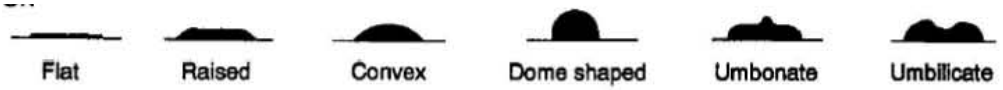
POLICY STATEMENT	Although exceptions may at times be required, it is necessary to establish a protocol for reporting cultures. Reporting is based upon what flora is cultured and from what source, and what significance these factors have on the majority of individuals. The physician and microbiologist alike should be aware of those individuals endogenously harboring organisms usually considered pathogenic, and those individuals in which indigenous flora may have initiated a disease process.
PURPOSE	The interpretation of primary cultures requires considerable skill. From initial observations, the microbiologist must assess the colonial growth and determine, depending on the source of the culture, whether any pathogens are present. Then proper steps should be taken to quantitate, identify the microorganisms, and perform appropriate antimicrobial susceptibility testing.
SCOPE	This procedure applies to technical personnel authorized to perform testing. This group includes, but is not limited to Medical Technologists as well as Leads and Supervisory personnel.
RESPONSIBILITY	All the above personnel are responsible for following this procedure. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.

PROCEDURE

1. Routine bacterial cultures are incubated for 24, 48 or 72 hours.
2. Examine all plates for macroscopic growth every 24 hours.
3. If no visible growth reincubate plates until final.
4. Examine growth for the following:

- Colony morphology – look at the plates from different angles and use direct illumination of the plate
- Hemolysis – use indirect illumination of the plate
- Pigment
- Growth/inhibition on selective media
- Reactions on differential media
- Odor – upon opening the plates (Do not directly smell the colony)
- Terms to describe gross colony morphology
  - Size (diameter in mm): Large = greater than 1 mm in diameter
    Medium = 1 mm in diameter
    Small = less than 1 mm in diameter
  - Shape:

  - Elevation



  - Margin (edge of colony)

  - Color: White, Black, Cream, Orange etc.
  - Surface Appearance: Glistening Dull
    Smooth Rough
    Granular Creamy
  - Density (ability to see through the colony):
    Opaque = cannot see through the colony
    Transparent = can see through the colony
    Translucent = only with light shining through
  - Consistency (best observed by picking up a colony with a loop or needle):
    Butyrous (buttery) Brittle
    Viscid (sticky) Membranous (pliable)
    Friable (crumbles easily)

5. Report colony counts according to following:
   - Examine all plates to distinguish suspected plate contamination from organisms actually in the culture.
   - Rare Growth = one to five colonies total
   - Light Growth = colonies in first quadrant only – ignoring a few colonies in second
   - Moderate Growth = colonies up to the second quadrant – ignoring a few colonies in third
   - Heavy Growth = colonies up to the third quadrant
   - Urine cultures = <10, 10-50, 50-100 or >100 CFU/ml
   - Catheter tip cultures = <15 CFU or >15 CFU
6. Perform Gram stains on colonies as needed
7. Perform appropriate initial rapid testing such as catalase, oxidase, indole, coagulase, etc
8. Isolate nonisolated colonies for further work up
9. See individual culture procedures for workup guidelines.
10. Set up definitive and susceptibility protocols on significant isolates and subculture.
11. Gram positive cocci in clusters → Staphylococcus
    ⇒ Catalase (positive)
    ⇒ Coagulase
    a. negative → Staph coag negative
       ⇒ Microscan - positive panel
    b. positive → Staph coag positive
       ⇒ Microscan - positive panel
       ⇒ PBP2
          - Perform on all Staph aureus isolates
          - positive → MRSA
          - negative → Staph aureus

12. Gram positive cocci in pairs and/or chains → Streptococcus

⇒ Catalase (negative)

⇒ Hemolysis (beta, alpha or nonhemolytic)

a. Beta hemolytic → Beta Strep

⇒ Strep Typing

⇒ Microscan – MicroStrep panel (performed only on request)

b. Alpha hemolytic →

⇒ Bile solubility (Desoxycholate)

⇒ Optochin (P) disc (≥ 14 mm)

⇒ PYR

- PYR positive; bile negative; P negative → Enterococcus species

  ⇒ Microscan - positive panel

- PYR negative; bile positive; P positive → Strep pneumoniae

  ⇒ Microscan – MicroStrep panel

  ⇒ If the Strep does not grow in the MicroStrep panel perform Etest.

- PYR negative; bile negative; P negative

  ⇒ Gram Stain

  - Gram positive cocci in pairs → Strep viridans group

    ⇒ Microscan – MicroStrep panel

    ⇒ If the Strep does not grow in the MicroStrep panel perform Etest.

