ANATOMICAL PATHOLOGY

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In 2009, nineteen laboratories participated in the histological staining program and fourteen laboratories joined the immunohistochemical staining program. The laboratories belong to various institutes, including Hospital Authority, government institutes/clinics, university laboratories as well as private hospitals.

I. Survey Format

Tables 1-2 summarise the various staining methods, cytopathology and antibodies assessed in this year QAP. A questionnaire was included in each survey asking details of the staining procedures done. These details allow the assessors to identify any erroneous step that caused the unsatisfactory staining results. The staining procedure of the top scored laboratory was compiled with the survey report for reference.

Table 1a. Histological Staining Program

Survey	Code Number	Staining Methods
One	HC0902	Massion Trichrome
Two	HC0908	Periodic Acid-Schiff
Three	HC0914	Congo Red
Four	HC0920	Perls' Prussian Blue Method

Table 1b. Cytopathology

Survey	Code Number	Results
One	HC0903	Negative for malignant cells
Two	HC0909	Malignant cells seen, favour adenocarcinoma
Three	HC0915	Negative for malignancy
Four	HC0921	Adenocarcinoma

Table 2. Immunohistochemical Staining Program

Survey	Code Number	Staining Methods
One	HC0904	S100
	HC0905	S100 (in house)
	HC0906	EMA
Two	HC0910	AE1/3
	HC0911	AE1/3 (in house)
	HC0912	EMA
Three	HC0916	CD 30
	HC0917	CD 30 (in house)
	HC0918	EMA
Four	HC0922	ER
	HC0923	ER (in house)
	HC0924	EMA

II. Method of Analysis

The staining performance was assessed with the following criteria (Table 3).

Table 3. Scoring System I

Staining	Scores
Little or no staining of the target substance / antigen	1
Very weak staining of the target substance / antigen	2-3
Weak staining of the target substance / antigen	4-5
Good staining of the target substance / antigen	6-7
Excellent staining of the target substance / antigen	8-10

Emphasis was placed on: i) crisp and intense positive staining with minimal or no background (good staining contrast), ii) there was no uneven or patchy staining and other unnecessary deposit and iii) the nuclear counterstaining were adequate. Score below 5 was considered as unsatisfactory.

To ensure objectivity in assessment, scores given by the assessors were averaged after excluding the highest and the lowest marks (Table 4). The average score, after rounding up to the nearest 0.5, constituted the final score of the laboratory

Table 4. Scoring system II

Participant	Scores given by Panel				Final Score
	Member A	Member B	Member C	Member D	
X	9	7	8	10	8.5
Y	6	4	5	7	5.5

Slide Return Summary

The slide return pattern of each survey was illustrated in Figure 1a and 1b and Figure 2.

