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LETTER TO EDITOR

Low cost immunohistochemistry bench for developing countries

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Dear Editor,

An essential pathology package for low- and middle-income countries has been recently proposed based on an integrated network of tiered laboratories.[1] With regard to anatomical pathology services, a tier-2 laboratory with basic histology services based on haematoxylin-eosin staining meets the needs of a district hospital. A model to set up such a low-cost, low-volume histology laboratory in a developing country has been previously reported.[2] Although majority of the pathological diagnoses could be rendered by good haematoxylin-eosin staining, immunohistochemistry has become an indispensable tool in aiding classification of undifferentiated neoplasms or identification of sites of origin for carcinomas of unknown primary site.[3] Immunohistochemistry for hormonal receptor and human epidermal growth factor receptor 2 (HER2) statuses also informs predictive and prognostic value in breast cancers.[4,5] We believe that patients in developing countries should be equally benefited from this diagnostic tool. Complementary to the proposed histology laboratory model, here we have constructed the list of resources of setting up a manual immunohistochemistry bench with minimal cost (Table 1) based on our previous knowledge transfer endeavour. With a simple protocol (Table 2 and FIG. 1) and proper tissue controls, high quality manual immunostaining is attainable. In our experience, a minimal panel of antibodies against pan-cytokeratin, cytokeratin 7, cytokeratin 20, leucocyte common antigen (CD45), S-100 protein, desmin, smooth muscle actin, estrogen receptor, progesterone receptor and HER2 could be ultilised to help in resolving many queries of the routine diagnostic pathology. With complementary immunohistochemistry services, a laboratory could be elevated to a tier-3 laboratory serving a regional or provincial hospital.[1]

TABLE 1: List of instruments and consumable reagents and estimated costs

Instruments	Estimated costs (US dollar)
1. Pressure cooker for heat-induced epitope retrieval	100
2. Slide box as a rack for incubation	4
Consumable reagents (400 immunohistochemistry tests)	
1. Glass slides coated with poly-L-lysine	400
2. Antigen retrieval buffer	400
3. Tris-buffered saline as washing buffer	200
4. Endogeneous peroxidase blocking solution	200
5. Polymer based detection system	900
Primary antibodies	
1. Primary antibodies (for 30 to 50 tests depending on dilution or ready-to-use)	150-500/ primary antibody
Total cost to set up immunohistochemistry service with a minimum 10 primary antibodies (500USD per primary antibody)	7204

Average cost for an immunohistochemistry test: 10.25 – 22.00 USD/slide

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TABLE 2: A simple protocol for immunohistochemistry

Steps	Time
1. Tissue sections are mounted onto poly-L-lysine coated glass slides	
2. The glass slides are placed on a hot plate at 60°C	1 hr
3. Deparaffinize and rehydrate the slides with xylene and graded ethanol to distilled water	
-immerse in xylene (two changes)	3 mins each
-immerse in absolute ethanol (two changes)	2 mins each
-immerse in 80% ethanol once	2 mins
-immerse in distilled water (two changes)	2 mins each
4. Heat-induced epitope retrieval in antigen retrieval solution using a pressure cooker (**FIG. 1**)	
-Place the glass slides into a heat resistant jar and immerse the slides with antigen retrieval solution	
-Place the heat resistant jar into a pressure cooker with some water in the pressure cooker	
-Secure the pressure cooker lid and boil the pressure cooker until full pressure is reached	
-Time for 3 minutes for full pressure	3 minutes
-Cool the pressure cooker immediately under running tap water	
5. Remove the glass slides and rinse with Tris-buffered saline (TBS) once at room temperature	
6. Incubate the glass slides with endogeneous peroxidase blocking solution in a slide box at room temperature (**FIG. 1**)	5 mins
7. Discard the endogenous peroxidase blocking solution and rinse with TBS three times	
8. Discard TBS and incubate the slides with primary antibodies	30 mins
9. Discard the primary antibodies and rinse with TBS four times	
10. Discard TBS and incubate the slides with peroxidase labelled polymer conjugated to secondary antibody	30 mins
11. Discard the secondary antibody and rinse with TBS once	
12. Rinse with distilled water once	
13. Incubate with DAB (3,3'-diaminobenzidine)	10 mins
14. Discard DAB and rinse the slides in water bath under running tap water	30 seconds
15. Counterstain with haematoxylin	1 min
16. Rinse the slides in water bath under running tap water	1 min
17. Dehydrate the slides with graded ethanol to xylene	
-95% ethanol (three changes)	15 dips
-Absolute ethanol (three changes)	2 mins each
-Xylene (three changes)	2 mins each
18. Mount the slides with cover slips	

Note: Incubation with reagents and rinsing with TBS could be done by flooding the reagents and TBS onto the tissue sections in the glass slides

Conflicts of interest: We do not believe that there is a conflict of interest that could potentially be construed to affect the material contained in the manuscript that is being submitted to the Journal.

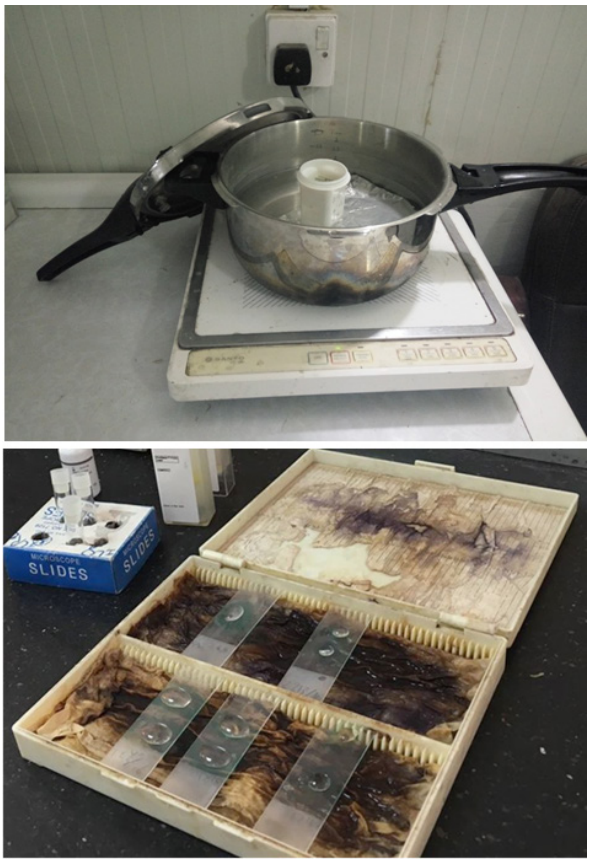

FIG. 1: (Top) Slides are subject to antigen retrieval in a pressure cooker. (Bottom) Slides are incubated with reagents on a slide box as a rack.

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