSH, the relevant Infectious Disease (ID) Team must be notified immediately if the hours are 0800-midnight. Notification of positives to ID team from midnight-0800 should be deferred until the next morning.

DO NOT notify the Infectious Disease team if:

- Patient is deceased,
- Patient is NOT admitted to an inpatient ward
- Patient is discharged from emergency department AND not admitted anywhere else

Notify the relevant Infectious Disease team by paging the on-call physician covering the team through locating as follows:

Note: *If the receiving ID team recognizes that a different ID team is the appropriate receive of the information being reported, the receiving ID team is responsible to relay the information received to the appropriate ID team*.

A. Positive Blood Cultures for *S. aureus*, yeast, or fungus <u>0800-midnight</u>:

Hospital	Infectious Disease (ID) Team (Monday-Friday):	Infectious Disease (ID) Team (Weekends/Holidays):	Locating Phone Number
TWH	TWH ID	TWH ID	14-3155
PMH	PMH Oncology ID	PMH Oncology ID	14-3155
MSH NICU	Sick Kids Infectious Disease*	Sick Kids Infectious Disease*	416-813-7500
MSH not NICU	MSH ID	MSH ID	14-3155
TGH Transplant floors - 7MA and 7MG	TGH Transplant ID	TGH Transplant ID	14-3155
TGH –all wards **other than** 7MA and 7MG	TGH ID	TGH ID	14-3155

*** Please provide the Sick Kids ID Team with the name of the person in NICU who was also notified by the lab about the result**

B. Positive Blood Cultures for *S. aureus*, yeast, or fungus <u>midnight-0800</u>:

No calls to any ID service during these hours. Call the next morning following the table above.

<u>BC BENCH WORKLISTS</u>

Resulting Worklists have been created to ensure all orders are accounted for. Technologist work-up benches must check the following pending lists daily:

Worklist 1: BC posted - No Isolate

This worklist includes all orders which had a positive bottle but do not have an isolate:

- Check if bottle is positive but NBS. These are okay and will remain on this list.
- Check to ensure all positives bottles with an organism seen in the gram stain have an isolate entered. This is to ensure a gram smear is not lost or missed for a new positive bottle.

Worklist 2: BC posted - with Isolate

This worklist includes all positive orders with isolates being worked on:

- Check the list for completeness of workup (culture not missed, culture plates missing, etc) result as needed.
- Use "FACOM, FNCOM…" to document completeness (ie. All checked & bottle completed with date)

Worklist 3: GRAM for BC

This worklist includes orders flagged as positive by Virtuo that require a GRAM result. Orders disappear once a GRAM result has been entered in the isolate window.
NOTE: only the first bottle to be flagged as positive from each set will appear

- Result GRAMS as required and check the list for completeness every hour
- If there is "no bacteria seen" in the GRAM/Acridine orange and the graph on the Virtuo is unremarkable, remove orders from the worklist by entering BC1 under BENCH

VIRTUO SYSTEM TROUBLESHOOTING

Virtuo troubleshooting guide is available on the Virtuo main screen or following this link: Virtuo User Manual

For service call BioMerieux at 1-800-361-7321

The Virtuo system is equipped with an alert system that notifies the user that attention is required by an audible alarm and yellow/red flashing light on top of the related Virtuo instrument, these should be addressed as they arise.
Note: There may be instances when the Virtuo does NOT alarm but there are issues to be resolved and these will be checked each morning by a bench MLT.

Expired BC bottles

1. Incubate all the bottles off-line
2. Sub the bottles according to the specimen processing manual
3. Make comment at the back of workcard to indicate the bottles are expired.
4. See Rejection manual for additional Test Comment requirement.

Anonymous bottles

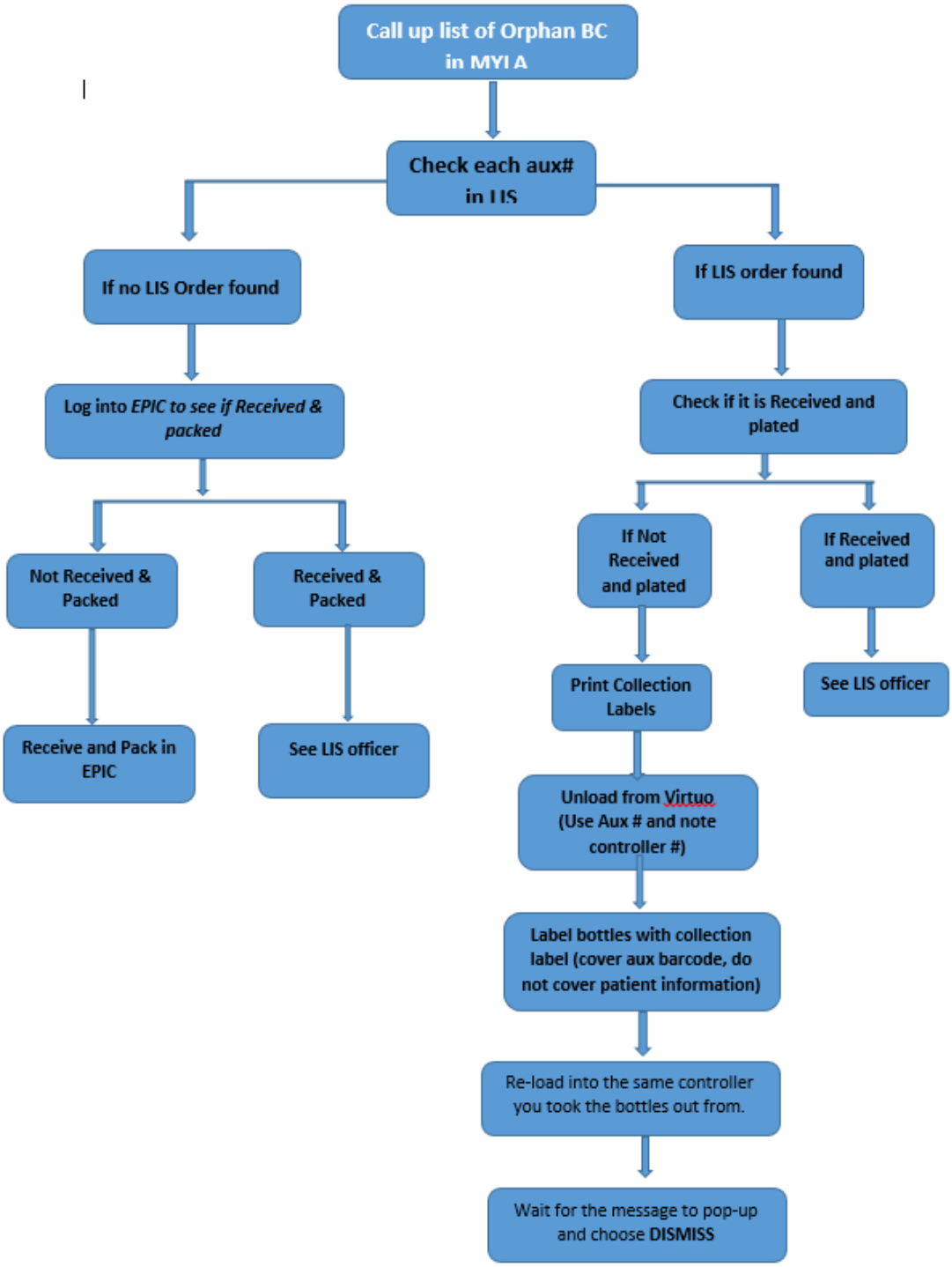
Theses bottles are missing BOTTLE IDs and are usually detected as soon as they are loaded, virtuo will alarm.