Figure 1a. Histological Staining

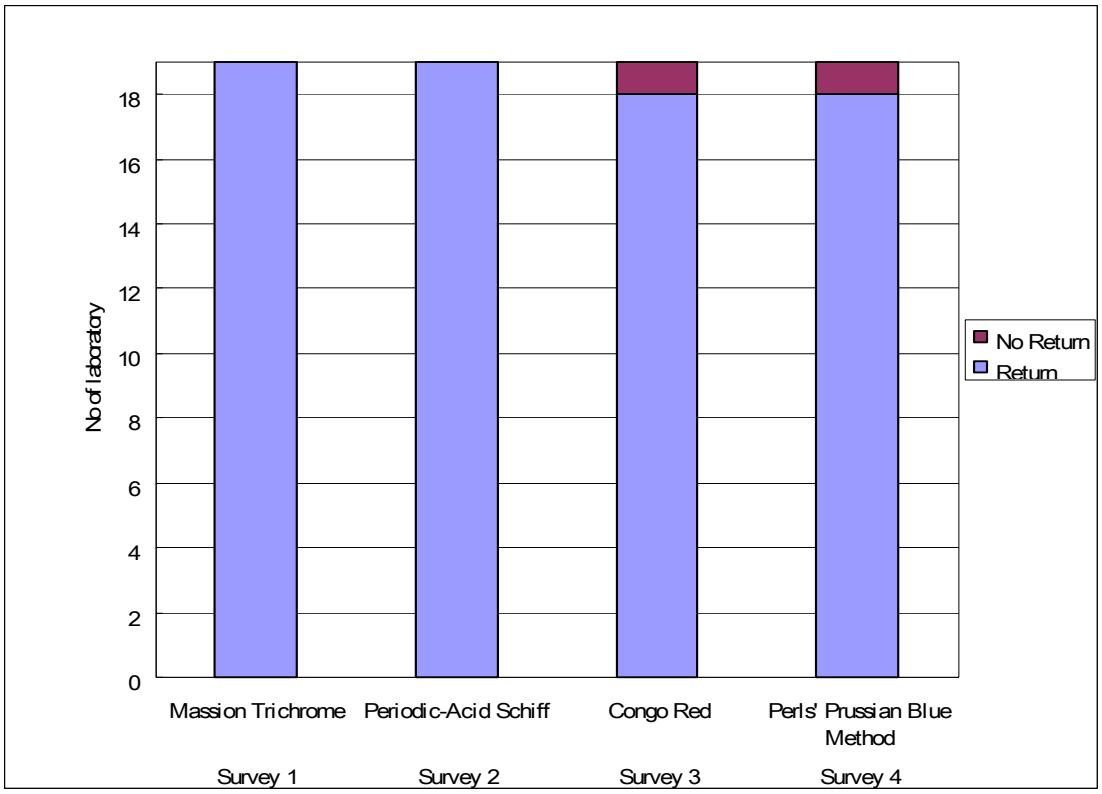


Figure 1b. Cytopathology

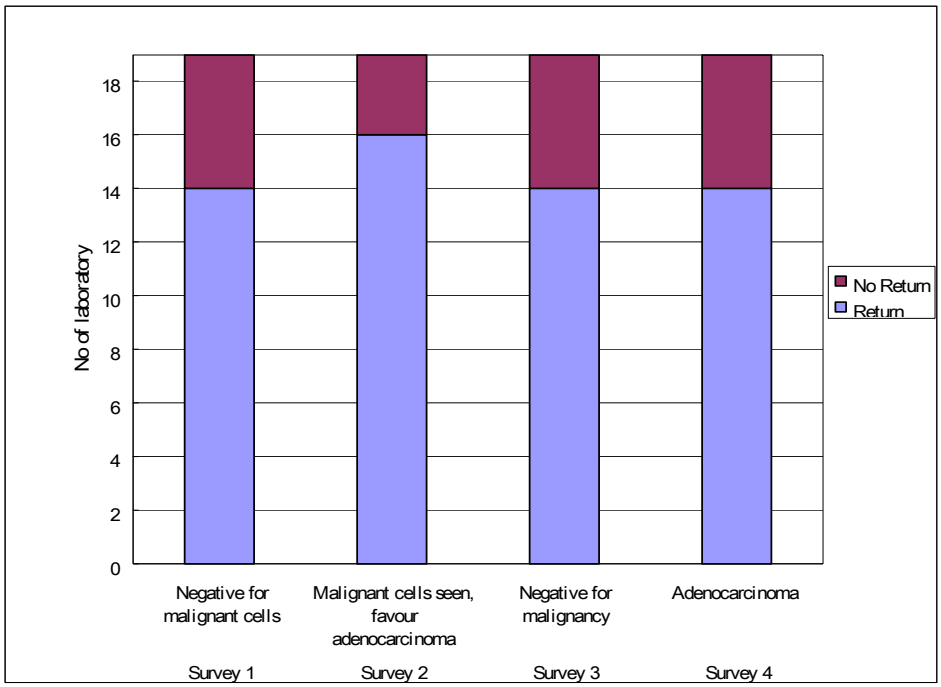
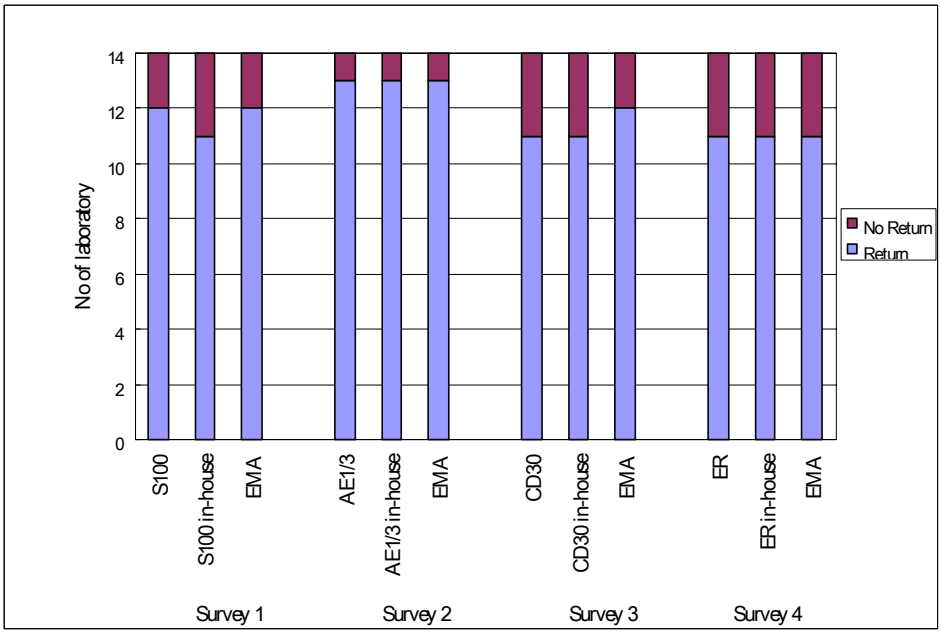


Figure 2. Immunohistochemical Staining



IV. Survey Analysis

i. Histological Staining Programme

Survey One material was a paraffin section of small intestine.

Figure 3: Survey one: HC0901, H&E

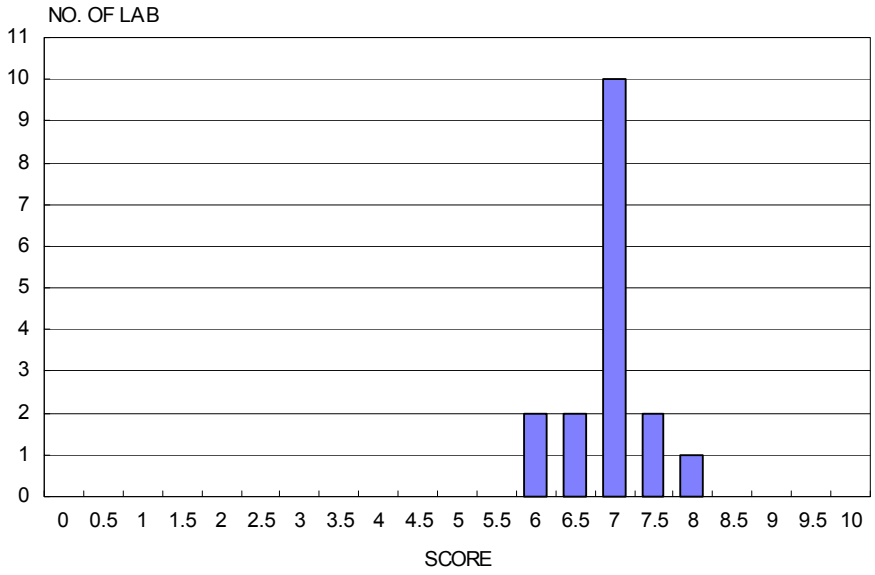
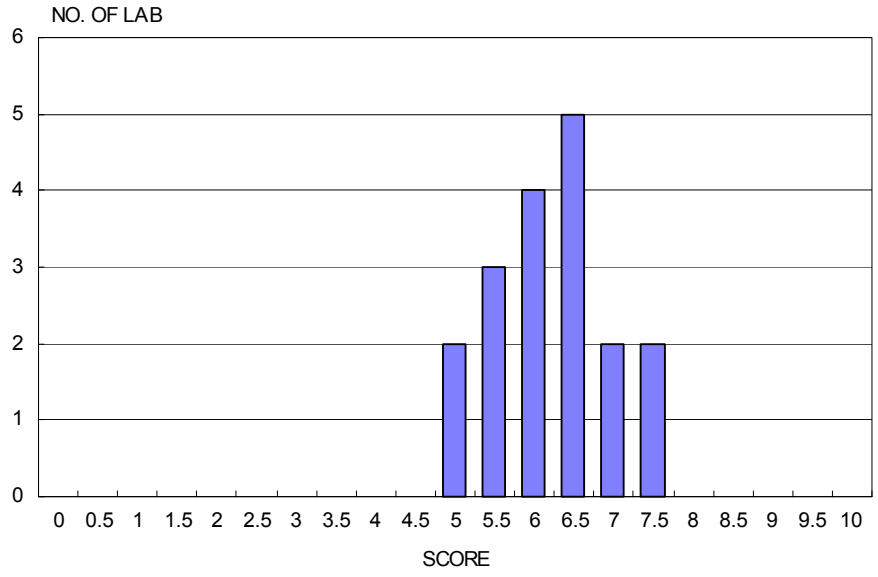