  - Gram posisitve cocci in clusters/tetrads → Aerococcus

    ⇒ Microscan - positive panel

c. If non hemolytic →

⇒ PYR

- positive → Enterococcus species

  ⇒ Microscan - positive panel

- Negative →Strep viridans group

  ⇒ Microscan – MicroStrep panel

⇒ If the Strep does not grow in the MicroStrep panel perform Etest.

⇒ Be aware of non hemolytic Group B Streps

⇒ Aerococcus – α-hemolytic strep colony, Gram positive cocci in cluster/tetrads

13. Gram negative rods →

⇒ MAC (Maconkey)

a. LF = lactose fermenting gram negative rod
   ⇒ Microscan – gram negative panel
   ⇒ Microscan Rapid ID panel is available for ID only
b. NLF = non lactose fermenting gram negative rod
   ⇒ Oxidase (enter result in MicroScan)
   ⇒ Microscan – gram negative panel
   ⇒ Microscan Rapid ID panel is available for ID only
c. Non Fermenting (glucose) gram negative rods
   ⇒ Oxidase (enter result in MicroScan)
   ⇒ Microscan – gram negative panel
   ⇒ Microscan Rapid ID panel and Remel RapID NF is available for ID only
d. No growth MAC and small gram negative rods → Hemophilus
   ⇒ RapID NH
   ⇒ Cefinase
   ⇒ Kirby Bauer (If sterile body site)
e. Small curved gram-negative rod (Seagull shaped) → Campylobacter
   ⇒ oxidase
   ⇒ catalase
   ⇒ hippurate hydrolysis
f. No growth on MAC and small gram negative rods from gential culture and
   ⇒ Pinpoint beta hemolytic growth on V agar → Gardnerella vaginalis

14. Gram positive rods

⇒ Catalase

a. small pleomorphic gram positive rods → Diptheroids
   ⇒ alpha or non hemolysis
   ⇒ catalase positive
b. small gram positive rods → Listeria
   ⇒ beta hemolysis
   ⇒ catalase positive
   ⇒ Microscan - positive panel
c. large boxy gram positive rods → Bacillus
   ⇒ beta hemolysis
   ⇒ motility – from a broth culture – either from the blood bottle or TSB
   ⇒ if non hemolytic and non motile notify DHMH
d. thin, elongated gram positive rods usually in chains → Lactobacillus
   ⇒ alpha hemolysis
   ⇒ catalase positive
   ⇒ PYR variable
   ⇒ RapID ANA

15. Gram negative diplococci → Neisseria or Moraxella
   ⇒ Oxidase (positive)
   ⇒ Catalase (positive)
   ⇒ Catarrhalis disk (positive for Moraxella)
   ⇒ RapID NH
   ⇒ Cefinase
16. Anaerobes
   ⇒ Gram stain
   ⇒ Sub culture areobically and anerobically
   ⇒ Greater than two anaerobes report as Mixed Anaerobic Flora
   ⇒ RapID ANA
17. Yeast

⇒ Gram stain

⇒ "feet"

d. "feet" → Candida albicans (If sterile body site, perform a Germ tube)

e. No "feet"

⇒ Germ Tube

- germ tube positive → Candida albicans
- germ tube negative
  - o routine culture
    - ⇒ gram stain and colony morphology consistent with Candia → Candida species not albicans
    - ⇒ in urines only – morphologically and microscopically small →Candida glabrata
    - ⇒ gram stain and colony morphology consistent with Cryptococcus sub to SAB w/ Emmons and perform RapID Yeast after 48 hours
  - o fungus culture or a sterile body site
    - ⇒ Sub to SAB w/ Emmons and perform RapID Yeast after 48 hours

