

MIXED CULTURE EXERCISE IN POST GRADUATE EXAMINATION

For pure culture refer to my previous article at www.microrao.com/micronotes/pg/pure_culture.pdf
The reader is strongly advised to read pure culture article before proceeding with mixed culture article.

When both pure and mixed cultures are given at the same time, students must proceed with mixed culture exercise first. This is because of possible interactions among the mixed population that may yield variable result. It is often seen that *Pseudomonas* whenever present tend to inhibit the other members in the mixed culture, hence a delay in processing may result in poor or no growth of the other members.

Usually mixed cultures are provided disguised as clinical specimen with relevant clinical findings. Sometimes these findings are vague and student may ask the examiner with specific questions that may be helpful. While some examiners appreciate this attitude, some others may scoff at it. The history and symptoms may give clue to the possible pathogen and contaminant/commensal. Students are expected to identify the pathogen and commensal or contaminant and issue a suitable report at the end. While some examiners expect all the isolates to be identified in detail, some others may expect identification in detail of only the pathogen. It is advisable to clarify what is expected of them by asking the examiners. Some examiners may expect identification of isolates using all possible media and tests, other may expect the students to use minimal media and tests (that are routinely performed) to identify the isolates. Once again, clarification with the examiners may be made.

The mixed cultures are often given in test tubes as suspensions that are prepared only a few minutes prior or rarely an overnight incubated suspension may be given to the student. If the suspension is recently prepared, it may be incubated for 30 minutes at 37°C. Some examiners don't entertain this idea and expect the mixed suspension to be processed immediately just as a clinical specimen is processed. Common samples that are given are urine, feces, csf, sputum or blood. Upper respiratory tract specimens and pus samples may be given in broth suspension or swabs. There is plenty of variation in the cases and the specimen given to the students.

DAY ONE:

It is vital that the students considers the mixed suspension as a real "clinical specimen" and process it accordingly. Depending on the provisional clinical diagnosis and the possible etiological agents suitable culture media must be chosen. Before the suspension is cultured, it is essential to perform microscopy examinations such as Gram stain and hanging drop preparation. While Gram stained smear is compulsory for all the specimens (except non-cholera fecal specimen), hanging drop is applicable only to fecal specimens. Strangely, some examiners insist on hanging drop on all liquid specimens. Even though gram smear on fecal specimen is not routinely performed, unfortunately some examiners insist upon it. Unstained wet mounts are useful in detecting pus cells, RBC and parasitic forms. A wet mount should be performed on fecal, urine and CSF specimens. Iodine wet mount on fecal specimen may be performed if dysentery is suspected. Methylene blue wet mount may be performed on fecal specimen if enteric fever is suspected. India ink wet mount may be performed on CSF samples if meningitis is suspected. Although it is impossible to find any cellular or parasitic forms in these wet mounts, the

negative observation must be reported as it is. Student must be aware of the importance of the Gram stained smear; it is possible that the student may miss one or two forms. It is also possible that the student may see only one form but subsequent cultures may yield more than one type of bacteria. The decision to centrifuge the sample may be taken according to the clinical specimen. Ultimately, it is important to project that the student is actually aware of the correct procedure of processing the sample.

Gram stained smear result should aid in selection of media. A simple medium, an enriched medium, a selective medium or/and an enrichment medium may be selected according to the case. The requirement of these media must be clearly written down in the answer script. The student should be able to explain convincingly the need for each of the selected medium as well as the reasons for omitting certain other media.

CASES & POSSIBILITIES:

- Non-cholera diarrhoea (children or adults): Nutrient agar (optional), MacConkey's agar, Blood agar (avoidable), XLD (optional)
- Cholera: Nutrient agar, MacConkey's agar, Blood agar (optional), TCBS agar, Alkaline peptone water
- Enteric fever (feces): MacConkey's agar, XLD/DCA/SS agar, Wilson & Blair's agar, Selenite F /tetrathionate broth, nutrient agar (avoidable/optional)
- Bacteremia (typhoid/endocarditis): MacConkey's agar and Blood agar
- Meningitis (CSF): MacConkey's agar and Blood/chocolate agar
- UTI: CLED agar/ MacConkey's agar and Blood agar
- Abscess (pus): MacConkey's agar and Blood agar
- Pharyngitis/Diphtheria (throat swabs): Blood agar/chocolate agar (variable), Loeffler's serum slope & Potassium tellurite agar (for diphtheria).
- Lower respiratory tract infection (sputum): MacConkey's agar, Blood/chocolate agar (accordingly)

The culture plates must be streaked with perfection to obtain as many isolated colonies. The turbidity of the suspension should be observed before streaking and the number of lines and the volume of the inoculum should be adjusted accordingly. Standard procedures for surface streaking must be followed. Success of mixed culture exercise depends on the students' ability to produce well isolated colonies on agar surfaces. The agar plates and enrichment broths (if any) must be incubated immediately at 37°C. If pneumococci is suspected, the plates may be placed in candle jar and incubated. If enrichment broth such as alkaline peptone water or selenite F broth is used, subculture on to MacConkey's agar must be made on the same day before leaving.

DAY TWO:

Upon arrival the student must observe all the inoculated plates carefully for the types of bacteria, their distribution and morphology. The student must label the individual type accordingly as (type 1, type 2 etc) and their appearance must be described (as mentioned in pure culture article) on all the media. It is possible that one medium may yield two (or more) types of colonies and another medium may yield one only type. More colonies may be seen on simple/low selective medium and fewer colonies on selective medium. The various colony types must be correlated on all the media used.

Example:

Colony morphology on Blood agar:

Type 1 colonies: Medium sized.....whatever!

Type 2 colonies: Large, irregular.....whatever!

Colony morphology on MacConkey's agar

Type 1 colonies: Medium sized.....whatever!
Type 2 colonies: Large, irregular.....whatever!

Gram stained smear, oxidase test, catalase test and hanging drop preparation should be performed accordingly depending on the type of bacteria isolated. Please refer to the pure culture article for more information on this. Once the organisms are distinctly isolated, each must be processed as if it were a pure culture. Some examiners expect the student to process only the likely/relevant pathogen and ignore the commensal/contaminant but some others expect the student to process all the types of bacteria grown. Student may ask the examiner for their preference. Antibiotic susceptibility of only the pathogen (if required) should be performed. The recent CLSI guidelines on its technique must be known.

DAY THREE:

This involves reading the results of biochemical tests, performance of additional test to identify/ subtype or confirm the isolate (as for pure culture) and interpret the antibiotic susceptibility report.

REPORT:

After the isolates are identified, the student must be able to name the pathogen and contaminant/commensal isolated. Although a routine report never mentions the commensal isolated, some examiners may expect the report to contain a mention of contaminants/ commensals too. The antibiotic susceptibility (if applicable) of only the pathogen must be presented along with the final report.

- This article may not be in its complete form and may need some edition.
- Since the procedure and methods followed in this exercise widely varies, student is advised to follow the pattern according to the center's own policies.
- The contents of this article is based on my own experience & may be used under ones own risk.
- Document prepared on 18th March 2009.