Figure 4: Survey one: HC0902, Massion Trichrome



Survey-Two material was a paraffin section of normal small intestine

Figure 5: Survey two: HC0907, H&E

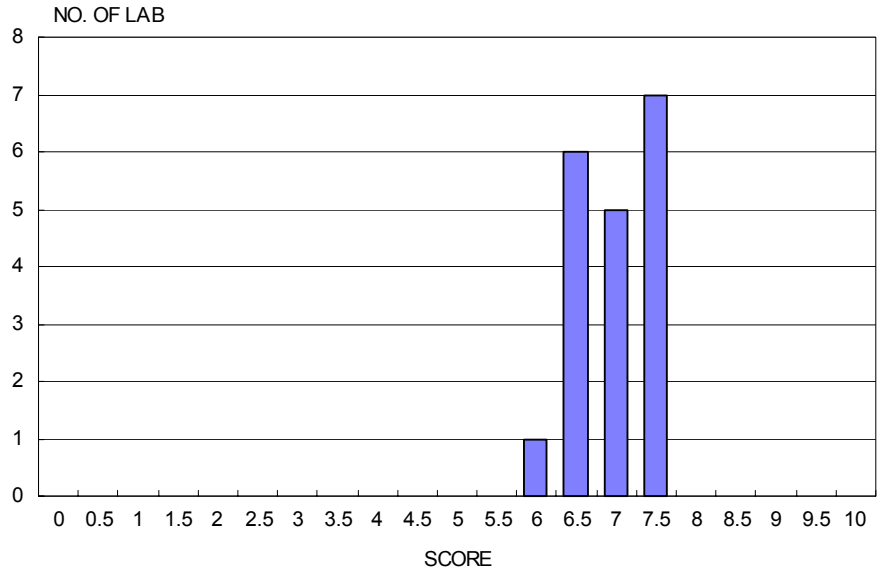
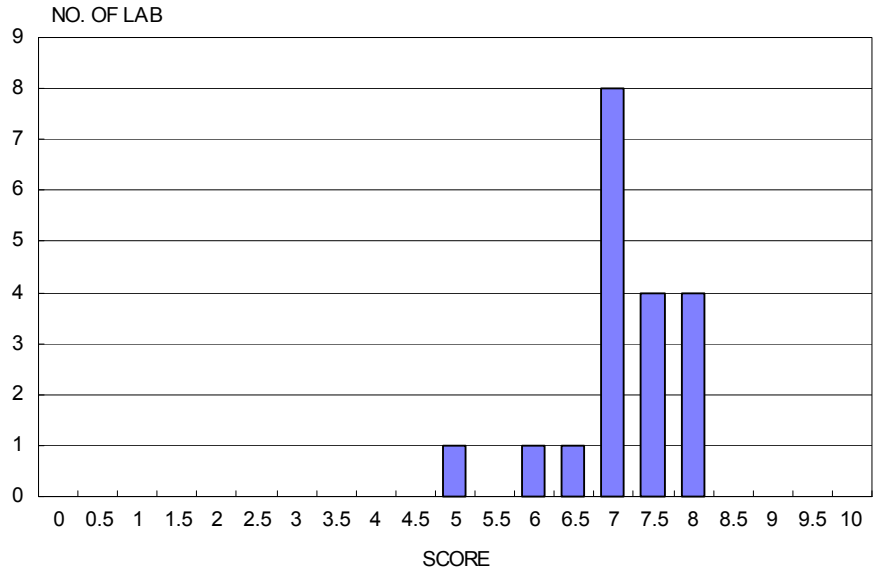


Figure 6: Survey two: HC0908, Periodic Acid-Schiff



Survey Three material was a kidney specimen with amyloidosis.

Figure 7: Survey three: HC0913, H&E

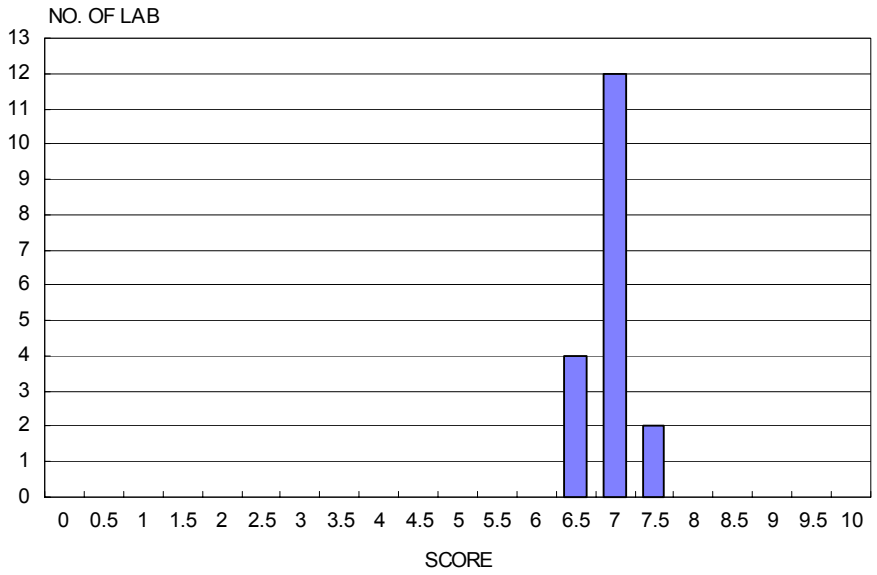
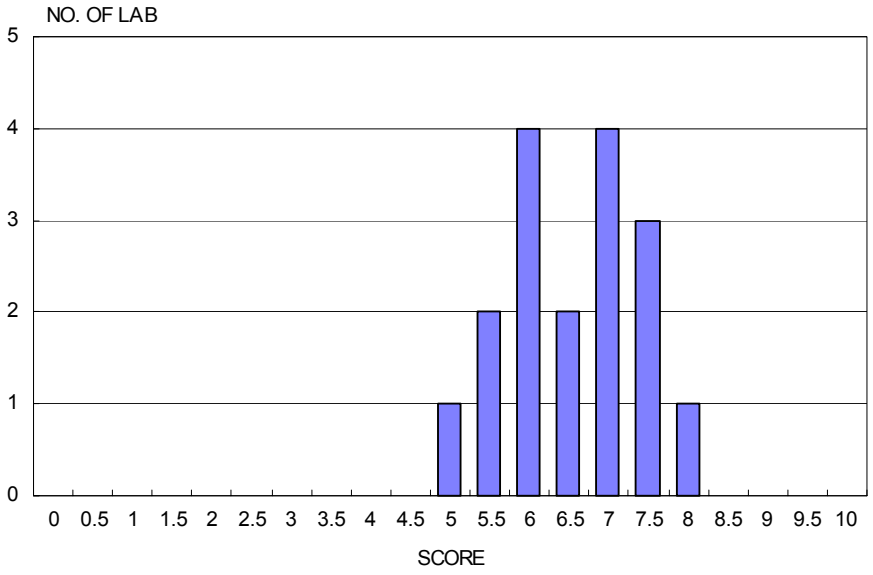


