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Topic Break Down

Topic	No. of Questions
Topic 1, General Microbiology	6
Topic 2, Bacteriology	67
Topic 3, Mycology	18
Topic 4, Parasitology	10
Topic 5, Virology	20
Topic 6, Molecular Microbiology	13
Topic 7, Laboratory Management	11
Topic 8, Public Health Microbiology	6
Total	151

QUESTION NO: 1

A clinical microbiology laboratory is using CRISPR-based technology for rapid and sensitive detection of specific bacterial DNA sequences directly from patient samples. This method relies on the ability of the Cas enzyme to:

- A. Reverse transcribe RNA into complementary DNA.
- B. Amplify target DNA sequences using specific primers and thermal cycling.
- C. Specifically bind to and cleave target DNA sequences guided by a short RNA molecule.
- D. Detect the presence of specific antibodies produced in response to bacterial infection.

ANSWER: C

Explanation:

“Specifically bind to and cleave target DNA sequences guided by a short RNA molecule” is correct because CRISPR-based detection uses programmable sequence recognition. A guide RNA contains a region complementary to the microbial nucleic-acid target and directs the associated Cas nuclease to that matching sequence. For DNA targets, enzymes such as Cas9 or Cas12 can recognize the guide-directed target only when the required adjacent sequence features are present. Target recognition activates nuclease activity. In diagnostic platforms, this activity may be measured directly through cleavage of a target-associated reporter or, with certain enzymes such as Cas12, through cleavage of labeled reporter molecules after target binding. The resulting fluorescence, colorimetric change, or other signal provides a rapid indication that the specific bacterial sequence is present in the patient specimen. This guide-directed recognition is what makes CRISPR assays highly specific and adaptable: changing the guide sequence can retarget an assay to a different organism, resistance determinant, or other clinically relevant DNA marker. The foundational mechanism of RNA-guided Cas nuclease targeting is described by the [National Center for Biotechnology Information review of CRISPR-Cas systems](#), and CRISPR diagnostic applications are reviewed in [this peer-reviewed diagnostic technology article](#).

QUESTION NO: 2

A clinical microbiology laboratory is performing quality control on antimicrobial susceptibility testing. The zone diameter for *Escherichia coli* ATCC 25922 tested against ciprofloxacin is consistently smaller than the acceptable range published by CLSI. The MOST likely cause of this discrepancy is:

- A. Use of expired antibiotic disks
- B. Incubation at an incorrect temperature
- C. Inoculation with a bacterial suspension that is too dense
- D. All of the above

ANSWER: D

Explanation:

All of the above is correct because each listed preanalytic or analytic condition can reduce the inhibition-zone diameter obtained with *Escherichia coli* ATCC 25922 and ciprofloxacin in disk diffusion quality control. Disk diffusion results depend on a standardized amount of active antimicrobial diffusing from the disk into Mueller-Hinton agar. Expired disks, or disks that have been stored in a way that permits deterioration of antimicrobial potency, may produce inadequate active drug concentrations and consequently smaller zones. Inoculating the plate with a suspension that is denser than the required 0.5 McFarland standard creates a heavier bacterial lawn; more organisms must be inhibited, which reduces the measured zone diameter. Incubation conditions must also be controlled because bacterial growth and antimicrobial activity are temperature dependent. A temperature outside the specified range can alter organism growth and diffusion-expression conditions sufficiently to shift QC results below the published limits. Repeated out-of-range QC results should prompt a documented investigation of disk storage and expiration, inoculum preparation, incubation equipment, medium, and procedural standardization before patient isolates are reported. CLSI disk-diffusion methodology emphasizes control of these variables in its [M02 standard](#). FDA also provides current antimicrobial susceptibility-test information and QC-related resources through its [Antibacterial Susceptibility Test Interpretive Criteria](#) page.

QUESTION NO: 3

A patient presents with symptoms suggestive of primary syphilis. Which diagnostic test typically becomes positive earliest in the course of infection?

