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MICROBIOLOGY EXTERNAL QUALITY ASSESSMENT SCHEME
Under the aegis of Indian Association of Medical Microbiologists
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MARCH 2020 104th EQAS PACKAGE – EVALUATION SCHEMA & ANSWER TEMPLATE

BACTERIOLOGY SMEARS:

Question: Carry out the appropriate staining procedure and document the relevant observation.
Evaluation format:
Presence and grading of host cells & debris (many/ moderate/few/no) To be described in terms of pus cells and epithelial cells **(1 mark)**
Presence & grading of organism/s gram stain finding**,** morphology (shape), arrangement and any other special characteristics observed **(2 marks)**
Interpretation: Probable organism or Impression- as asked in the question **(1 mark)**

Exercise Number	Question	Report	Marks & Comment
SM1	Please carry out a Gram stain on the given fixed smear prepared from a voided URINE specimen of a 63-year old diabetic lady with a history of mild dysuria and increased frequency for 2 days.	**Presence of host cells & debris** (1 mark): Occasional pus cells, Many epithelial cells. **Description of Organism/s** (2marks: each organism description 0.5 marks): Many Gram positive spherical cocci in pairs, chains, groups. Many Gran negative bacilli (slender and thick). Many Gram positive bacilli, Moderate oval budding yeast like organisms. **Impression/comment** (1 mark): Improperly collected specimen. Suggest repeat appropriately collected mid-stream clean catch specimen for culture.	4 marks No marks deducted for an incorrect grading
SM2	Please carry out a Gram stain on the given fixed smear prepared from an EXUDATE specimen of a 23-year old man presenting with an exudative lesion on the right leg associated with high grade fever, chills and myalgia for 2 days	**Presence of host cells & debris** (1mark): Many pus cells. **Description of Organism/s** (2 marks): Many Gram positive spherical cocci in pairs and chains. **Possible organism** (1 mark): Streptococcus species (*Probably S. pyogenes*)	4 marks No marks deducted for an incorrect grading

SM3	Please carry out a Gram stain on the given fixed smear prepared from an ENDOTRACHEAL ASPIRATE specimen of a 69-year old gentleman admitted in the ICU with worsening saturation.	**Presence of host cells & debris** (1mark): Many pus cells. **Description of Organism/s** (2 marks): Many Gram negative cocco-bacilli **Possible organism** (1 mark): Probable NFGNB- *Acinetobacter* spp	4 marks No marks deducted for an incorrect grading

BACTERIOLOGY CULTURE:

Question: A freeze-dried (lyophilized) culture of an organism isolated from a clinical specimen is given. Carry out the appropriate techniques for each exercise and identify the pathogen. Carry out the antimicrobial susceptibility testing according to the panel given below.

EVALUATION FORMAT:

Culture microscopy& identification: (7 marks) Microscopy (1mark), culture characteristics (2 mark), Biochemical key identification characteristics (2 marks), Final identification (2 marks)

Susceptibility testing: (2 marks per drug)

For culture identifications or susceptibility tests that have been performed by automated systems, the printouts of the automated report MUST be attached along with the report for the evaluator's reference.

Susceptibility interpretation errors:

Minor error (mE) : Susceptible / resistant isolate reported as intermediate susceptible (1 mark deducted)

Major error (ME) : Susceptible isolate reported as resistant (2 marks deducted)

Very major error (VME) : Resistant isolate reported as susceptible (3 marks deducted)

- ✓ VITEK/ E-test MIC will be awarded the complete mark if the interpretation is consistent with the expected report.
- ✓ NEW: Only ONE FINAL susceptibility report for each drug tested should be reported. If two reports with discrepant interpretations are reported, they will be marked as an incorrect answer. (eg. one DD and one automated/MIC report)

☞ NOTE: Incomplete exercises : NOT evaluated.

CU1: Isolated from a FECES specimen of a 26year old gentleman with a 2-day history of diarrhea and vomiting associated with abdominal pain.