Figure 8: Survey three: HC0914, Congo Red



Survey-Four material was a paraffin section of liver specimen

Figure 9: Survey four: HC0919, H&E

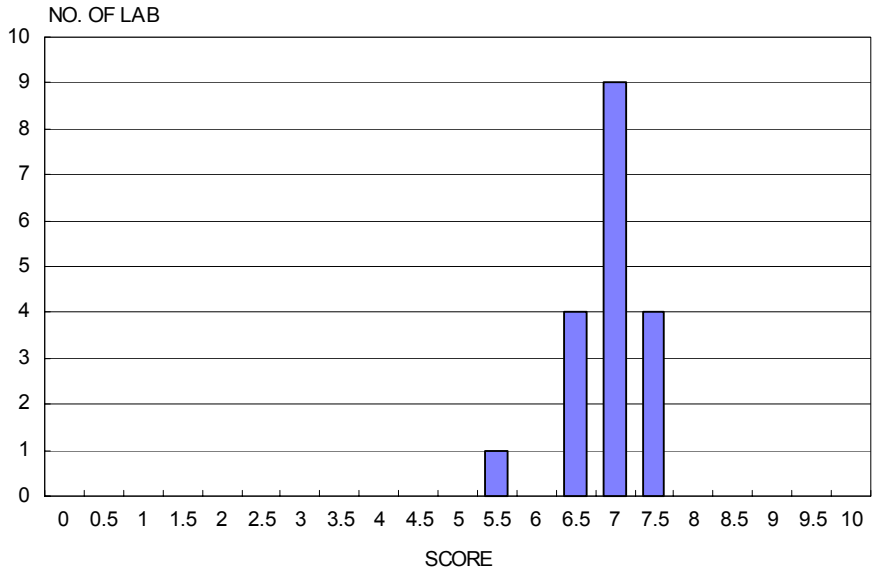
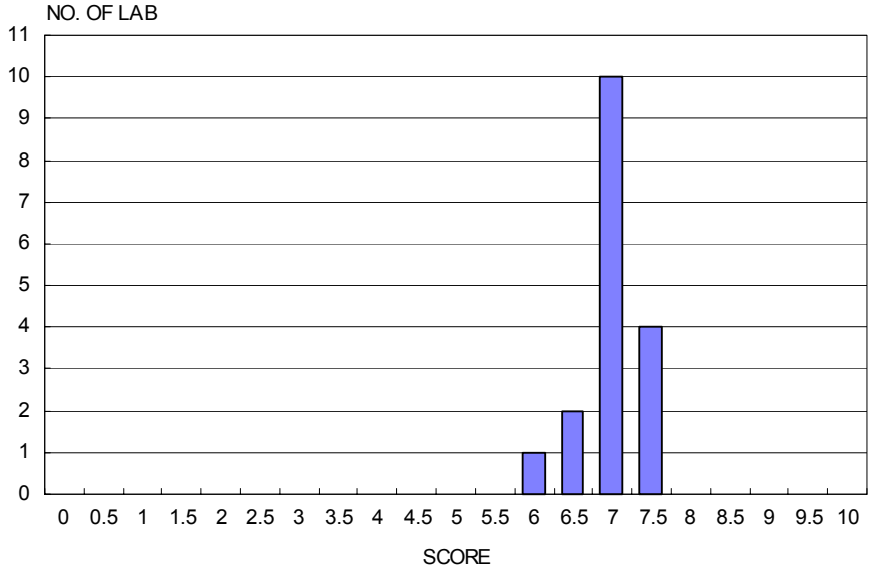


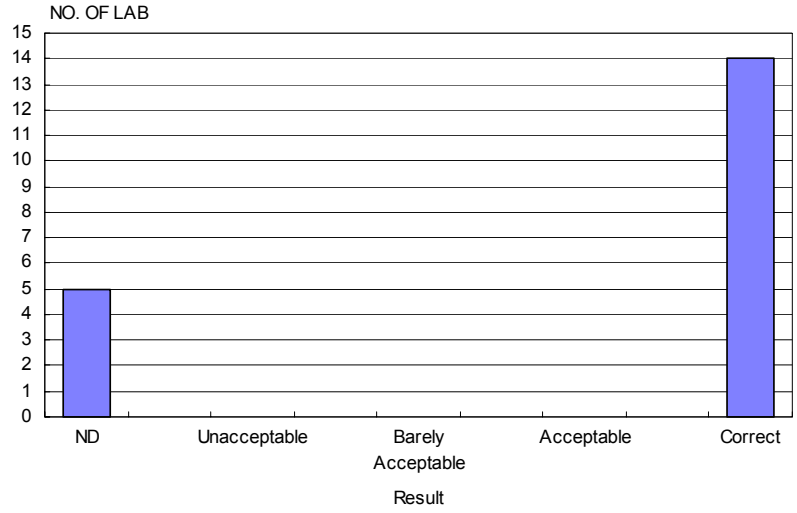
Figure 10: Survey four: HC0920, Perls' Prussian Blue Method



ii. Cytopathology

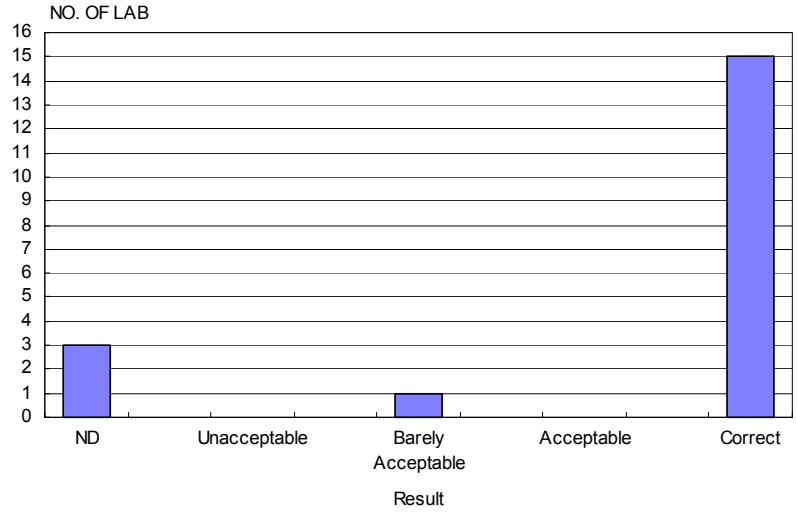
For Survey One only 74% participants returned their results for assessment, all with correct results.