- A. Rapid Plasma Reagin (RPR)
- B. Venereal Disease Research Laboratory (VDRL) test on CSF
- C. Darkfield microscopy of lesion exudate
- D. Treponema pallidum particle agglutination (TP-PA)

ANSWER: C

Explanation:

Darkfield microscopy of lesion exudate is correct because it can directly demonstrate motile *Treponema pallidum* organisms in material collected from a compatible chancre or other moist lesion, before the patient has necessarily developed a detectable antibody response. In primary syphilis, the organism is present locally at the lesion site, making direct detection a particularly early diagnostic approach when appropriate specimen collection and experienced microscopy are available. This is especially useful during the early window in which serologic tests may still be nonreactive despite clinical disease. The CDC states that direct detection of *T. pallidum* from lesion exudate or tissue by darkfield microscopy is a definitive method for early syphilis diagnosis; molecular testing can also support direct detection where validated. Darkfield examination requires prompt examination of a properly collected specimen and should not be used for oral lesions because nonpathogenic treponemes in the mouth can resemble *T. pallidum*. See the CDC's [Syphilis—STI Treatment Guidelines](#) and its [Syphilis testing guidance for clinicians](#).

QUESTION NO: 4

Which pattern on a Kirby-Bauer disk diffusion plate, showing resistance of *Staphylococcus aureus* to erythromycin but susceptibility to clindamycin with a flattening of the clindamycin zone adjacent to the erythromycin disk (D-zone test positive), indicates inducible clindamycin resistance?

- A. Indicates the strain possesses the *msrA* gene
- B. Indicates the strain should be reported as clindamycin susceptible
- C. Indicates the strain possesses an *erm* gene and should be reported as clindamycin resistant
- D. Indicates the strain is tolerant to clindamycin

ANSWER: C

Explanation:

Indicates the strain possesses an *erm* gene and should be reported as clindamycin resistant is correct. A positive D-zone test is characterized by blunting or flattening of the clindamycin inhibition zone on the side nearest the erythromycin disk, producing the characteristic "D" shape. This demonstrates inducible resistance to the macrolide-lincosamide-streptogramin B antimicrobial group. In staphylococci, this phenotype is most commonly mediated by an *erm* methylase gene. Erythromycin acts as an inducer of methylase expression; once induced, methylation of the 23S rRNA target prevents clindamycin from binding effectively. Although routine clindamycin testing may initially produce a susceptible result, clindamycin therapy can select for constitutive expression of this resistance mechanism and lead to treatment failure. Therefore, according to standardized antimicrobial-susceptibility testing interpretation, an erythromycin-resistant, clindamycin-susceptible *S. aureus* isolate with a positive D-zone test must be reported as clindamycin resistant. The D-zone test is specifically used to identify this clinically important inducible phenotype and guide accurate reporting. See the CLSI [M100 antimicrobial-susceptibility testing standard](#) and the original description of the disk approximation test at [PubMed](#).

QUESTION NO: 5

A clinical trial is evaluating the efficacy of a new antifungal agent against invasive aspergillosis. Serum galactomannan levels are being used as a biomarker to monitor the infection. A decrease in galactomannan levels during treatment would indicate:

- A. Development of resistance to the antifungal agent.
- B. Successful clearance or reduction of the *Aspergillus* burden.
- C. A false-positive result in the galactomannan assay.
- D. Concurrent infection with a non-*Aspergillus* mold.

ANSWER: B

Explanation:

Successful clearance or reduction of the *Aspergillus* burden. Galactomannan is a polysaccharide cell-wall component released by *Aspergillus* species during active growth. In a patient with invasive aspergillosis and an initially positive serum galactomannan result, serial measurement can provide supportive evidence about the infection's trajectory. A declining galactomannan index during appropriately administered antifungal therapy is consistent with reduced fungal activity and decreasing organism burden, making it a useful adjunct marker of therapeutic response in a clinical trial.

Biomarker trends should be interpreted alongside the patient's clinical status, imaging findings, antifungal drug exposure, and baseline assay result. Nonetheless, when the same assay is followed over time, a sustained decrease is biologically consistent with less circulating galactomannan as invasive *Aspergillus* infection comes under control. The Infectious Diseases Society of America notes that serial serum galactomannan monitoring can be used to help assess response in patients with invasive aspergillosis. Further diagnostic and monitoring context is available in the [IDSA Aspergillosis Clinical Practice Guideline](#) and the [ASM Clinical Microbiology Reviews article on aspergillosis diagnosis](#).