Microscopy: Gram stain and motility (1 MARK)	**Culture characteristics (2 MARKS)**	**Biochemical identification (2 MARKS) Main/Key characteristics required for identification of the organism. (Minimum:3 key characteristics)**	**Method used**
Gram stain: Gram negative comma shaped bacilli Dil Carbol Fuchsin: Comma shaped pink bacilli Actively motile: Darting motility	NA: Non-mucoid small opaque greyish-white colonies BA: Beta hemolytic grey colonies MA: Non-mucoid non-lactose fermenting, smooth glistening colonies TCBS: Non-mucoid, smooth flat yellow colonies	Oxidase: Positive Catalase: Positive Nitrates : Reduced to nitrites OF Glucose: Fermentative MMTPC: ++ +/+ + + (No gas/H2S) Decarboxylase test: Lysine & Ornithine decarboxylated. Arginine not dihydrolysed. Sucrose: Fermented Arabinose: Not fermented Mannose: Fermented Voges Proskauer: Positive Griegs test: Positive PB50: Resistant *Special tests:* String tests, Nitroso-indole test	Methods applicable: 1. Manual conventional biochemical identification 2. Automated methods - Eg: Vitek 2 /MALDI-TOF Optional/ confirmatory method: PCR- 16S rRNA
FINAL IDENTIFICATION (2 MARKS)	Genus ***Non-agglutinating Vibrio***	Species ***cholerae***	Serotype (if applicable) **Non O1- Non O139**

Antibiotics	**Ampicillin (10µg)**	**Tetracycline (30µg)**	**Co-trimoxazole (25µg)**	**Cefotaxmine (30µg)**	**Ciprofloxacin (5µg)**
Zone size (mm) **MIC(µg/ml)**	**≥17** **≤8**	**≥15** **≤4**	**≥16** **≤2-38**	**≥26** **≤1**	**≥21** **≤1**
Interpretation (S / MS /R)	**S**	**S**	**S**	**S**	**S**

(Abbreviations: NA nutrient agar, BA blood agar, MA MacConkey agar, TCBS Thiosulphate citrate sucrose bile salt sucrose agar, MMTPC preliminary screening media- mannitol motility medium, triple sugar iron agar, peptone water, citrate)

CU2: Isolated from a URINE specimen of a 35year old gentleman with hemiplegia on a long-term urinary catheter

Microscopy: Gram stain and motility (1 MARK)	**Culture characteristics (2 MARKS)**	**Biochemical identification (2 MARKS) Main/Key characteristics required for identification of the organism. (Minimum:3 key characteristics)**	**Method used**
Slender Gram negative bacilli Motile	NA: Pinkish-orange colonies BA: Non hemolytic smooth pigmented colonies MA: Non-mucoid non-lactose fermenting pigmented colony Prodigiosin (reddish- orange pigment) +	Oxidase: Negative Catalase: Positive MMTPC: ++ -/+ - + (No gas/H2S) SUCROSE: Fermented Sorbitol: Fermented Lysine iron agar: Decarboxylated Decarboxylase test: Lysine & Ornithine decarboxylated. Arginine not dihydrolysed DNAase test: Positive Gelatin: Liquefied Pb300: Resistant	Methods applicable: 1. Manual conventional biochemical identification 2. Automated methods - Eg: Vitek 2 /MALDI-TOF Optional/ confirmatory method: PCR- 16S rRNA PCR: Positive for *amp* C & ESBL -*veb* genes
FINAL IDENTIFICATION (2 MARKS)	Genus ***Serratia***	Species ***marcescens***	Serotype (if applicable)

Antibiotics	**Cefpodoxime (10µg)**	**Co-trimoxazole (25µg)**	**Amoxicillin-clavulanate (20/10µg)**	**Levofloxacin (5µg)**	**Meropenem (10µg)**
Zone size (mm) **MIC(µg/ml)**	**18-20** **4**	**≥16** **≤2-38**	**6** **>32**	**≥21** **≤0.5**	**≥23** **≤1**
Interpretation (S / MS /R)	**Caution use**	**S**	**Intrinsic resistance**	**S**	**S**

(Abbreviations: NA nutrient agar, BA blood agar, MA MacConkey agar, MMTPC preliminary screening media- mannitol motility medium, triple sugar iron agar, peptone water, citrate)

CU3: Isolated from an EXUDATE specimen from a 22year old lady with a history of extensive burns to her right forearm.