Figure 11: Survey one: HC0903, Target Answer: Negative for malignant cells



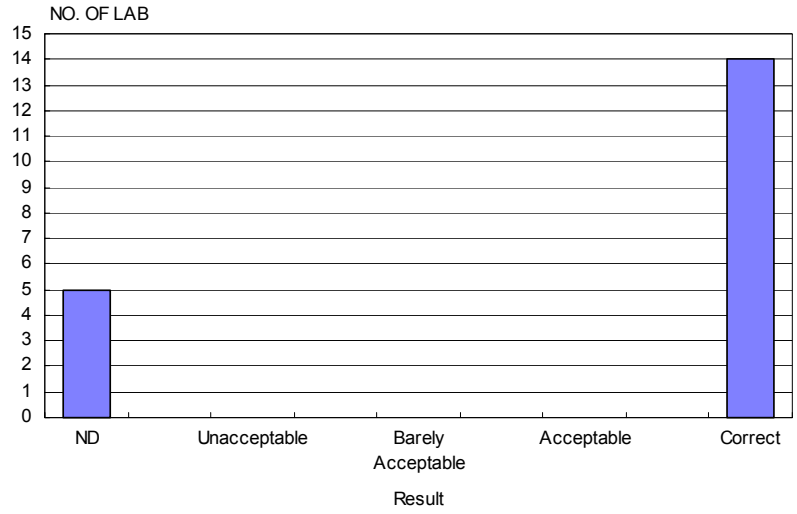
For Survey two about 84% participants returned their results. All except one answered correctly.

Figure 12: Survey two: HC0909, Target Answer: Malignant cells seen, favour adenocarcinoma



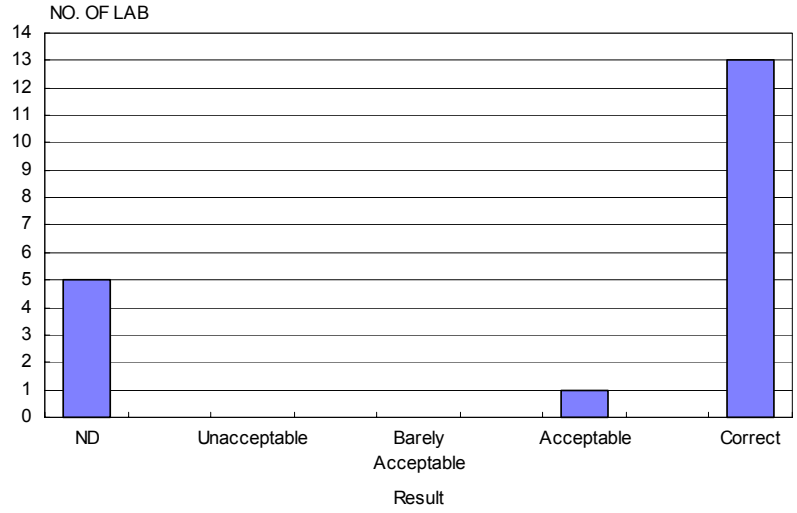
With Survey Three 74% laboratories returned their results. All got correct results.

Figure 13: Survey three: HC0915, Target Answer: Negative for malignancy



In Survey Four 74% participants returned their results and all gave correct answers.

Figure 14: Survey Four: HC0921, Target Answer: Adenocarcinoma



iii. Immunohistochemical Staining Programme

a. Survey One

S100

The antibody detects cells that express S100 Ca^{2+}-binding proteins. The S100 proteins comprises 19 members that are differentially expressed in a various cell types; such as in glial cells of the central and peripheral nervous system, in melano-cytes, chondrocytes, adipocytes, cardio-myocytes, salivary epithelial cells, renal cells and fibroblasts etc.

S100 is useful in the identification of S100-positive neoplasms, such as malignant melanoma (Orchard GE, 2000), Langerhans' histiocytosis (Ye F, Huang S-W, Dong H-J, 1990), chondroblastoma (Edel G et al, 1992), and schwannoma (Gould VE et al, 1986).

The survey material for the S100 demonstration was a section of appendix. The best stained section showed intense staining of nerve bundles and nerve fibres innervating smooth muscle layers and the lamina propria between epithelial crypts of the appendix. The inter-digitizing cells in lymphoid follicles are clearly stained, while nerve bundles and fat cells in the stroma are also well demonstrated. Little or no background staining of connective tissue and lymphoid follicles was demonstrated

One out of twelve laboratories failed in the HC0904 (supplied antibody) and no laboratory failed in the HC0905 (in-house antibody). The median score of HC0904 and HC0905 were found to be 8.0 and 7.5 respectively. The major cause of the failure was weak signal demonstration with heavy background. The distributions of score were shown in Figure 15 and 16:

Figure 15: Survey one: HC0904, S100

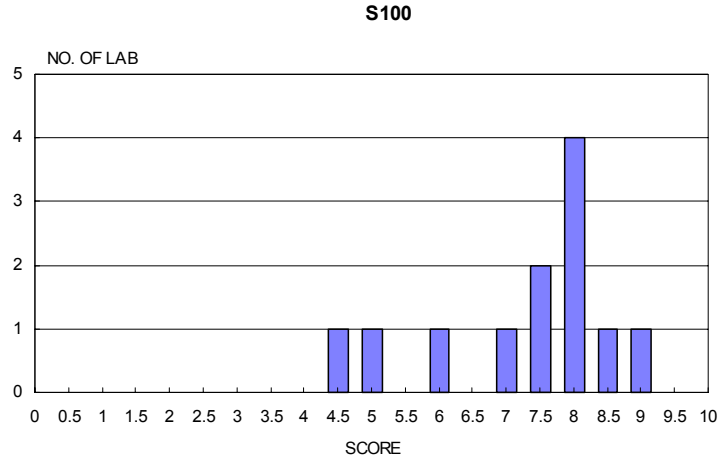


Figure 16: Survey one: HC0905, S100 in-house

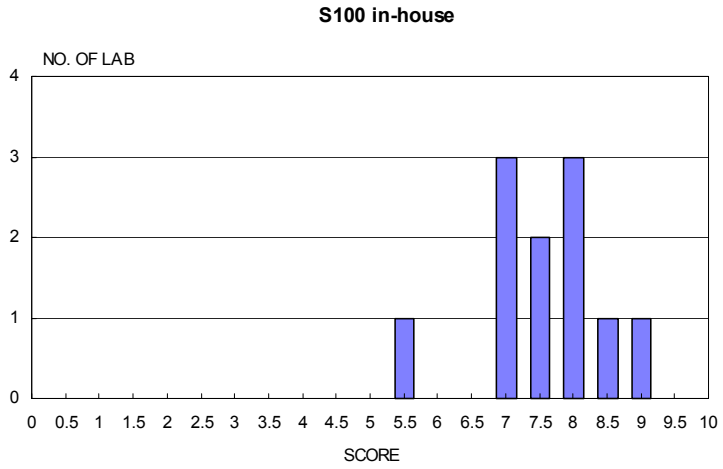


Table 5. The Best Method

STEP	***HC0904*** ***(S 100 Supplied)***	***HC0905*** ***(S 100 in-house)***
Supplier	Dako	Dako
Dilution	1:1200	1:3000
Peroxidase Blocking	10 min	10 min
Antigen retrieval:	Pressure cooking pretreatment 3.5 min.	Pressure cooking pretreatment 3.5 min.
Detection System	Dako Envision HRP	Dako Envision HRP
Duration of Colour Development	DAB 10 min.	DAB 10 min.