QUESTION NO: 6

Under the Clinical Laboratory Improvement Amendments (CLIA), participation in an approved external Proficiency Testing (PT) program serves primarily to:

- A. Fulfill requirements for laboratory accreditation by The Joint Commission only.
- B. Provide an objective, ongoing assessment of the accuracy and reliability of the laboratory's testing compared to its peers.
- C. Validate the performance characteristics (e.g., sensitivity, specificity) of newly implemented test methods.
- D. Determine the reference intervals (normal ranges) for quantitative assays performed by the laboratory.

ANSWER: B

Explanation:

Provide an objective, ongoing assessment of the accuracy and reliability of the laboratory's testing compared to its peers. Under CLIA, proficiency testing is an external quality-assessment process in which a laboratory tests specimens distributed by an approved PT program and reports results for comparison with the expected result and with results from other participating laboratories using comparable methods. This provides an independent measure of the laboratory's routine analytic performance over time and helps demonstrate that patient-testing results are accurate and reliable. For regulated analytes and specialties, laboratories must enroll in a CMS-approved PT program and successfully participate according to CLIA requirements. PT specimens must be handled and tested in the same manner as patient specimens, supporting an assessment that reflects ordinary laboratory practice rather than a special exercise. PT performance is therefore a central ongoing quality tool and a CLIA condition for laboratories performing moderate- or high-complexity testing in specialties for which PT is required. CMS describes PT as a means to evaluate a laboratory's performance and accuracy through external comparison. See the [CMS CLIA Proficiency Testing](#) page and the applicable federal requirements at [42 CFR Part 493, Subpart H](#).

QUESTION NO: 7

A clinical microbiology laboratory encounters a Gram-negative bacillus isolated from a bloodstream infection. The isolate exhibits resistance to multiple antibiotics, including carbapenems. Further phenotypic testing reveals the production of a metallo-beta-lactamase (MBL). Which of the following antibiotic combinations might still retain activity against this isolate?

- A. Ceftazidime-avibactam
- B. Piperacillin-tazobactam
- C. Aztreonam
- D. Aztreonam-avibactam

ANSWER: D

Explanation:

Aztreonam-avibactam might retain activity against an MBL-producing Gram-negative bacillus because aztreonam is structurally stable to hydrolysis by metallo-beta-lactamases, including clinically important NDM, VIM, and IMP enzymes. In practice, MBL-producing Enterobacterales often also produce additional serine beta-lactamases, such as ESBLs or AmpC enzymes, that can inactivate aztreonam. Avibactam inhibits many of these accompanying serine beta-lactamases, thereby protecting aztreonam and restoring its potential activity against the isolate. This complementary mechanism is the rationale for using the aztreonam-avibactam combination when an MBL is identified. Susceptibility testing remains essential because activity depends on the organism's complete resistance mechanism profile and should guide treatment of a bloodstream infection. The Infectious Diseases Society of America identifies aztreonam combined with avibactam as a preferred approach for infections caused by NDM and other MBL-producing Enterobacterales. Aztreonam-avibactam also has an FDA-approved indication for complicated intra-abdominal infection, reflecting its role against susceptible Gram-negative organisms with limited beta-lactam treatment options. See the [IDSA antimicrobial-resistant Gram-negative infection guidance](#) and the [FDA approval announcement for aztreonam-avibactam](#).

QUESTION NO: 8

A patient develops profuse watery diarrhea after recent broad-spectrum antimicrobial therapy. A stool nucleic acid amplification test is positive for toxigenic *Clostridioides difficile*, but a toxin enzyme immunoassay is negative. The patient has received a laxative within the previous 24 hours and has no abdominal pain, fever, or leukocytosis. The MOST appropriate interpretation is:

- A. Colonization with toxigenic *C. difficile* is possible, and the result should be correlated with clinical criteria for testing
- B. The negative toxin assay definitively excludes carriage of toxigenic *C. difficile*
- C. The nucleic acid amplification result proves toxin-mediated colitis requiring treatment
- D. The result indicates that the stool specimen was unsuitable for molecular testing

ANSWER: A

Explanation:

Colonization with toxigenic *C. difficile* is possible, and the result should be correlated with clinical criteria for testing is correct. Nucleic acid amplification tests detect toxin genes and are highly sensitive, but they do not establish that toxin is being produced in sufficient quantity to cause disease. Recent laxative use provides an alternative explanation for diarrhea, while the negative toxin assay and absence of supporting clinical findings lower the likelihood of active *C. difficile* infection. Testing should generally be restricted to patients with otherwise unexplained, clinically significant diarrhea to reduce detection of colonization.