1. Select “Resolve” from the main screen of the alarming unit.
    a. For bottles loaded within 10 minutes
        i. Unload and remove patient label obscuring bottle ID
        ii. Scan bottle barcode and save (NOTE: If unable to scan bottle barcode, place a generic label on the bottle, ensure to select bottle type)
        iii. Reload bottle in same controller it originated from
    b. For bottle loaded for >10 minutes or unknown amount of time
        i. Do not unload, all bottle readings will be lost
        ii. Scan a generic label into the virtuo, ensure to select bottle type
        iii. Save
        iv. Affix the generic label onto the bottle and reload into originating controller

Orphan Bottles

These bottles are missing PATIENT DATA (accession numbers and/or patient info)
Refer to flowchart below for workflow.
Note: Ensure patient inconsistencies are fixed before proceeding.

1. At Virtuo home screen, select "search" from the main screen (do separately for each controller)
    a. Under load status, select "Loaded"
    b. Under missing information, select "patient", then search. (if no items appear, there are no problems)
        i. If items appear on the list, **ensure that they all have bottle ID numbers before proceeding**
    c. If items have bottle IDs but are missing the accession number, either because the label is unreadable or unscannable.
        i. Select the bottles by touching the box next to each one
        ii. select unload (note the originating controller, search one at a time by scanning bottle ID, do not type)
        iii. scan accession number (print new label in LIS if needed)
        iv. reload bottle in same controller it originated from
    d. For all others that have bottle ID and accession number
        i. Choose one bottle of each pair and select "unload" (note originating controller)
        ii. Take unloaded bottles and check EPIC that they are collected and accessioned. See Appendix V.
        iii. reload bottle in same controller it originated from
        iv. If bottle has been collected and accessioned in EPIC and there is no patient information. Download the Patient Information from the LIS to the VIRTO using the Instrument Menu. See Appendix IV.



Load List

1. At virtuo home screen, select "Reports"
2. Select "load list", print using icons on screen or check the list onscreen
3. Scan to see what bottle types are associated with each lab number (can only have one FA, FN, or PED)
    a. If there are multiple bottles of one type under one lab number, print LIS label and auxiliary number label. (note originating controller)
    b. If two FN under one order, two FA under second order
        i. Search in virtuo by accession number
        ii. Highlight all bottles and unload
        iii. Check all bottles have same collection date/time/site in LIS
        iv. If so, exchange one bottle of each set to make matched pair
        v. Generate an LIS label for bottles that you are changing, affix LIS label
        vi. At the originating controller, select search, scan each bottle ID separately to edit the accession number
        vii. Scan in new LIS label, save, then reload
        viii. In LIS, document by selecting "PULL", "BOTTLE", choose "FOR double FA/FN", input original accession number
    c. If three or four bottle are under one order,
        i. Search in virtuo by accession number
        ii. Highlight all bottles and unload
        iii. Check all bottles have same collection date/time/site in LIS
        iv. If so, make one matched pair and reload
        v. The extra bottles need a new order created:
            1. For MSH, CAMH, GRACE use addnext in LIS,
            2. For UHN, use EPR
            3. Baycrest, Bridgepoint and WCH, need new orders created by their own labs
        vi. Generate an LIS label for bottles that you are changing, affix LIS label
        vii. At the originating controller, select search, scan bottle ID. Edit the accession number by scanning the new LIS label, save
        viii. Scan in new LIS label then reload
        ix. In LIS, document by selecting "PULL", "BOTTLE", choose "FOR double FA/FN", input original accession number

VIRTUO DAILY CHECKS

Order Entry comments daily printouts

- Check comments for query Brucella, PUO/FUO, endocarditis (SBE/IE)
- Change all BC specimens currently in process in the Virtuo on the same patient to 21 day incubation following procedure to changing incubation times. See Appendix III for detailed instructions.
- Remember to document date you changed the incubation time in LIS.

BC Receiving worklist

- Complete all BC bench worklists checks and Virtuo troubleshooting prior to investigating BC receiving worklist.
- Blood cultures from ALL sites (MSH, UHN etc.) not received after 24 hours must be investigated and **finalized**.
- Ensure the following are done at minimum as part of the investigation:
    - Check in MYLA if bottles were loaded
    - Check Virtuo loaded bottles for mislabeling/duplicate/relabeling errors
    - Call submitter:
        - For UHN, bring to a senior to send a "LMP Escalation Alert" for missing sample
        - For Sinai, call ward/provider to check if sample was actually taken/sent.
        - For interfaced orders, call associated laboratory
- Note: document all communications done as part of the investigation

Resulting worklists

1. BONNB new 7 days
    - Change incubation of any bottles in virtuo to 7 days following procedure to Changing Incubation Times
    - Remember to document change date
2. BC cancelled by HIS at MSH
    - For each order in worklist, highlight and copy order number into Myla to search if bottle is loaded.
    - For bottles found loaded, go to virtuo, search by accession number at each controller. Unload
    - In order entry "add next" to create a new order using original collection and received information. Affix labels to bottle
    - At virtuo, search by bottle ID, select edit then scan new LIS number
    - reload bottle in same controller it originated from

