JOURNAL-BASED LEARNING EXERCISES

Each article's contents should be read, researched and understood, and you should then come to a decision on each question. The pass mark is 17 out of 20 questions answered correctly. JBL exercises may be completed at any time until the published deadline date. Please select your choice of correct answers and complete the exercises online at: **www.ibms.org/cpd/jbl**

DEADLINE WEDNESDAY 3 JANUARY 2018

	Comparison of the fast track diagnostics respiratory 21 and Seegene Allplex multiplex polymerase chain reaction assays for the detection of respiratory viruses. Barrett K, Anderson TP, Fahey JA, Jennings LC, Werno AM, Murdoch DR. *Br J Biomed Sci* 2017; **74** (2): 85–9. Assessment No: V101017		**miR-199a-3p downregulation in thyroid tissues is associated with invasion and metastasis of papillary thyroid carcinoma.** Liu C, Xing M, Wang L, Zhang K. *Br J Biomed Sci* 2017; **74** (2): 90–4. Assessment No: H101017
01	Viral respiratory infections are an infrequent cause of admission to hospital.	01	Papillary thyroid carcinoma (PTC) is the least common type of thyroid malignancy, accounting for just 8% of thyroid cancers.
02	Real-time multiplex polymerase chain reaction (PCR) assays are increasingly used for respiratory virus detection, offer automated analysis in a closed-tube system, and have the advantage of high throughput.	02	The prognosis in patients with PTC is generally relatively poor.
03	A total of 199 consecutive respiratory samples were tested by the FTD and Seegene assays within one day of collection.	03	Clinicopathological factors such as extrathyroidal extension, tumour size, multifocality, lymphatic invasion and desmoplastic reaction are associated with poor prognosis in PTC.
04	All swab samples were collected in 2.5 mL viral transport medium (VTM) and stored at 4°C until tested.	04	In the human genome, the precursors miR-199a-1 and miR-199a-2 map to different loci on chromosomes 1 and 19, respectively.
05	Only the Seegene assay included a synthetic RNA internal control, and this was spiked into the Easymag lysis buffer to control for PCR inhibition.	05	miR-199a-3p is upregulated in several human malignancies, such as ovarian, hepatocellular, colorectal and prostate cancers.
06	The FTD assay had five, fourplex RT-PCR pools per sample which required 10µL extracted sample in each pool.	06	miR-199a-3p restoration in PTC cells impairs migration and proliferation, which suggests that it acts as a tumour suppressor.
07	The Seegene assay consisted of three pools each with six- to eightplex reactions per sample.	07	A total of 136 PTC and 52 normal thyroid samples snap frozen in liquid nitrogen and stored at –80^{0}C were studied.
08	For rhinovirus discrepant testing, a rhinovirus-specific assay and a picornavirus assay were performed to include as many rhinovirus genotypes as possible, and an enterovirus assay was also performed to exclude any enteroviruses.	08	Expression levels of miR-199a-3p were measured using miRNA sequence-specific primers.
09	Pool 2 targets included parainfluenza viruses 2, 3 and 4.	09	Total extracted RNA resuspended in 60µL preheated (95°C) nuclease-free water.
10	One Seegene result that was pan-influenza-positive but influenza H3-negative was included as a pan-influenza-negative sample.	10	A cut-off value of 8.24 distinguished high miR-199a-3p value patients from low value patients.
11	In this study, analysis was restricted to the 10 respiratory viral pathogen targets common to both assays.	11	He *et al.* were the first to apply fine-needle aspiration testing to the assessment of PTC.
12	The Fast-Track assay also included parechovirus and *Mycoplasma pneumoniae*.	12	A tissue biomarker is unhelpful in the diagnosis and discrimination of PTC.
13	A respiratory pathogen was detected in 123 (61.8%) samples by the FTD assay and in 127 (63.8%) samples by the Seegene assay.	13	Upregulation of miR-21 has been associated with good clinicopathological characteristics in patients with osteosarcoma.
14	The FTD assay detected more bocavirus-positive samples than the Seegene assay, and almost all of these additional results were low-level positives.	14	Serum miR-124 levels have shown significant increases in patients with pancreatic ductal adenocarcinoma.
15	Nine of the 12 bocavirus-positive results were in samples for which other viruses were also detected.	15	The association of miR-199a-3p levels with clinicopathological parameters in PTC patients was assessed by chi-squared test.
16	The presence of adenoviral DNA at low concentration is of uncertain clinical significance in respiratory samples as adenovirus can be detected in asymptomatic patients.	16	Tumour size was greater or equal to 2 cm in 32 cases of PTC studied.
17	The nucleic acid extraction step for the FTD assay utilises a smaller volume of sample and elutes into a larger volume.	17	Multicentricity (>2) of PTC was seen in 103 of the cases in this study.
18	One advantage provided by the FTD assay is that the PCR amplification step is 30 minutes faster using the Roche Lightcycler 480 thermal cycler.	18	Fine-needle aspiration biopsy (FNAB) and cytological assessment have been a cornerstone of diagnostic thyroid nodule management since the 1980s.
19	The FTD assay had the advantage of automated result calling, but only when using the dedicated Bio-Rad real-time thermal cycler.	19	The *BRAF*^{V600E} mutation has been shown to be associated with aggressive clinical behaviour in PTC.
20	Both assays utilised real-time PCR and dye-labelled probes.	20	miR-199a is a phylogenetically conserved miRNA.

REFLECTIVE LEARNING

01	Real-time multiplex PCR methods are the most commonly used commercial methods but have low throughput due to multiplexing limitations. Discuss.	01	MicroRNAs (miRNAs) are small, non-coding RNAs that have important functions in development, cell differentiation and in regulation of the cell cycle and apoptosis. Perform a literature search and discuss current applications in the diagnosis of cancer.
02	In which other areas, and for what, are real-time multiplex PCR methods being used?	02	Point-of-care testing and its application to the detection of microRNAs in serum. Discuss.