b. Survey Two

AE1/3

The antibody is a cocktail of two monoclonal antibodies; AE1 and AE3. Antibody AE1 reacts with an antigenic determinant of the subfamily A cytokeratins, including cytokeratins 10, 13, 14, 15 16 and 19 (Sun TT et al, 1984). Antibody AE3 reacts with the subfamily B cytokeratins including cytokeratins 1 and 2, 3, 4, 5, 6, 7 and 8 respectively (Eichner R, Bonitz P, Sun TT, 1984).

The antibody is useful clinically for the identification of tumours as carcinoma or epithelial origin and for differential identification of undifferentiated carcinomas.

The survey material for the AE1/3 demonstration was a case of undifferentiated carcinoma of caecum. The undifferentiated cancer cells were in sheet or nest form and in the lymphoid cell rich stroma. The tumour cells contain vesicular nuclei and eosinophilic cytoplasm forming syncytial sheets morphologically similar to lymphoepithelioma-like carcinoma. Immunohistochemical study shows that the tumour cells are positive for AE1/3.

All laboratories were well-performed in both HC0910 (supplied antibody) and HC0911 (in-house antibody) assessment. The median score of HC0910 and HC0911 were found to be 8.0 and 8.0 respectively.

The distributions of score were shown in Figure 17 and 18:

Figure 17: Survey two: HC910, AE1/3

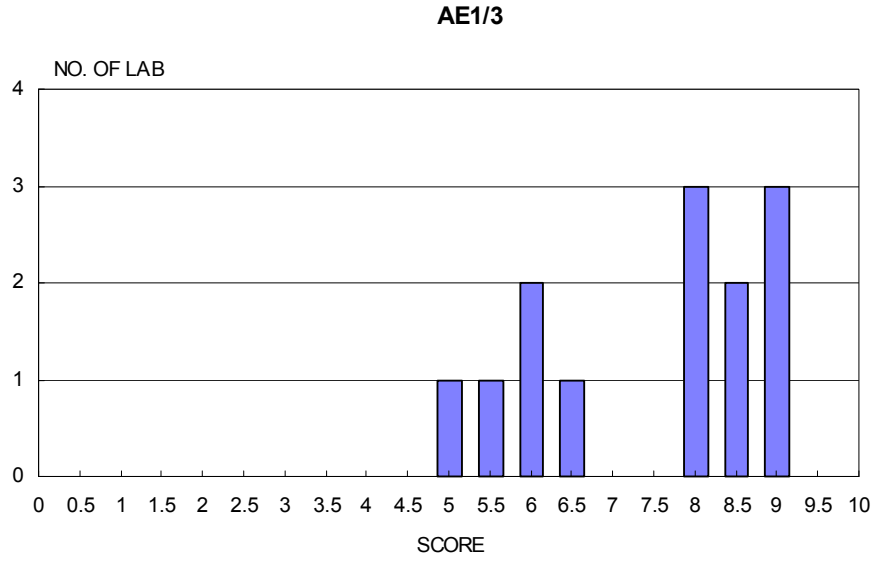


Figure 18: Survey two: HC0911, AE1/3 in-house

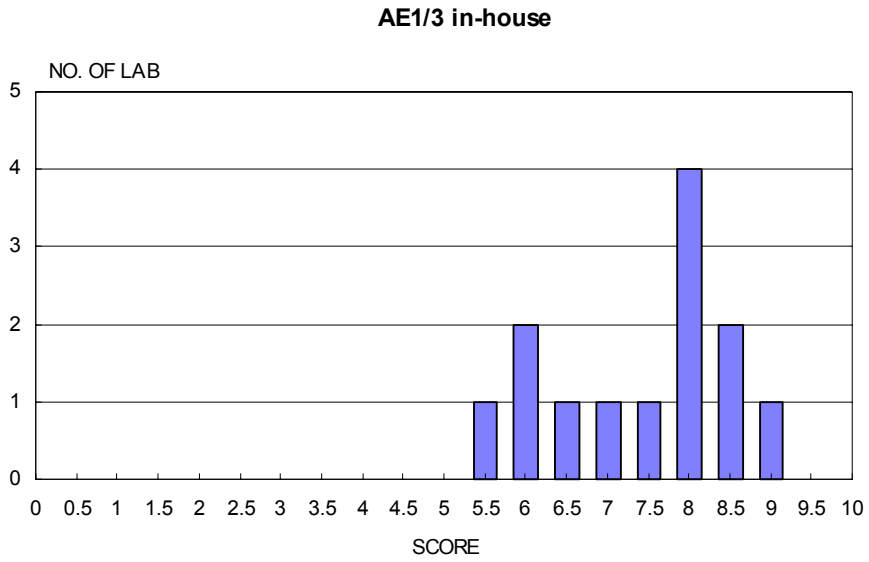


Table 6. The Best Method

STEP	***HC0910 (AE1/3 Supplied)***	***HC0911 (AE1/3 in-house)***
Supplier	Dako	Dako
Dilution	1:50	1:50
Peroxidase Blocking	unspecified	unspecified
Antigen retrieval:	Pressure cooking pretreatment 15 min.	Pressure cooking pretreatment 15 min
Detection System	i-view Detection Kit System	i-view Detection Kit System
Duration of Colour Development	DAB 5 min.	DAB 5 min.

c. Survey Three

CD30

CD30 is a transmembrane cytokine receptor belonging to the tumour necrosis factor (TNF) receptor superfamily. CD30 expression is found on Hodgkin and Reed-Sternberg (H-RS) cells, anaplastic large-cell lymphoma (ALCL) cells, and on activated B and T lymphocytes (de Bruin PC et al, 1995). In non-lymphoid tissues and neoplasms, CD30 expression has been confirmed in embryonal carcinomas, seminomas, decidual cells and mesotheliomas (Dürkop H, Foss H-D, Eitelbach F, 2000). The antibody is a useful tool for the identification of ALCL and as a secondary marker for Hodgkin's disease (Schwarting R et al, 1989)

The survey material for the CD30 demonstration is a case of lymphocyte-rich Classical Hodgkin Lymphoma. Vague small nodules of small B cells are seen, with small clusters of T cells identified within these B cell nodules. Some of these T cells are seen surrounding isolated large cells. Mummified cells and rare cells with Reed-Sternberg cell morphology are also present. Immunohistochemical study shows that the scattered large cells are positive for CD30.