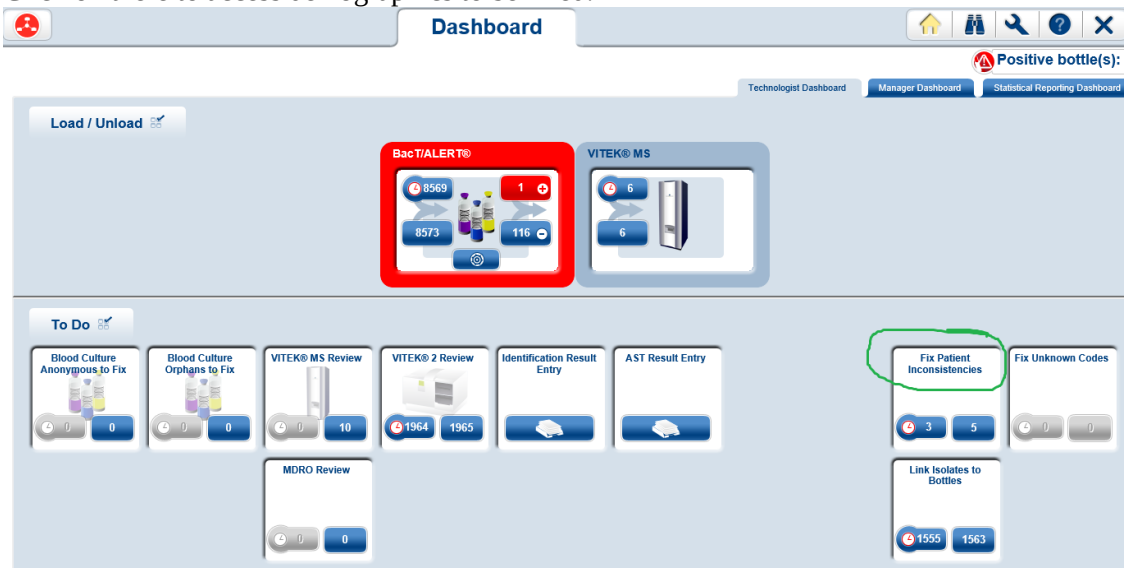
- document in LIS – “PULL”, “BOTTLE”, “processing BC cancelled by HIS” include original cancelled accession number

3. PD effluent new 21 days
   - Change incubation of any bottles in virtuo to 21 days following procedure to Changing Incubation Times
   - Remember to document change date
4. Fluid in BC bottles
   - Sort list by oldest received date
   - Look at all specimens received 5 or more days ago that are NOT PD effluents
   - Check that workup is completed, if primary plates and bottles are negative results as “No growth after 5 days”
5. BC Inception (cord bloods)
   - check all orders ensuring workup is complete

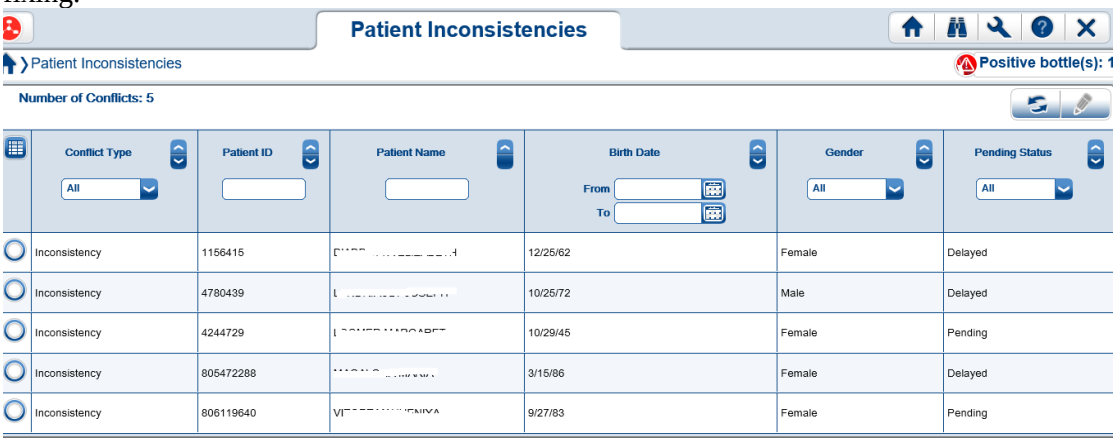
How to fix patient inconsistencies in the MYLA

These fixes need to be done consistently because it will prevent results from transferring. Inconsistencies relates to any demographic changes that the system has noticed such as a D.O.B or name change etc.

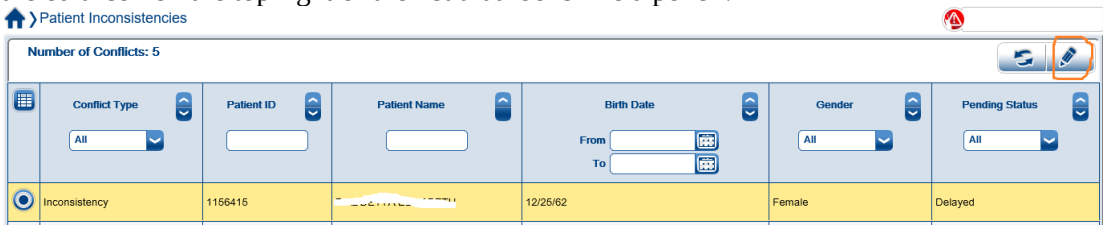
1. From the MYLA Dashboard on the bottom right there is a box called “Fix Patient Inconsistencies”
   The number on the left indicates new additions. The number on the right indicates the total that need fixing.
   Click on there to access demographics to be fixed.



