1. A 60-year-old man with a history of refractory chronic lymphocytic leukemia treated with BTK and PI3K inhibitors presents with a two-month history of headaches, eye blurriness, memory issues, and word-finding difficulties, and was found on brain MRI to have multiple ring enhancing lesions suspicious for abscess. Tissue from surgical resection showed scattered, narrow, hyphal forms with irregular branching and occasional septations. The clinical infectious diseases team requests molecular testing for further classification of the observed organisms. Of the gene targets below, which is the most likely to provide the requested information?

- a. 16S rRNA
- b. B1
- c. hsp65
- d. ITS
- e. rpoB

2. A 50-year-old woman with a history of acute myeloid leukemia, recently admitted for induction chemotherapy, complicated by typhlitis and pleural effusion, presented with progressive decline of mental status and MRI showed numerous small ring-enhancing lesions in the brain concerning for infection versus malignancy. Surgical biopsy of temporal lobe lesions showed microabscess with predominantly yeast-like and occasional filamentous fungal forms. Gram and Warthin-Starry stains were negative. Cultures were positive for Gram-negative rods in liquid media only, unable to be further classified. Molecular testing identified *Candida albicans* and *Cutibacterium acnes*. What is the most likely interpretation of these results?

- a. *Candida albicans* is false positive; *Cutibacterium acnes* is false positive
- b. *Candida albicans* is false positive; *Cutibacterium acnes* is true positive
- c. *Candida albicans* is true positive; *Cutibacterium acnes* is false positive
- d. *Candida albicans* is true positive; *Cutibacterium acnes* is true positive

3. Which of the following is an advantage of unbiased metagenomic next-generation sequencing assays?

- a. Ability to detect novel pathogens
- b. Faster turn-around time than targeted RT-qPCR testing
- c. Lower cost compared to cultures and histology
- d. Wide availability to academic and non-academic medical centers
Scroll to Page 3 for Answers
**Question 1: Correct answer and rationale:** D – Internal transcribed spacer (ITS) sequencing, encompassing the region between the 28S and 18S rRNA genes in the fungal genome, and sequencing of the D1/D2 region of the 28S rRNA gene, have proven to be effective targets for speciating the majority of pathogenic fungi due to sequence diversity and well curated reference databases. The other options are most useful for bacteria (16S rRNA), mycobacteria (hsp65, rpoB), and *Toxoplasma gondii* (B1).

**Question 2: Correct answer and rationale:** C - Histology shows fungal forms (yeast and pseudohyphae) compatible with the molecular findings of *Candida albicans*. No histological correlates of *Cutibacterium acnes* (a Gram-positive organism) were identified, and cultures were only positive for a Gram-negative organism in liquid media, suggesting contamination in this case.

**Question 3: Correct answer and rationale:** A - While metagenomic NGS is not yet widely available in all practice settings, takes more time than targeted assays, and is more expensive due to personnel and equipment requirements, it does provide the opportunity to diagnose novel or unexpected pathogens.