Microscopy: Gram stain and motility (1 MARK)	**Culture characteristics (2 MARKS)**	**Biochemical identification (2 MARKS) Main/Key characteristics required for identification of the organism. (Minimum:3 key characteristics)**	**Method used**
Spherical Gram positive cocci arranged in groups Non-motile	NA: Cream colonies BA: Beta hemolytic cream colonies MA: small opaque colonies MSA: Small yellow colonies	Catalase: Positive Clumping factor: Positive Tube coagulase test: Positive Mannite: Fermented OF Glucose: Fermentative Trehalose: Fermented Ornithine: Not decarboxylated DNAase test: Positive Gelatin: Liquefied Voges Proskauer: Positive Furazolidone: Susceptible Novobiocin- Susceptible PB300: Resistant	Methods applicable: 1. Manual conventional biochemical identification 2. Automated methods - Eg: Vitek 2 /MALDI-TOF Optional/ confirmatory method: PCR- 16S rRNA PCR: *mec* A gene +
FINAL IDENTIFICATION (2 MARKS)	Genus **(MRSA) *Staphylococcus***	Species ***aureus***	**Serotype (if applicable)**

Antibiotics	**Cefoxitin (30µg)**	**Erythromycin(15µg)**	**Co-trimoxazole (25µg)**	**Clindamycin(2µg)**	**Linezolid(30µg)**
Zone size (mm)	**≤21**	**≥23**	**≥16**	**≥21**	**≥21**
MIC(µg/ml)	**≥8**	**≤0.5**	**≤2-38**	**≤0.5**	**≤4**
Interpretation (S / MS /R)	**R**	**S**	**S**	**S**	**S**

(Abbreviations: NA nutrient agar, BA blood agar, MA MacConkey agar, MSA mannitol salt agar)

Serology:

Marking System

Parameter	Accepted unit of measurement	Qualitative		Quantitative / Semi - Quantitative	
CRP	mg/L	Observation: Positive/Negative	Report: Positive or Negative (2 marks)	Observation: Value in mg/L (1 mark)	Report: Positive or Negative (1 mark)
RF	IU/mL	Observation: Positive/Negative	Report: Positive or Negative (2 marks)_	Observation: Value in IU/mL (1 mark)	Report: Positive or Negative (1 mark)

104th EQAS SEROLOGY EXPECTED RESULTS – C-Reactive Protein

Samples	SE1	SE2	SE3	SE1	SE2	SE3	SE1	SE2	SE3	SE1	SE2	SE3
Method	Turbidimetry			Nephelometry			Latex Agglutination			Others		
Expected Result	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Positive
Values for z Score (-2 to +2 ranges)	19.61 - 54.40	0.00 - 5.00	4.8 - 16.6	17.8 - 44.96	0.02 - 3.40	5.78 - 13.7	0.024 - 40	Not Applicable	Not Applicable	24.7	0.20 - 9.00	7.40 - 23.0
Values for z Score (-2 to -3 ranges)	10.8 - 17.5	-	2.37 - 4.7	9.61 - 15.41	-	4.21 - 5.05	-	Not Applicable	Not Applicable	14.2 - 19.38	-	1.2
Values for z Score (+2 to +3 ranges)	55.2 - 60.0	5.14 - 6.32	17.0 - 19.0	45.97 - 47.7	3.56	13.8	46-48	Not Applicable	Not Applicable	-	9.8 - 10	24.7
Observed range of values from Participants	1.00 - 312	0.00 - 39.65	0.22 - 241.8	0.40 - 53.3	0.0 - 30.2	0.13 - 70.0	0.02 - 240	0 -12	0 - 96	2.20 - 61.50	0.20 - 14.3	1.20 - 24.7

104th EQAS SEROLOGY EXPECTED RESULTS – Rheumatoid Factor

Samples	SE1	SE2	SE3	SE1	SE2	SE3	SE1	SE2	SE3	SE1	SE2	SE3
Method	Turbidimetry			Nephelometry			Latex Agglutination			Others		
Expected Result	**Negative**	**Negative**	**Positive**	**Negative**	**Negative**	**Positive**	**Negative**	**Negative**	**Positive**	**Negative**	**Negative**	**Positive**
Values for z Score (-2 to +2 ranges)	0.9 - 22.6	-2.7 to 16.71	26.1 - 65.1	-	-	18.31 - 61.83	-	-	-	4.04 - 17.43	3.5 - 10	10.43 - 102
Values for z Score (-2 to -3 ranges)	0.00 - 0.39	-	16.3 - 23.12	-	-	10 - 10.53	-	-	-	2.4	0.3- 0.34	-
Values for z Score (+2 to +3 ranges)	23 - 27.3	17.61 - 20	67 - 76.2	-	-	-	-	-	-	-	17	200
Observed range of values from Participants	0.00 - 230	-2.7 to 389.96	0.0 - 268.8	8 - 49.4	0.8 - 20	10 - 160	0 - 80	0 - 30	8 - 192	2.4 - 59	0.3 - 20	10.43 - 381

Note: values beyond -3 & +3 are given "0"marks