QUESTION NO: 9

An immunocompromised patient develops a disseminated fungal infection. Blood cultures are negative, but a serum galactomannan assay is strongly positive. A CT scan of the chest reveals multiple nodular lesions with a halo sign.

Bronchoalveolar lavage (BAL) fluid is analyzed by PCR, yielding a positive result for *Aspergillus fumigatus*. The halo sign observed on the CT scan is primarily attributed to:

- A. Extensive granuloma formation surrounding fungal hyphae.
- B. Angioinvasion by *Aspergillus* hyphae leading to localized hemorrhage and necrosis.
- C. Formation of thick-walled cysts containing fungal conidia.
- D. A hypersensitivity reaction in the lung parenchyma.

ANSWER: B

Explanation:

Angioinvasion by *Aspergillus* hyphae leading to localized hemorrhage and necrosis is correct. In invasive pulmonary aspergillosis, particularly in severely immunocompromised patients, hyphae invade pulmonary blood vessels. This vascular invasion causes thrombosis and tissue infarction/necrosis in and around a pulmonary nodule. The characteristic CT halo sign represents a central nodular area of fungal infection and infarcted lung surrounded by a rim of ground-glass attenuation, which reflects perinodular alveolar hemorrhage. Thus, the sign is an early radiographic marker of angioinvasive disease and should prompt timely antifungal evaluation and treatment in an appropriately high-risk clinical setting. The concordant strongly positive serum galactomannan result and detection of *Aspergillus fumigatus* in BAL fluid further support invasive aspergillosis in this scenario. The Infectious Diseases Society of America identifies the halo sign as a typical CT feature of angioinvasive aspergillosis, and radiology references similarly describe the surrounding ground-glass opacity as hemorrhage caused by vascular invasion. See the [IDSA clinical practice guideline for aspergillosis](#) and the [Radiopaedia overview of the pulmonary CT halo sign](#).

QUESTION NO: 10

A patient with a history of travel to a developing country presents with chronic diarrhea, abdominal pain, and weight loss. Stool examination reveals the presence of cysts containing four nuclei and chromatoidal bars with rounded ends. The MOST likely causative organism is:

- A. *Giardia lamblia*
- B. *Entamoeba histolytica*
- C. *Cryptosporidium parvum*
- D. *Cyclospora cayentanensis*

ANSWER: B

Explanation:

Entamoeba histolytica is the most likely causative organism because its mature cysts characteristically contain four nuclei and may contain chromatoidal bodies, which appear as cigar-shaped or rod-like inclusions with rounded ends. These cysts are the environmentally resistant, infective stage transmitted by ingestion of fecally contaminated food or water, making exposure during travel to areas with inadequate sanitation epidemiologically compatible. Intestinal amebiasis may range from asymptomatic colonization to prolonged diarrhea, abdominal discomfort, and weight loss; invasive disease can produce amebic colitis and, in some cases, extraintestinal infection. Microscopic identification of cyst morphology supports an *Entamoeba* diagnosis, although routine microscopy cannot reliably distinguish the morphologically identical *E. histolytica* and *E. dispar*. In clinical practice, a species-specific stool antigen assay or molecular test is therefore useful when determining whether pathogenic *E. histolytica* is present. The combination of compatible symptoms, travel-associated fecal–oral exposure risk, and the described mature cyst morphology makes *Entamoeba histolytica* the best answer. CDC DPDx provides diagnostic morphology and testing guidance for amebiasis: [CDC DPDx: Amebiasis](#). Clinical information is also available from the [CDC amebiasis overview](#).