One out of eleven laboratories failed in both HC0916 (supplied antibody) and HC0917 (in-house antibody) assessment. The median score of HC0916 and HC0917 were found to be 5.5 and 5.0 respectively.

The distributions of score were shown in Figure 19 and 20:

Figure 19: Survey three: HC0916, CD30

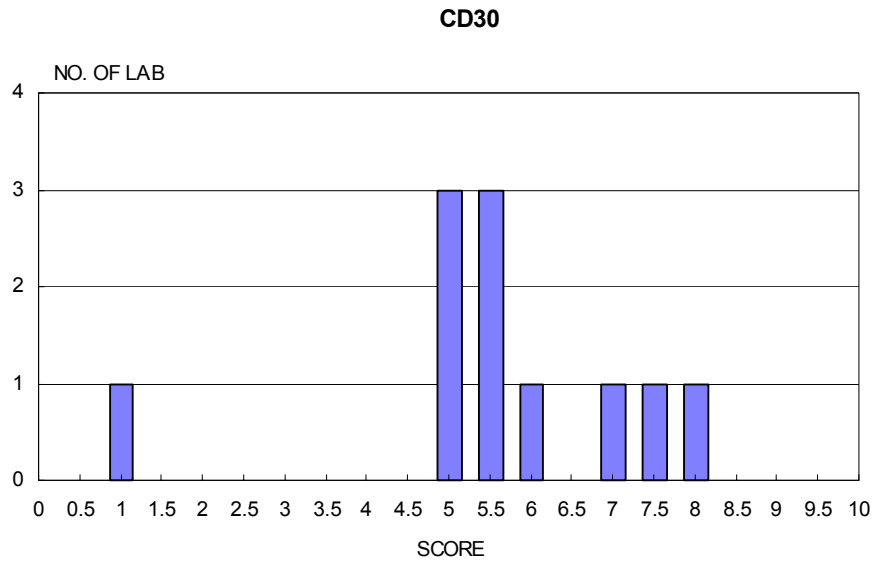


Figure 20: Survey three: HC0917, CD30 in-house

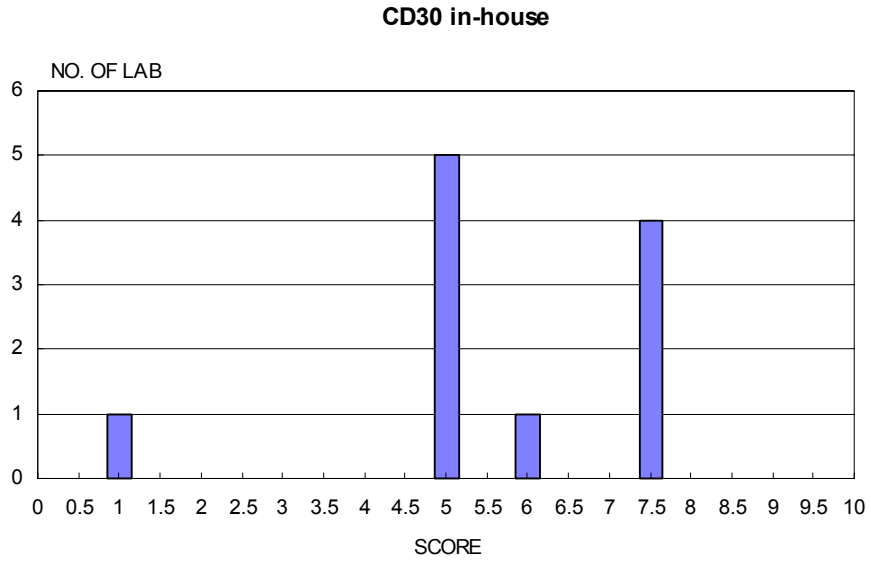


Table 7. The Best Method

STEP	***HC0916*** ***(*CD30 *Supplied)***	***HC0917*** ***(*CD30 *in-house)***
Supplier	Dako	Dako
Dilution	1:20	1:15
Peroxidase Blocking	10 min	10 min
Antigen retrieval:	Pressure cooking pretreatment 3.5 min	Pressure cooking pretreatment 3.5 min
Detection System	Envision Detection System	Envision Detection System
Duration of Colour Development	DAB 10 min.	DAB 10 min.

d. Survey Four

ER

Estrogens have been found to be preferentially concentrated in the estrogen target organs of human breast cancers and it is documented that the breast cancer growth rate is dependent on the presence of estrogen or progesterone or both in most breast cancers (Elledge RM & Fuqua SAW, 2000, Ch.31). Thus, estrogen receptor status in mammary carcinomas is considered to be a validated prognostic and predictive factor for patient management for anti-hormonal therapy (Fitzgibbons FK et al, 2000). The antibody is useful in the semi-quantitative detection of human estrogen receptor in tissue sections of human breast cancer by immunohistochemistry

The survey material for the ER demonstration is a case of lymphocyte-rich Classical Hodgkin Lymphoma. Vague small nodules of small B cells are seen, with small clusters of T cells identified within these B cell nodules. Some of these T cells are seen surrounding isolated large cells. Mummified cells and rare cells with Reed-Sternberg cell morphology are also present. Immunohistochemical study shows that the scattered large cells are positive for CD30.

One out of eleven laboratories failed in HC0922 (supplied antibody) and no laboratory failed in HC0923 (in-house antibody) assessment. The median score of HC0922 and HC0923 were found to be both 8.0 in score.

The distributions of score were shown in Figure 21 and 22:

Figure 21: Survey four: HC0922, ER

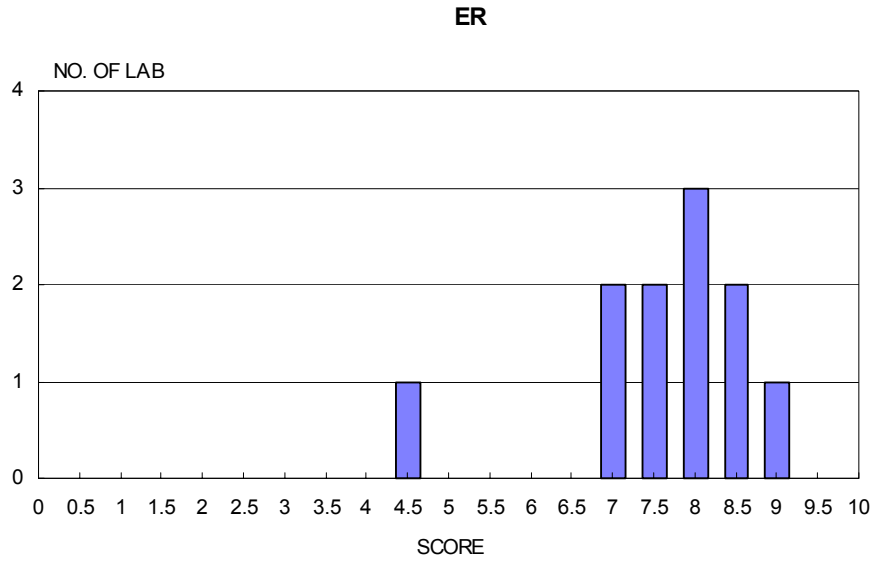


Figure 22: Survey four: HC0923, ER in-house

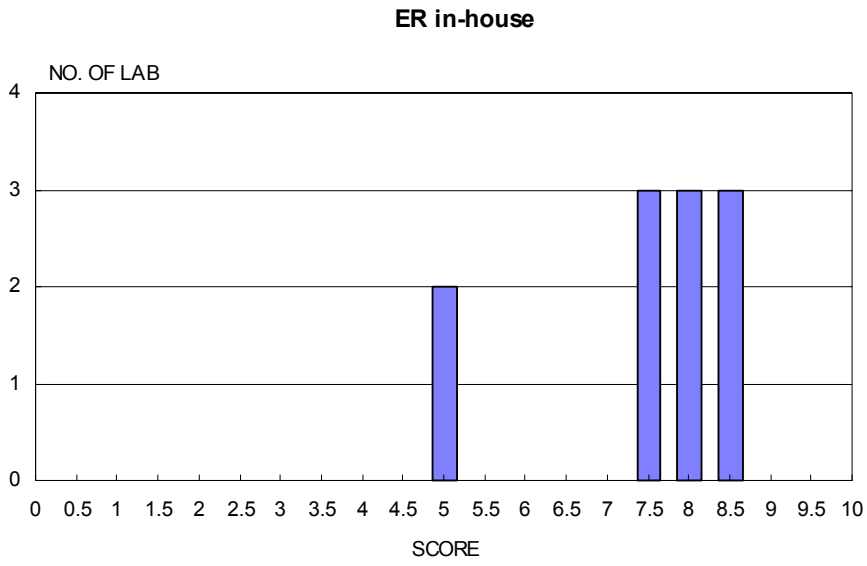


Table 8. The Best Method

STEP	***HC0922*** ***(*ER *Supplied)***	***HC0923*** ***(*ER *in-house)***
Supplier	Dako	Dako
Dilution	1:20	1:15
Peroxidase Blocking	10 min	10 min
Antigen retrieval:	Pressure cooking pretreatment	Pressure cooking pretreatment
	3.5 min	3.5 min
Detection System	Envision Detection System	Envision Detection System
Duration of Colour Development	DAB	DAB
	10 min.	10 min.

e. Continue Assessment of Laboratory Performance: EMA

EMA

Epithelial membrane antigen (EMA) proteins are present in a variety of epithelia of both normal and neoplastic types. The antibody is useful in the detection of breast carcinoma metastases in histological sections of liver, lymph node, and bone marrow, in the differentiation of anaplastic carcinoma from malignant lymphomas, and for the recognition of spindle cell epithelial malignancies (Cordell J et al, 1985, Sloane JP & Ormerod MG, 1981).

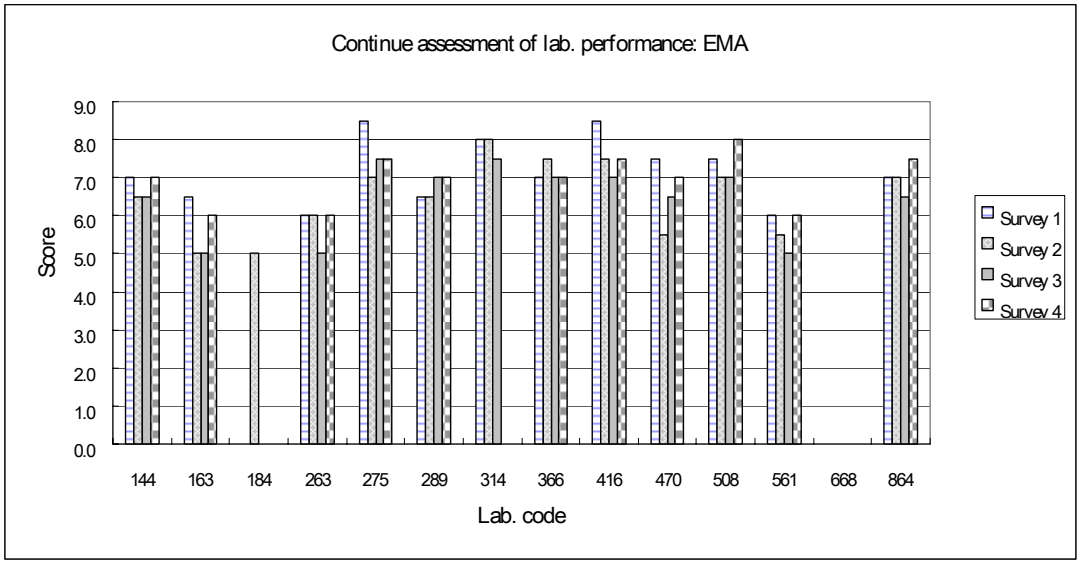
The survey material for the HC0924 EMA demonstration is a case of undifferentiated carcinoma of caecum. The undifferentiated cancer cells are in sheet or nest form and in the lymphoid cell rich stroma. The tumour cells contain vesicular nuclei and eosinophilic cytoplasm forming syncytial sheets morphologically similar to lymphoepithelioma-like carcinoma. Immunohistochemical study shows that the tumour cells are positive for EMA.

To monitor the performance consistency, the EMA antibody and sections from the same tissue block were sent to the participants for all four surveys. The returned slides were assessed as usual. The median scores of the four surveys were tabulated in table 9 and the distribution of scores was shown in Figure 23.The calculated means of all the returns was 6.8 and the standard deviation was 0.91. All the laboratories who had returned the slides passed the assessment.

Table 9. Median Score Summary

EMA	Survey 1	Survey 2	Survey 3	Survey 4
Median	7	6.5	7	7

Figure 23. Distribution of Scores



Two laboratories; 184 and 668, that had not return their EMA slides for more than two surveys were excluded from the continue assessment.

Eight laboratories; 144, 263, 289, 314, 366, 508, 561 and 864, with "score spread" of less than or equal to 1 were considered as consistence.

Five laboratories; 163, 184, 275, 416 and 470, with "score spread" of greater than 1 were considered as inconsistence.

References

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