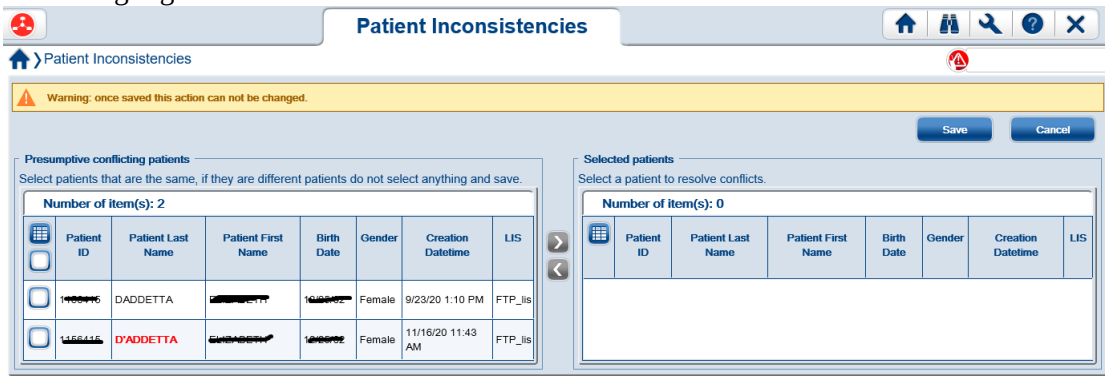
2. You will now be on the Patient Inconsistencies page where you will see a list of patients that need fixing.



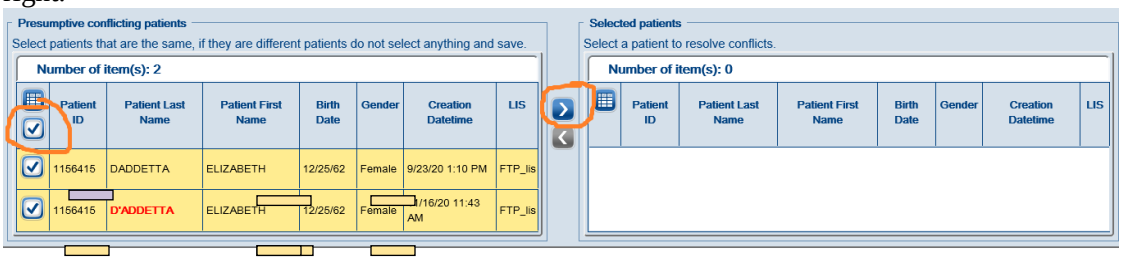
3. These should be fixed one at a time. Click on the first one to be fixed. This will allow you to use the edit icon on the top right of the list that looks like a pencil.



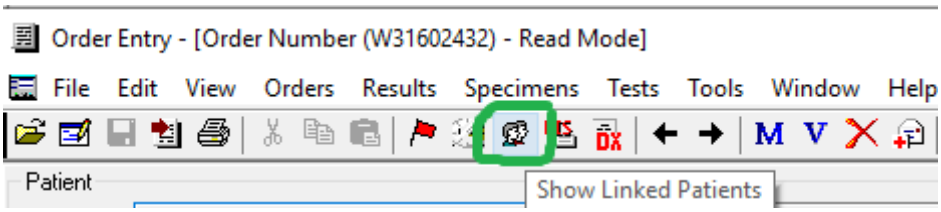
4. Click on the Edit "Pencil" icon 
5. This will take you to a new screen. The information that has changed and what is was changed to will be highlighted in red.



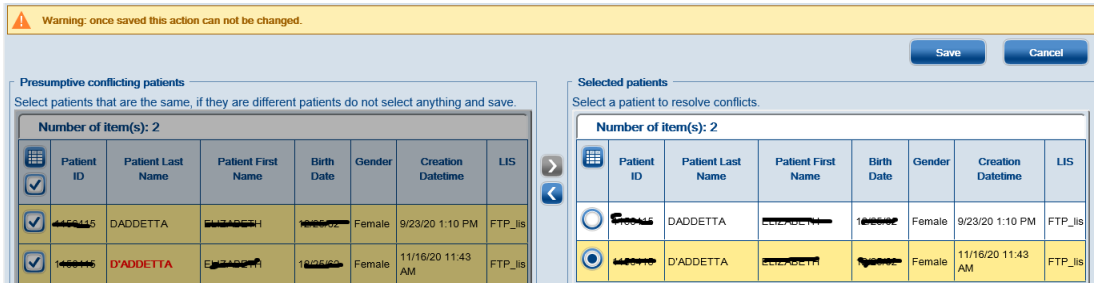
6. The object is to combine everything into the correct demographic. You will want to only include patients that are obviously alike. At this point if they all need to be merged then you can click on the box at the top of the list to choose all and then use the > arrow to move them to the box on the right.



7. As indicated above the first box, if they are different patients do not select anything and save. Go to Order Entry in the LIS and go to the patients MRN number, go into an order and then click on the Show Patient Links "two heads" icon. It will show you the history of the patient demographics.



8. When you click on the > arrow both patients will move to the right. Choose the patient you want to merge everything to which will be the bottom one that was highlighted in the red originally. Click Save.



9. Usually there are only two patients on the left side but sometimes there can be a lot more in a case usually when a patient goes from an Unidentified male or female to an actual name.

10. You should now be back to the list page of patients that need to be fixed. Click on the next one and repeat steps 3 to 8.

11. If you are not sure please ask someone with more experience or ask Nancy Hinricks for a demo.

Reference

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

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S. Mirrett, M.P. Weinstein, L. Reimer, M.L. Wilson and B. Reller. 2001. Relevance of the number of positive bottles in determining clinical significance of Coagulase-Negative *Staphylococci* in blood cultures. J. Clin. Microbiol. 39:3279-3281.

CMR 1995 8(4):447-483 and QMPLS Broadsheet on ESBL and ampC Resistance in GNB (updated 2007-12-10)

ISOLATER 10 BLOOD CULTURE SYSTEM FOR DIMORPHIC FUNGI

Introduction

The Isolator 10 blood culture system should be used for the isolation and detection of Cryptococcus and dimorphic fungi such as Histoplasma and Blastomyces.

If BacT/Alert bottles are received with a request for dimorphic fungi, notify the ward / ordering physician that they must use the Isolator 10 collection tubes. The BacT/Alert bottles should only be processed as per routine blood cultures.

Collection and Transport

See Specimen Processing Procedure MI_SM_PROC

Reagents / Materials / Media

See Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001

Procedure

A. Processing of Isolator 10 Microbial Tubes:

See Specimen Processing Procedure MI_SM_PROC

B. Interpretation of Fungal Culture Plates:

Refer to.

Reporting Results

Refer to Mycology Manual..

Reference

1. Isolator 10 Product Insert.

APPENDIX I - Quality Control (QC) of the Virtuo System

Daily

When entering the "Blood culture Posted-No Iso" or "GRAMB for BC" worklist, the LIS will prompt "QC pending: Would you like to bridge to QC?" Enter "Y".

i) Document gram stain QC

ii) Document Bench Top cleaning

iii) Document completion of daily system checks and worklist review for the Virtuo.