18. Physicians may request additional work up by ordering "MICRO ADD ON TO CULTURE WORKUP" and specifying their request.

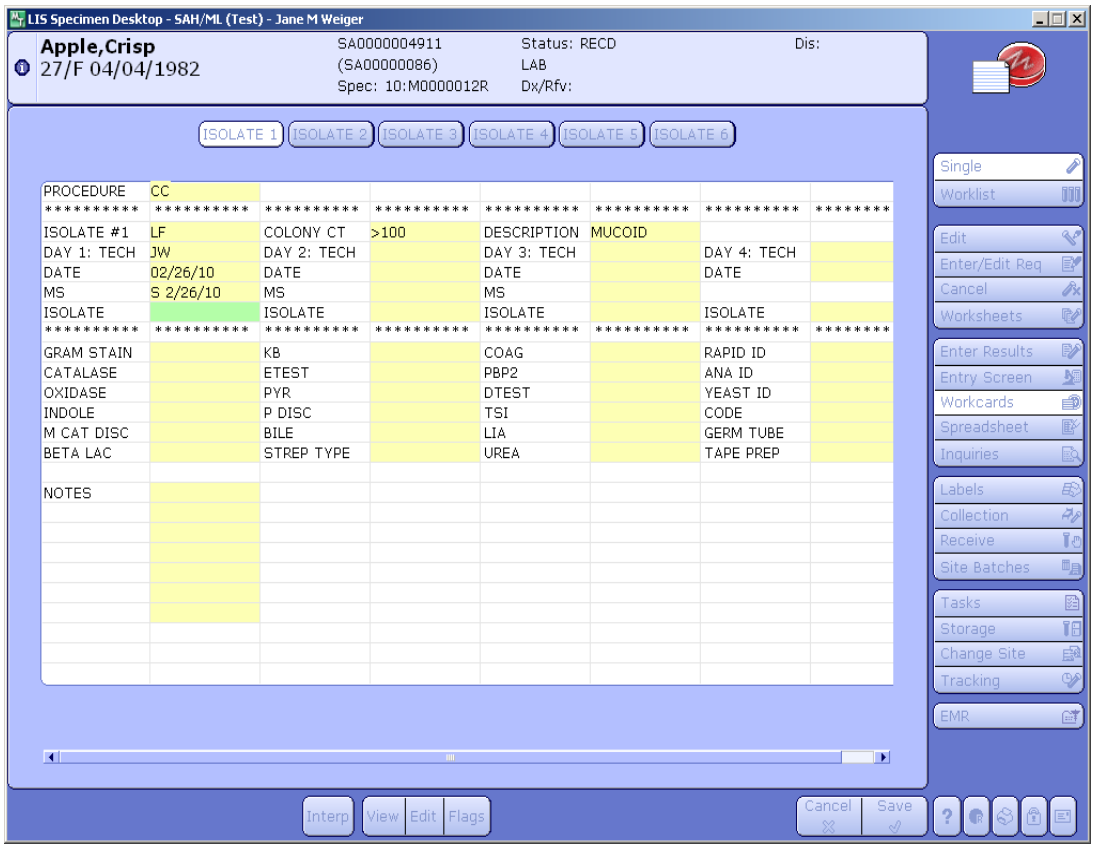
19. <u>MicroScan</u>

- Check the status of the **MSCAN interface** in Meditech under the MIC Analyzer Desktop to make sure the interface is running. Then select Process.
- To Download Specimens to the Microscan, select the Add/Remove, add specimens and enter the panel data and save.
- To Accept Transmission from the Microscan, select Transmissions, result and verify the organism and susceptibility results. Select Preliminary/Verified or Final/Verified and Save.

20. Refer susceptibility testing up to 3 days on same organism from same or simular sources. For isolates of Staph coag negative that meet the criteria for workup; workup each isolates from each culture and report the susceptibilities.
21. Notify the charge tech of problematic cultures immediately. Delays in identification can affect patient care.
22. Follow up identification and susceptibility testing procedures until all relevant isolates have been identified.

REPORTING

1. Enter preliminary reports on cultures updating them daily until final.
2. If the culture was processed after 4pm, enter preliminary report of “No growth < 24 hours” or “Culture in Progress.
3. Document all work in the workcard in the spaces provided. The isolate and quantity will show externally.



4. Update the workcard as testing is performed.
5. Enter the final identification when completed.

RELATED DOCUMENTS

- MICR 6140 R Specimen Processing
- MICR 6200 R Gram Stain
- MICR 6300 R Anaerobic cultures
- MICR 6310 R Body Fluid cultures
- MICR 6330 R Enteric cultures
- MICR 6335 R Environmental cultures
- MICR 6350 R Genital Cultures
- MICR 6370 R Respiratory Cultures
- MICR 6380 R Urine Cultures
- MICR 6390 R Wound Cultures
- MICR 6395 R Surveillance Cultures
- MICR 6405 R PathoDx Strep Grouping
- MICR 6410 R Bile Solubility
- MICR 6416 R Catalase
- MICR 6420 R Cefinase
- MICR 6425 R Coagulase
- MICR 6435 R LIA (Lysine Iron Agar)
- MICR 6440 R Catarrhalis Disk
- MICR 6445 R Optochin (Taxo P Disks)
- MICR 6450 R DrySlide Oxidase
- MICR 6455 R PBP2 Latex Agglutination Test
- MICR 6460 R PYR disks
- MICR 6465 R RapID ANA II System
- MICR 6469 R RapID NF PLus System

- MICR 6470 R RapID NH System
- MICR 6475 R Hippurate
- MICR 6478 R Spot Indole
- MICR 6480 R Staph Latex Test
- MICR 6485 R TSI (Triple Sugar Iron Agar)
- MICR 6490 R Urea Slants
- MICR 6510 R Etest
- MICR 6530 R Kirby Bauer
- MICR 6540 R Microscan Panels
- MICR 6570 R Microscan LabPro
- MICR 6730 R Germ Tube
- MICR 6790 R RapID Yeast Plus

REFERENCES

Garcia, L. Clinical Microbiology Procedure Handbook. 3nd Edition, Volume 1, American Society for Microbiology, Washington, D.C.