Troubleshooting Guide of PT Failures for Microbiology Programs

After looking at the Performance Report for failed results, it is important to go over the possible errors that could have happened throughout the testing process. This guide may help determine sample handling errors when it comes to testing Microbiology based PT samples.

Below are some of the items that could possibly affect sample integrity and the result reported for a sample:

Condition of samples upon receipt

- What temperature did you receive the samples at?

Storage of Samples upon receipt

- What temperature did you store the samples at upon receipt?

Handling instructions

- Did you read the handling instructions for the program before testing the samples?

Background/Patient History Information on Worksheets

- Is the organism you identified in the sample considered a pathogen for the sample source and/or case history information indicated on the Worksheet?

Sample Preparation

- Was the sample equilibrated to a specific temperature before testing?
- Was the sample reconstituted correctly?
- Was the sample diluted correctly?

Media and Culture Conditions

- Should you have used primary culture media or selective culture media?
- Did you read the sample source and case history information on the Worksheet to determine what media and growing conditions to use?

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Sample Handling Errors

If there was a problem with the reporting option you submitted for a sample, on your Performance Report, please review the following information for probable causes and/or solutions based on the type of testing.

- **Acid Fast Stain – Brightfield Microscopy**
    - Participants are to submit a qualitative reporting option for the quantity of Acid Fast Bacilli (AFB) using a Ziehl Neelsen stain and brightfield microscopy. The reporting options are based on the WHO/IUATLD recommended grading of sputum smear microscopy results which involve reading the slide with the 100x oil immersion objective.
    - Results for AFB using auramine stain with fluorescent microscopy cannot be reported at this time.

Problem	Probable Cause and/or Solution
If you reported negative for a sample that was positive for AFB, using Ziehl Neelsen stain	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o Slide was wiped instead of blotted during the drying step o Wrong side of the slide was stained o Carbol fuchsin prepared from poor quality reagents o Carbol fuchsin reagent has expired or was stored in direct sunlight o Carbol fuchsin staining time was less than 10 minutes o Slide was over decolorized o Check results reported for the positive control organism with the same lot of carbol fuchsin stain
If you reported positive for a sample that was negative for AFB, using Ziehl Neelsen stain	o The wrong sample was tested o Decolorization time was not long enough o Decolorizing solution has expired and could be contaminated o Carbol fuchsin has dried on the smear o Check results reported for the negative control organism with the same lot of carbol fuchsin stain

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- **Antimicrobial Susceptibility Testing**
  - Participants' results for antimicrobial susceptibility testing are evaluated based on the suggested antimicrobial groupings in the current version of the CLSI document "Performance Standards for Antimicrobial Susceptibility Testing: Informational Supplement, M100-SXX.
  - Based on the organism you isolated and the sample source indicated on the Worksheet, refer to the table in the CLSI document containing the suggested antimicrobial groupings for fastidious organisms or the table containing the suggested antimicrobial groupings for non-fastidious organisms. Results for other antimicrobials not listed in the CLSI document will be considered unacceptable (UNACC).

Problem	**Probable Cause and/or Solution**
If the antimicrobial reported is on the list of CLSI acceptable antimicrobials, and Sensitive was reported instead of Resistant	o Check your performance report to determine if you correctly identified the organism. The MIC interpretations may be different depending on the organism you identified. o Check the age of the inoculum source plate. Plate used to prepare inoculum should be 18-20 hours. o Check the concentration of organism used to inoculate the broth or agar MIC medium was at a high enough concentration for testing. o Check to make sure the antimicrobial used for testing is not expired and the QC organism tested with the same antimicrobial has a MIC within the acceptable range for the agar or broth MIC assay. o Check pH of the MIC media (pH should be 7.2 to 7.4) o Ca++ and/or Mg++ concentration too high or too low (for Tetracyclines and Aminoglycosides)
If the antimicrobial reported is on the list of CLSI acceptable antimicrobials, and Resistant was reported instead of Sensitive	o Check performance report to determine if you correctly identified the organism. The MIC interpretations may be different depending on the organism you identified. o Check to make sure the concentration of organism used to inoculate the broth or agar MIC medium was not above the acceptable concentration for testing. o Check to make sure the antimicrobial used

	for testing is not expired and the QC organism tested with the same antimicrobial has a MIC within the acceptable range for the agar or broth MIC assay. o Check pH of the MIC media (pH should be 7.2 to 7.4) o Ca++ and/or Mg++ concentration too high or too low (for Tetracyclines and Aminoglycosides)

- **Bacterial Culture and Identification**

The sample material sent out for the Bacterial Identification program will consist of aerobic, anaerobic or microaerophilic organisms. You must review the case history information and sample source information for each sample on your Worksheet in order to determine the media and culture conditions to use.