APPENDIX II - Sterility Testing of Blood for Media

Initial testing (performed by blood culture bench)

On receipt in the laboratory, each bottle is assigned a letter (A, B, C, etc.). Aerobic BacT/Alert bottles are labelled with corresponding letters. The smaller portion of the barcode is attached to the original bottle of blood.

Enter the data in the LIS as follows:

MRN: 7 7 7 7 7 7 7 7 7

TESTS: BLOOD CULTURES

SOURCE: BFA

SITE: HORSE / SHEEP, LOT #, EXP DATE

With a needle and syringe, 2.5 mL of blood is aseptically transferred from each bottle of blood and inoculated into separate BacT/Alert bottles. The original bottles of blood are immediately refrigerated and the BacT/Alert bottles loaded and processed as routine specimens.

If any BacT/Alert bottle gives a positive reading, the QA technologist must be informed ASAP and the original bottle of blood is removed from use. The BacT/Alert bottle is Gram stained and subcultured to BRUC ($An0_2$) and CHOC (CO_2). Identification to the species level (e.g. staphylococcus, diphtheroid, etc.) will be sufficient.

After Use (performed by media preparation and the QA technologist)

As each bottle of blood is used, the last few drops of blood are inoculated onto a BA plate which is labelled with the lot # and letter. This plate is incubated at 35°C for 48 hours, then at RT for 48 hours. The results are recorded as a QC item in the LIS for the medium that the blood has been added to.

Appendix III – Changing Incubation Times

Follow this procedure for any specimens which requires >5 days incubation:

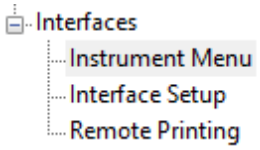
1. In the LIS result entry, on the line for BCBC - right click "R" column (on the right hand side of the line), select "Result Media" or double click for red check mark
2. From BCBC media, add new media BC21, Record the date incubation was modified in Virtuo.
3. Change Virtuo Incubation time
    a. Print label with the aux number (UHN) or LIS nuber (for all other hosptials), note the originating controller
    b. using this number, locate bottle in Virtuo instrument using SEARCH, VIEW
    c. EDIT to change incubation time

NOTE: you will need to repeat this process for all specimens currently incubating in Virtuo for that patient.

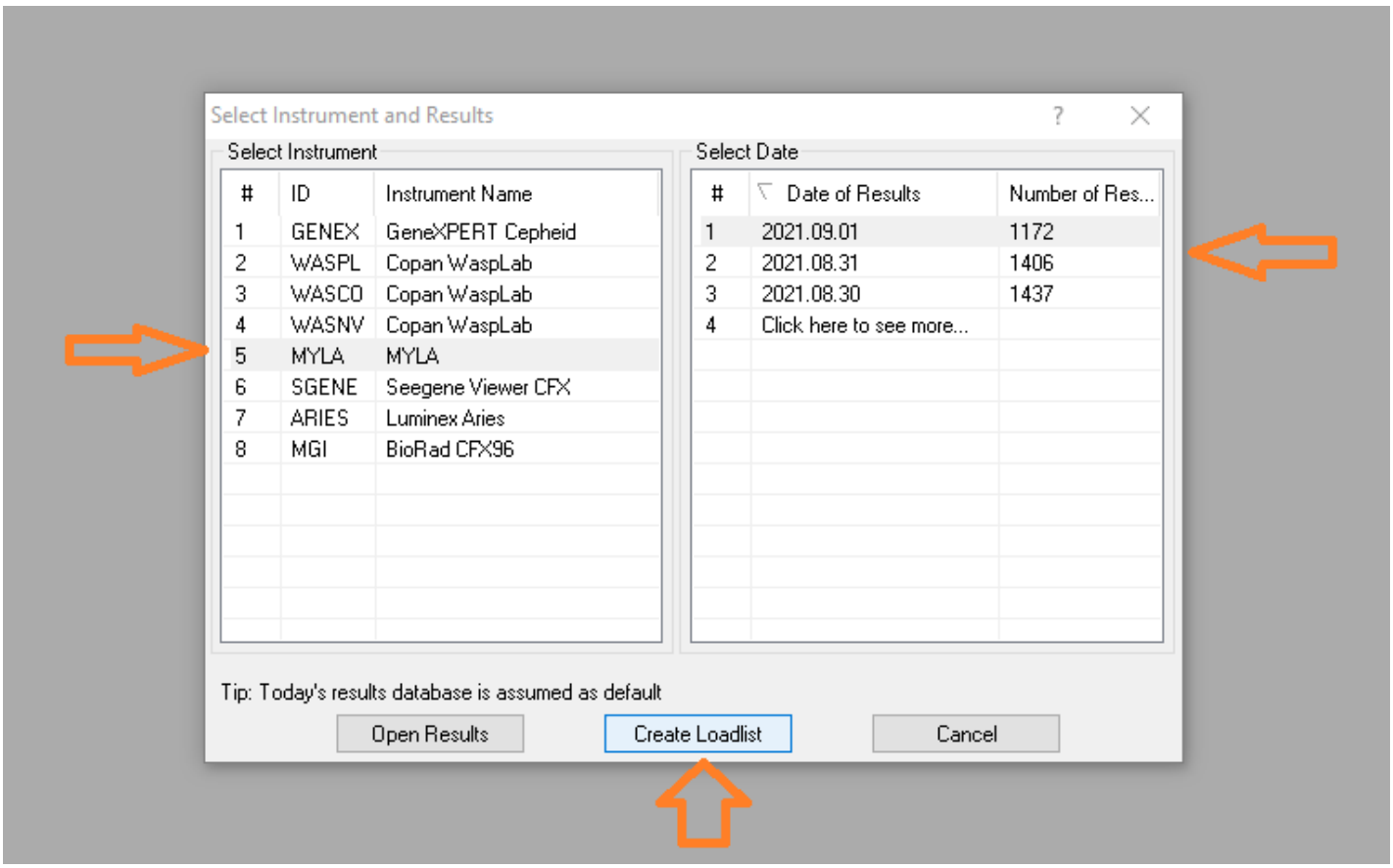
Appendix IV – How to fix orphan bottles sending patient information from LIS