QUESTION NO: 11

An immunocompromised patient returning from a trip to the southwestern United States presents with fever, cough, and chest pain. Chest X-ray shows diffuse pulmonary infiltrates. Sputum culture grows a dimorphic fungus that forms spherules containing endospores at 37°C. The MOST likely causative agent is:

- A. *Histoplasma capsulatum*
- B. *Blastomyces dermatitidis*
- C. *Coccidioides immitis*
- D. *Paracoccidioides brasiliensis*

ANSWER: C

Explanation:

Coccidioides immitis is the most likely agent because the defining tissue-phase morphology of *Coccidioides* is a large, thick-walled spherule filled with endospores. In the environment, this fungus grows as a mold that produces arthroconidia; inhalation of arthroconidia can initiate pulmonary coccidioidomycosis. Once in host tissue at body temperature, the arthroconidia enlarge into spherules, which mature, rupture, and release endospores that can develop into new spherules. This distinctive cycle is diagnostically important in direct specimens and histopathology.

The epidemiologic setting strongly supports this identification. *Coccidioides* is endemic in arid regions of the southwestern United States, where soil disruption and dust exposure can aerosolize infectious arthroconidia. Primary infection commonly presents with fever, cough, pleuritic chest pain, and pulmonary infiltrates. Immunocompromised patients have an increased risk of severe diffuse pulmonary disease and extrapulmonary dissemination. Culture handling requires particular care because the mold phase can produce readily aerosolized arthroconidia; laboratories should follow appropriate biosafety procedures and promptly communicate suspicion of this organism. The CDC clinical overview describes the endemic geography, pulmonary presentation, and risk for severe disease, while the CDC laboratory guidance discusses diagnostic and laboratory considerations for coccidioidomycosis. See [CDC: Clinical Overview of Valley Fever](#) and [CDC: Testing and Diagnosis for Valley Fever](#).

QUESTION NO: 12

For epidemiological tracking and outbreak investigations of *Clostridioides difficile* infection (CDI), which molecular typing method based on PCR amplification targeting repetitive elements within the pathogenicity locus (PaLoc) has historically been widely used and designated by specific nomenclature (e.g., NAP1/BI/027)?

- A. Multilocus sequence typing (MLST)
- B. Pulsed-field gel electrophoresis (PFGE)
- C. PCR Ribotyping
- D. Whole-genome sequencing (WGS) SNP analysis

ANSWER: C

Explanation:

PCR Ribotyping is the best answer because it has historically been one of the most widely used typing approaches for *Clostridioides difficile* surveillance and outbreak investigation. It generates characteristic banding profiles after PCR amplification of the variable intergenic spacer regions between bacterial ribosomal RNA genes. These profiles are assigned numerical ribotype designations, including the internationally recognized ribotype 027. The epidemic strain commonly described as NAP1/BI/027 combines names derived from several historical typing systems: “027” is the PCR-ribotype designation and is therefore the designation directly associated with PCR ribotyping. This strain became particularly important in healthcare-associated CDI epidemiology because of its association with severe disease and widespread outbreaks. PCR ribotyping offered a practical, reproducible way for laboratories to compare isolates and communicate results during the period when this lineage emerged and spread. Although whole-genome approaches now provide substantially higher resolution for transmission analysis, PCR ribotyping remains central to the historical nomenclature and to interpretation of much of the published CDI epidemiology. For further background, see this [review of *C. difficile* typing methods](#) and the [CDC clinical overview of *C. difficile*](#).

QUESTION NO: 13

A patient with a history of neutropenia develops a severe sinus infection. Tissue biopsy reveals broad, ribbon-like, non-septate hyphae with right-angle branching. The MOST likely causative organism belongs to the order:

- A. Mucorales
- B. Aspergillales
- C. Eurotiales
- D. Onygenales

ANSWER: A

Explanation:

Mucorales is correct because the tissue morphology is characteristic of invasive mucormycosis, an angioinvasive infection that is particularly important in patients with substantial neutrophil dysfunction or neutropenia. Organisms in this order, including *Rhizopus*, *Mucor*, *Lichtheimia*, and related genera, typically appear in tissue as broad, irregular-width, ribbon-like hyphae that are pauciseptate (often described as nonseptate) and branch at wide, approximately right angles. Rhinocerebral or sinonasal disease can progress rapidly, with invasion of blood vessels causing thrombosis, tissue infarction, and necrosis; therefore, recognition of this histopathologic pattern should prompt urgent clinical evaluation and management. Although hyphal morphology can be distorted in necrotic tissue or by tissue processing, the combination of broad pauciseptate hyphae, wide-angle branching, and severe sinus infection in an immunocompromised host strongly supports Mucorales. The CDC describes mucormycosis as a serious mold infection caused by molds in the order Mucorales and notes its occurrence in people with weakened immune systems. The ECMM/Mycoses Study Group global guideline further emphasizes early diagnosis and prompt management for suspected mucormycosis. See the [CDC overview of mucormycosis](#) and the [ECMM/MSG global guideline](#).

QUESTION NO: 14

A clinical microbiology laboratory is using isothermal nucleic acid amplification techniques for rapid detection of *Mycobacterium tuberculosis* in sputum. Compared to conventional PCR, isothermal methods offer the advantage of:

- A. Higher sensitivity and specificity for detecting low levels of target DNA.
- B. Faster reaction times and the ability to be performed without sophisticated thermocyclers.
- C. The ability to simultaneously amplify multiple targets in a single reaction.
- D. Easier integration with downstream analysis techniques such as Sanger sequencing.

ANSWER: B

Explanation:

Faster reaction times and the ability to be performed without sophisticated thermocyclers is correct. Conventional PCR requires precisely repeated temperature changes for denaturation, primer annealing, and extension, so it relies on a programmable thermocycler. Isothermal amplification methods instead carry out target amplification at one sustained temperature. Depending on the method and assay design, this can substantially simplify instrumentation, reduce power and workflow requirements, and support near-patient or decentralized molecular testing. These operational features are especially useful for rapid tuberculosis testing in settings where access to complex laboratory infrastructure is limited.

Isothermal assays can also produce detectable amplification products quickly because their enzymatic mechanisms continuously amplify nucleic acid without waiting for thermal cycling steps. For example, loop-mediated isothermal amplification uses strand-displacing polymerase activity at a constant temperature and has been developed for detection of *Mycobacterium tuberculosis* complex. The practical benefit in this question is therefore workflow and instrument simplification paired with rapid amplification, rather than an inherent universal improvement in analytical performance. Assay sensitivity, specificity, and multiplexing capability remain dependent on the individual assay's target selection, primer design, specimen preparation, and validation. See the review of isothermal methods in [Clinical Microbiology Reviews](#) and the World Health Organization's guidance on tuberculosis molecular assays at [WHO](#).

QUESTION NO: 15

A patient returning from a mission trip to Africa presents with fever, headache, and a characteristic relapsing pattern of symptoms. Peripheral blood smear reveals the presence of extracellular flagellated protozoa. The MOST likely diagnosis is African trypanosomiasis caused by:

- A. *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense*
- B. *Trypanosoma cruzi*
- C. *Leishmania donovani*
- D. *Plasmodium vivax*

ANSWER: A

Explanation:

Trypanosoma brucei gambiense or *Trypanosoma brucei rhodesiense* is correct because these organisms cause human African trypanosomiasis (African sleeping sickness). They are extracellular hemoflagellates transmitted by tsetse flies in sub-Saharan Africa. During the hemolymphatic stage of infection, parasites circulate in blood and lymph, producing intermittent or relapsing fever, headache, malaise, pruritus, and lymphadenopathy. Antigenic variation of the surface glycoprotein coat allows waves of parasitemia to recur, accounting for the characteristic relapsing clinical pattern. Blood smear examination may demonstrate motile, extracellular trypomastigotes with an undulating membrane and a flagellum. *T. b. rhodesiense* generally causes a more acute illness in eastern and southeastern Africa, whereas *T. b. gambiense* usually produces a more chronic disease in western and central Africa. Both can progress to central nervous system involvement, with sleep disturbance and other neurologic manifestations, if untreated. The Centers for Disease Control and Prevention describes African trypanosomiasis and its vector, geographic distribution, and clinical stages at [CDC DPDx: African Trypanosomiasis](#). Additional clinical information is available from the [CDC African Trypanosomiasis overview](#).