Review your records for that sample, noting your findings about gram stain and morphology (if performed), morphologic characteristics on growth media, results for biochemical tests etc.

Problem	Probable Cause and/or Solution
If an organism was reported and it was a negative sample	o There may be a contamination problem o The wrong sample was tested
If the wrong genus and/or species of organism was reported	o The wrong sample was tested o Improve ability to differentiate organisms based on growth patterns and biochemical tests ➢ Review your records for the sample, noting your findings about gram stain and morphology (if performed), morphologic characteristics on growth media, results for biochemical tests etc.
If no growth was reported and the sample contained a pathogen	o The sample was stored at the wrong temperature upon receipt o Check gram stain slide to see if organism was missed o Review Handling instructions to determine if sample was stored at the indicated temperature upon receipt and if sample was reconstituted correctly.

	o Review Worksheet for sample source and case history information for the sample. The PT provider may have indicated for you to set up the culture to microaerophilic or anaerobic conditions. If you don't do microaerophilic or anaerobic cultures at your location, submit a reporting option that indicates you don't do this type of testing at your location. Failure to do so will result in an UNACC for that sample on your performance report.

- **Blood Parasites**

The sample material sent out for the Blood Parasite program or Malaria program consist of Giemsa stained thick or thin blood smears. The 2 sample format Blood Parasites program (BLPA432) only consists of Giemsa stained thin blood smears.

Problem	**Probable Cause and/or Solution**
If the wrong genus and/or species of parasite was reported	Improve ability to differentiate parasites at different stages of growth. ➢ Compare parasite morphology with those described in a reference textbook. If unsure of the species, only report to the genus level. Failure to do so might result in an unacceptable grade. ➢ In thick films, organisms tend to be more compact and denser than in thin films, which might affect identification.
If parasite(s) were reported and the sample was negative for parasites	o The wrong slide was reviewed o Identified an element as a parasite ➢ Review the slide again and refer to reference textbooks to review the morphology of the parasite you thought you observed on the slide.
If no parasite(s) were reported and the sample was positive for parasite(s)	o The sample was stored at the wrong temperature upon receipt o The blood film wasn't scanned on a low power objective before going to a high power objective. ➢ First screen the smear at a low magnification (10× or 20× objective), to detect large parasites (microfilaria) then examine the

	smear using oil immersion objective. CLSI recommend scanning at least 300 oil immersion fields for the determination of "No Parasite Seen". o The sample may have contained a low concentration of parasite.

- **Dermatophyte Testing**

The DERS435 program is designed for participants to identify the presence/absence of dermatophytes on Dermatophyte Test Medium (DTM). Any other type of media is not suitable for this program.

In addition to observing for a red color change, the interpretation of DTM growth should include a review of the gross and microscopic colony morphology. Dermatophytes grown on DTM are usually lightly pigmented (white, tan, or cinnamon-colored), whereas common saprophytes are often darkly pigmented. Some organisms will grow on DTM after 48 hours but will change the DTM to a yellow color, which does not indicate the presence of a dermatophyte.

Problem	**Probable Cause and/or Solution**
If positive was reported and the sample was negative for dermatophytes	o There may be a contamination problem o The wrong sample was tested
If negative was reported and the sample was positive for dermatophytes	o The sample was stored at the wrong temperature upon receipt o The sample was reconstituted incorrectly o The wrong sample was tested o There may be a problem with the lot of DTM used for culture ➢ Review your records to confirm growth of the QC organism was acceptable on the lot of DTM you used for testing. o The culture conditions were not suitable for growth o There may have been a low concentration of dermatophyte in the sample

- **Genital Culture**

The sample material for the Genital Culture program consists of normal flora and/or pathogens isolated from the genital tract. Review the sample source information on your Worksheet to determine which media and culture conditions to use.