How to fix Orphan Bottles from the 1st of the month

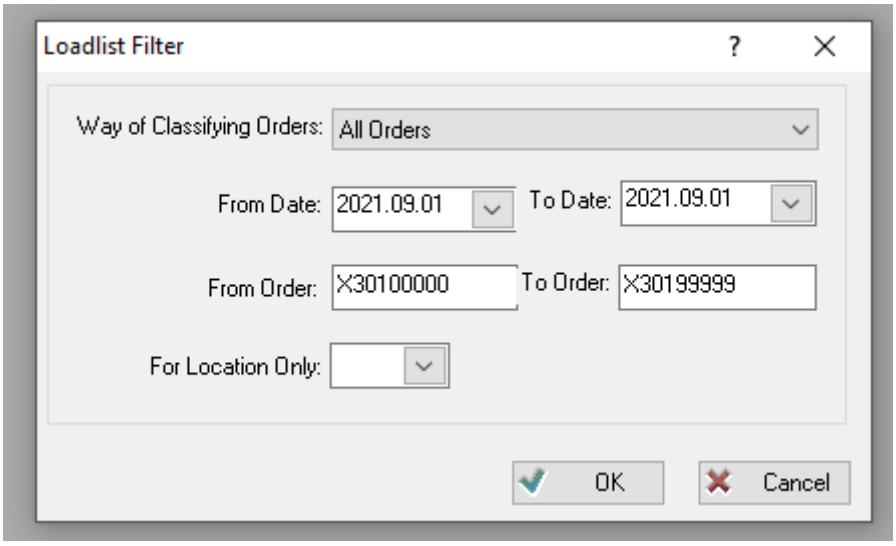
Instrument menu



Choose Myla
Create Loadlist from the first of the month (some may also be in the last day of the previous month)

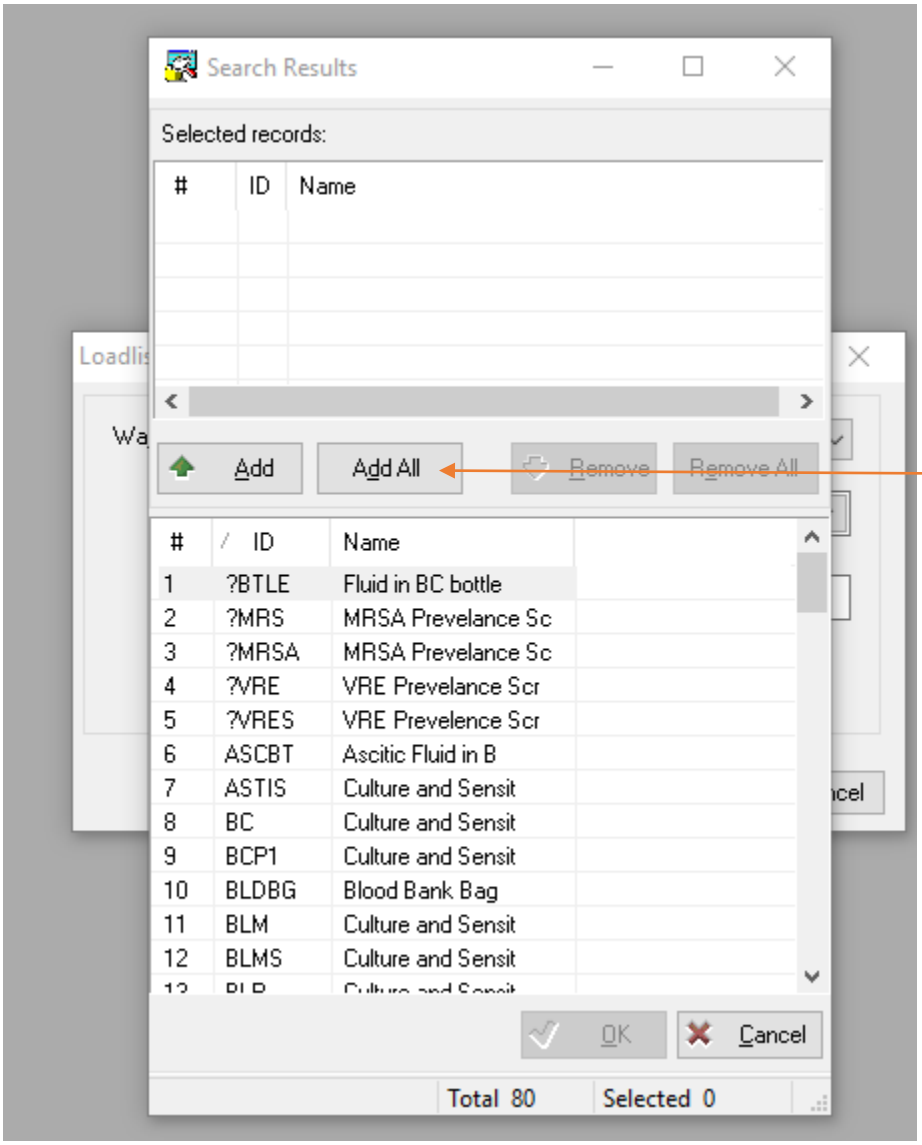


When you click on Create Loadlist the next screen will come up. Make sure the date you want shows.

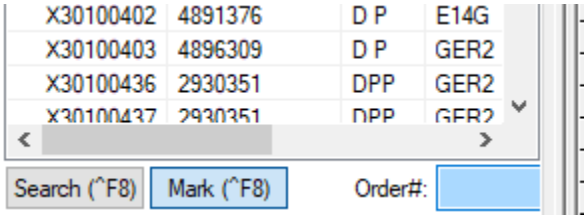


Click OK

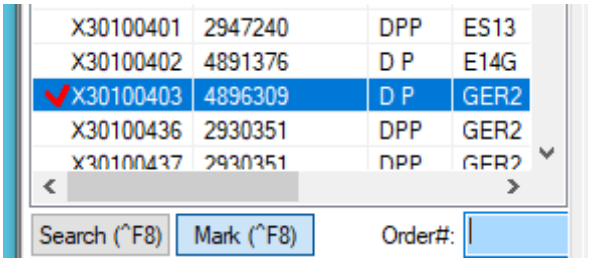
Add all. OK (Ideally choose less i.e. BC, BC1, C&S, ASCBT and ASTIS and just add those)



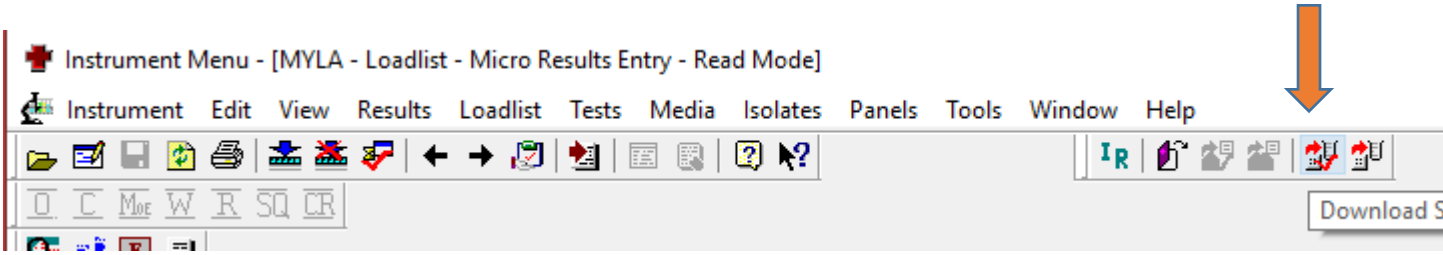
You must search at the bottom by Order number. It has to be a Soft Order number so if the BC only has an auxiliary Order number from UHN on it, look up the Soft number first.



If it finds the order there will be a red check mark on the left.



Download ONE at a time. This is important. It will crash the interface if you try to do more than one at a time. Hovering over the arrow will show you what you are on.
It will also show you what you are on at the bottom left of the screen.



If all of the above does not solve the orphan bottle issue, and it is a UHN specimen, a merge of MRNs may be required.

Appendix V – Orphan troubleshooting in EPIC

After logging into EPIC, to investigate the problem

1. Choose "Specimen Inquiry" - by specimen
    a. Scan UHN barcode
    b. Check to see if receiving and packing list are done
2. If packing & receiving is needed, close windows, then choose "Specimen Lookup"
    a. Scan barcode
    b. Click "Receive"
    c. Enter reason if needed
3. To put on packing list, choose "Packing List editor"
    a. Click "Create"
    b. Find UHN Sinai Packlist (ex. 123456789), then "Accept"
    c. Under "add specimen", scan barcode
    d. Click "+add"
    e. Click "Ready", and "Picked up"

Record of Edited Revisions

Manual Section Name: Blood Culture Manual

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	May 30, 2001	Dr. T. Mazzulli
Annual Review	May 30, 2002	Dr. T. Mazzulli
Annual Review	May 30, 2003	Dr. T. Mazzulli
Page 15, reporting of *S. aureus* to ID resident	July 27, 2004	Dr. T. Mazzulli
Handling of special request for *Brucella*	December 17, 2004	Dr. T. Mazzulli
Reload all false positive bottles regardless of the number of times flagged.	December 17, 2004	Dr. T. Mazzulli
See Bacteria work-up manual for isolate work-up	December 17, 2004	Dr. T. Mazzulli
Refer to previous isolate up to 72 hours; same with *Enterococcus*	December 17, 2004	Dr. T. Mazzulli
Refer to previous isolate up to 72 hours for freezing.	December 17, 2004	Dr. T. Mazzulli
Do not report the number of bottles positive	December 17, 2004	Dr. T. Mazzulli
New troubleshooting Reports 6 and 7	December 17, 2004	Dr. T. Mazzulli
Remove subculture on SAB for yeast page 7	December 17, 2004	Dr. T. Mazzulli
Annual Review	December 17, 2004	Dr. T. Mazzulli
CHC/Ajax No need to call ward with sensitivities and ID Page 12	April 13, 2005	Dr. T. Mazzulli
Call ID physician including S. aureus and SPICE bugs	September 21, 2005	Infectious Disease Physician
Annual Review	December 17,1005	Dr. T. Mazzulli
Blood collection procedure	January 15, 2006	Dr. T. Mazzulli
UHN Bone Marrow accessioning	January 15, 2006	Dr. T. Mazzulli
Annual Review	July 23, 2006	Dr. T. Mazzulli
Set up DENKA as soon as presumptive ID of S. aureus and enough growth	February 14, 2007	Dr. T. Mazzulli
CTRL U for false positives to remove LIS Positive flag	February 14, 2007	Dr. T. Mazzulli
Modify Mycology plates for ISOLATOR 10	June 29, 2007	Dr. T. Mazzulli
Annual Review	July 16, 2007	Dr. T. Mazzulli
Do not need to call ID and sensi to MSH wards	May 16, 2008	Dr. T. Mazzulli
Changed reporting positive gram as “isolate”	January 30, 2008	Dr. T. Mazzulli
Added SPICE bug reference and remove Cedecea from	January 30, 2008	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
SPICE list		
CNST Sensi change	February 13, 2008	Dr. T. Mazzulli
Appendix VI - Ajax Blood Culture processing changed to RVHS Blood culture Processing	April 01, 2008	Dr. T. Mazzulli
Discharged ER patients with S. aureus or SPICE bugs – stop call to ID.	May 16, 2008	Dr. T. Mazzulli
Annual Review	May 16, 2008	Dr. T. Mazzulli
Annual Review	June 01, 2009	Dr. T. Mazzulli
Change PD fluid incubation to 21 days per Hemodialysis Unit request	June 01, 2009	Dr. T. Mazzulli
Positive blood with yeast; add direct germ tube	June 01, 2009	Dr. T. Mazzulli
Positive blood with yeast or fungus, page ID resident/physician	July 24, 2009	Dr. T. Mazzulli
Annual Review	June 01, 2010	Dr. T. Mazzulli
Expanded TDNA reading instructions	November 23, 2011	Dr. T. Mazzulli
Annual Review	November 23, 2011	Dr. T. Mazzulli
Clarify Denka refer back, page 9	October 09, 2012	Dr. T. Mazzulli
Removed Appendix VI – Processing RVHS blood Cultures	October 09, 2012	Dr. T. Mazzulli
Updated Autopsy Blood work-up, reporting statement	December 05. 2012	Dr. T. Mazzulli
Annual Review	December 05. 2012	Dr. T. Mazzulli
CNST reporting phrase changed	May 13, 2013	Dr. T. Mazzulli
CNST reporting phrase changed back	July 16, 2013	Dr. T. Mazzulli
Removed CNST not S. lugdunensis susceptibility testing Set up susceptibilities on CNST in BC if isolated from patients with endocarditis	November 10, 2013	Dr. T. Mazzulli
CNST reporting phrase changed	November 10, 2013	Dr. T. Mazzulli
Annual Review	December 05, 2013	Dr. T. Mazzulli
Change to work up to MS	March 28, 2014	Dr. T. Mazzulli
Freeze **ALL** isolates from blood culture including Autopsy isolates	March 28, 2014	Dr. T. Mazzulli
Annual Review	March 28, 2014	Dr. T. Mazzulli
BC positive for S. aureus and yeast/fungi should be	July 20, 2014	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
called to Sick Kids ID as of Aug 1, 2014		
Host Not Responding Message	August 9, 2014	Dr. T. Mazzulli
-Autopsy blood: -Single isolate: minimum work up or MS & specified Mixed culture is > or equal to 2. -False positive cultures: - Moved last paragraph to work up of false positive → culture ~p.23 (this section is work up of initial pos bottle accessioning bloods side) -Processing of sub-culture: -successful ID from maldi is GREATER than or equal to 98% -Removed susceptibility comments; refer to manual and vitek ms isolate manual -removed reminder for DENKA, refer to sensitivity manual. - Updated AT LIS section of download problem with patient demographics. Removed section: APPENDIX V - Handling of Bone Marrow in Blood Culture Bottles from UHN is gone	September 10, 2014	Dr. T. Mazzulli
Revised sections for: **For Gram stain results** **For Culture results** **Infection Control Team Reporting** **Infectious Diseases Team Reporting**	December 1, 2014	Dr. T. Mazzulli
Revised: Infectious Diseases Team Reporting: (p.14)	March 4, 2015	Dr. T. Mazzulli
Reorganize Annual Review	March 11, 2015	Dr. T. Mazzulli
p.16 Modified Isolater processing to : Centrifuge blood at 3000g	November 12, 2015	Dr. T. Mazzulli
Annual Review Positive cultures: additional media added incubation criteria to table	March 4, 2016	Dr. T. Mazzulli
Manual bottle procedure modified to include further macroscopic examination: Examine all bottles for macroscopic growth twice daily for day 1 and day 2, record results in the LIS. For the remainder of the incubation period check bottles macroscopically at least daily and record in the LIS	June 16, 2016	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Annual Review Processing of positive BC moved to Specimen Processing Manual. Updated procedures from BacTAlert to Virtuo procedures.	February 23, 2017	Dr. T. Mazzulli
Updated Error reports for the Virtuo Added Report: • Bottles with missing patient information Removed Reports: • Bottle with wrong accession # • Negative to Date unloaded by mistake • Loading problem - bottle ID without accession number • Accession number with wrong bottle	March 7, 2017	Dr. T. Mazzulli
Added Virtuo Troubleshooting appendix with customer service information, and appendix for worklists/daily checks. Removed appendix I QC of Virtuo system, information added to BC manual under section BC Bench Pending Worklists and troubleshooting appendix.	May 08, 2017	Dr. T. Mazzulli
Annual Review Removed BC Rec'd/Plat'd worklist Added a step to identify orphan bottles within Virtuo Added BONNB New resulting worklist	March 09, 2018	Dr. T. Mazzulli
Updated note regarding ID notification: Anaerobes to be frozen. Removed from exception list. Old: DO NOT notify the Infectious Disease team if the patient is deceased, was seen in an outpatient clinic, discharged from the emergency department. New:DO NOT notify the Infectious Disease team if the patient is deceased, was seen in an outpatient clinic, discharged from the emergency department, or discharged from a ward (unless the patient had a previous positive during that admission).	April 13, 2018	Dr. T. Mazzulli
Removed new fluids from worklists. No need to add prelim status, now down automatically.	December 31, 2018	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Updated BC receiving worklist instructions with added troubleshooting instructions. Updated names of worklists to match LIS worklist names.		
Annual Review	April 23, 2019	Dr. T. Mazzulli
Annual Review	June 05, 2020	Dr. T. Mazzulli

Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
Updated E coli freezing instruction Referred phoning guideline to Internal Manual	November 19, 2020	Dorna Zareianjahromi
Added section on generating orphan report	February 11, 2021	Dorna Zareianjahromi
Updated Virtuo troubleshooting, other minor updates	March 11, 2021	Wayne Chiu
Updated Culture calling for ID and SENS	March 17, 2021	Wayne Chiu
Added section for handling expired bottles, minor formatting updates	March 30, 2021	Wayne Chiu
Minor formatting change	April 11, 2021	Jessica Bourke
Added S aureus auto comment, added note regarding vanco dependent enterococci	June 7, 2021	Wayne Chiu
Updated wording in Load List section, minor formatting	July 9, 2021	Wayne Chiu
Updated wording in appendix III, added hyperlink	July 14, 2021	Wayne Chiu
Specified instructions for checking worklist for fluid in BC bottles	Sep 1, 2021	Wayne Chiu
Added note – do not notify ID team for hemodialysis patient unless they are admitted	Mar 26, 2022	Wayne Chiu
Added section – how to fix patient inconsistency	June 6, 2022	Wayne Chiu
Updated LIS to EPIC, added Appendix IV for fixing orphan bottles	June 13, 2022	Wayne Chiu
Added Appendix V for EPIC troubleshooting	July 6, 2022	Wayne Chiu
Added note regarding aminopeptidase test to help differentiate overdecolorized GPB such as Bacillus sp	July 18, 2022	Wayne Chiu
Updated the vitro daily check for the resulting worklist of fluid in BC bottles		

Page Number / Item	Date of Revision	Edited by:
Added “notify MOH for reportable” to reporting section	Aug 19, 2022	Wayne Chiu
Specified do not report # of positive bottles or bottle type when notifying positive gram smears	Aug 23, 2022	Wayne Chiu
Updated Virtuo daily checks, BC receiving worklist to check samples not received after 24hrs. Added flowchart for orphan bottle troubleshotting	Sep 21, 2022	Wayne Chiu
Clarified the list of do-not notifying the Infectious Disease team (page 10)	June 23, 2023	Qin Liu
p.17 BC Receiving worklist procedure updated	September 18, 2023	Jessica Bourke
Added link to Virtuo user manual in Virtuo troubleshooting section	May 13, 2024	Jessica Bourke
P10 the clinical ID service has been updated to streamline the ID paging procedure.	July 2, 2024	Qin Liu