Problem	Probable Cause and/or Solution
If growth was reported and the sample was negative for organisms	o There may be a contamination problem o The wrong sample was tested
If the wrong genus and/or species of organism was reported	o Improve ability to differentiate organisms based on growth patterns and biochemical tests. ➢ Review your records for the sample, noting your findings about gram stain and morphology (if performed), morphologic characteristics on growth media, results for biochemical tests etc. o If unsure of the species, only report to the genus level. Failure to do so might result in an unacceptable grade.
If no growth was reported and the sample was positive for a pathogen	o The sample was stored at the wrong temperature upon receipt o The sample was incorrectly reconstituted o Review Handling instructions to determine if sample was stored at correct temperature upon receipt and if sample was reconstituted correctly. o Review Worksheet for sample source information. Based on the sample source, determine if correct media and incubation was used for culture.

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- **Gram Stain and Morphology**

The sample material for the Gram Stain program consist of unstained heat fixed smears.

Problem	**Probable Cause and/or Solution**
If "gram negative microorganism" was reported and the sample contained a "gram positive microorganism"	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o The slide was over-decolorized o Failure to include the mordanting (iodine) step
If "gram positive microorganism" was reported and the sample contained a "gram negative microorganism"	o The wrong sample was tested o The slide was under-decolorized o The decolorization step was too short or was omitted o The gram stain dried on the smear
If "no microorganisms observed" was reported and the sample contained a "gram negative microorganism" and/or "gram positive microorganism"	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o The slide was wiped instead of blotted during the drying step o The wrong side of the slide was stained o The slide wasn't reviewed on low power (10x objective). Look at many microscopic fields and in different areas of the slide.

- **Mold Culture**

Problem	**Probable Cause and/or Solution**
If growth was reported and there was no organism present in the specimen	o There may be a contamination problem o The wrong sample was tested
If the wrong genus and/or species of organism was reported	You need to improve your ability to differentiate organisms based on growth patterns and microscopic features. Macroscopic examination ▪ Colonial morphology ▪ Surface pigment ▪ Reverse pigment ▪ Growth on cycloheximide containing agar

	Microscopic examination ▪ tease mount or scotch tape preparation using Lactophenol Blue (LPCB) ➢ Under light microscope, examine the slide for the presence, shape, size and attachment of conidia. Compare features with those described in a reference textbook
If no growth was reported and the sample was positive for a pathogen	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o Review Handling instructions to determine if sample was stored at correct temperature upon receipt and if sample was reconstituted correctly. o Review the lactophenol cotton blue stained smear (if used at your location) to see if the organism was missed on the slide o Review the Worksheet for the sample source and case history information for the sample. Based on the case history and sample source information, determine if the correct media and incubation conditions were used for culturing the sample.

➢ **Neisseria gonorrhoeae Screening**

Problem	**Probable Cause and/or Solution**
If positive for N. gonorrhoeae was reported, and the sample was negative for N. gonorrhoeae	o There may be a contamination problem o The wrong sample was tested
If negative for N. gonorrhoeae or no growth was reported, and the sample was positive for N. gonorrhoeae	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o The sample was reconstituted incorrectly o The chocolate agar or selective media, such as modified Thayer-Martin, Martin Lewis or New York City medium, wasn't equilibrated to room temperature before inoculating with the sample. o The inoculated plate was not incubated in a

	carbon dioxide atmosphere. Check your QC records for the lot # of media and QC organism used to determine if there was a problem with the levels of CO_2.

- **PVA Smear**

The sample material for the PVA Smear program consists of mercury fixed PVA fecal smears. Refer to the case history information on the Worksheet for details regarding the history of the patient.

Problem	**Probable Cause and/or Solution**
If the wrong genus and/or species of parasite was reported	Need to improve ability to differentiate parasites at the different stages of growth. ➢ Compare the parasite morphology with those described in a reference textbook. ➢ If unsure of the species, only report to the genus level. Failure to do so might result in an unacceptable grade.
If a parasite was reported and the sample was negative for parasites	o There may be a contamination problem o The wrong sample was tested o There may be an artifact in the sample. Artifacts should be considered on the basis of size, shape, lack of organelles and defining feature, and variable reactivity with common stains ➢ Compare the morphology with those described in a reference textbook
If no parasites were reported and the sample was positive for parasites	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o The entire coverslip wasn't scanned on the 10x objective at low light before going to the high power objective. ➢ If you see something suspicious on the low power objective, go to the 40x objective to see more detailed morphology of the element in question. High dry power examination should include at least one third of the smear area.

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- **Streptococcus A Screening**

Problem	Probable Cause and/or Solution
If positive for Group A Streptococcus was reported, and the sample was negative for Group A Streptococcus	o There may be a contamination problem o The wrong sample was tested
If negative for Group A Streptococcus was reported, and the sample was positive for Group A Streptococcus	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o The sample was reconstituted incorrectly o The lot number of selective media used may have expired o The culture conditions were not suitable for growth

- **Urine Colony Count**

Problem	Probable Cause and/or Solution
If a result above or below the acceptable value was reported	o The wrong sample was tested o Review the handling instructions for the program, to make sure you reconstituted and/or diluted the sample properly. o Make sure you reported your results in the same units as the reporting options in OASYS. The reporting options for urine colony count on the OASYS website are in colonies/mL.
If growth was reported and the sample was negative for organisms	o The wrong sample was tested o There may be a contamination problem ➢ Check growth of the QC organism on the lot number of media you used ➢ The lot of media used may have expired
If no growth was reported for a positive sample	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o The culture conditions were not suitable for growth o The sample was reconstituted incorrectly ➢ Review Handling instructions

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- **Urine Culture**

Problem	Probable Cause and/or Solution
If growth was reported and the sample was negative for organisms	o There may be a contamination problem o The wrong sample was tested
If the wrong genus and/or species of organism was reported	Improve ability to differentiate organisms based on growth patterns and biochemical tests ➢ Review your records for the sample, noting your findings about gram stain and morphology (if performed), morphologic characteristics on growth media, results for biochemical tests etc.
If no growth was reported for a positive sample	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o The culture conditions were not suitable for growth o The sample was reconstituted incorrectly ➢ Review Handling instructions

- **Wet Mount**

The sample material for the Wet Mount program consists of formalin preserved fecal suspension. Refer to the case history information on the Worksheet for details regarding the history of the patient.

Problem	Probable Cause and/or Solution
If the wrong genus and/or species of parasite was reported	Improve ability to differentiate parasites at different stages of growth. ➢ Compare the parasite morphology with those described in a reference textbook. ➢ If unsure of the species, only report to the genus level. Failure to do so might result in an unacceptable grade.
If parasite(s) was reported and the sample was negative for parasites	o There may be a contamination problem o The wrong sample was tested o There may be an artifact in the sample. Artifacts should be considered on the basis of size, shape, lack of organelles and defining feature ➢ Compare the morphology with those described in a reference textbook

If no parasite(s) was reported and the sample was positive for parasites	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o The entire coverslip wasn't scanned on the 10x objective at low light before going to the high power objective. ➢ If you see something suspicious on the low power objective, go to the 40x objective to see more detailed morphology of the element in question. High dry power examination should include at least one third of the coverslip area.

- **Yeast Culture**

Problem	Probable Cause and/or Solution
If growth was reported for a negative sample	o There may be a contamination problem o The wrong sample was tested
If the wrong genus and/or species of organism was reported	Improve ability to differentiate organisms based on growth patterns on selective media, results from germ tube test, urease test and other biochemical tests.
If no growth was reported for a positive sample	o The sample was stored at the wrong temperature upon receipt o The wrong sample was tested o Review Handling instructions to determine if sample was stored at correct temperature upon receipt and if sample was reconstituted correctly. o Review gram stain slide (if performed) to see if the organism was missed o Review Worksheet for sample source and case history information. Based on case history and sample source information, determine if the correct media and incubation conditions were used for culture.