ANATOMICAL PATHOLOGY

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In 2008, eighteen and fourteen laboratories participated in the histological staining and the immunohistochemical staining program respectively. The laboratories originated from hospitals of the Hospital Authority, government institutes/clinics, universities and private hospitals.

I. Survey Format

Table 1 and 2 summarised the various staining methods and antibodies assessed in this year program. A questionnaire was included in each survey asking details of the staining procedures done. These details allow the assessors to identify any erroneous step causing unsatisfactory staining results. The staining procedure of the top scored laboratory was compiled with the survey report for reference.

Table 1. Histological Staining Program

Survey	Code Number	Staining Methods
One	HC0802	Warthin-Starry's Silver
Two	HC0808	Gomori's Methenamine Silver (Grocott)
Three	HC0814	Von Kossa
Four	HC0820	Ziehl Neelsen

Table 2. Immunohistochemical Staining Program

Survey	Code Number	Staining Methods
One	HC0804	Cadherin E
	HC0805	Cadherin E (in house)
	HC0806	Cyclin D1
Two	HC0810	CD 99
	HC0811	CD 99 (in house)
	HC0812	Cyclin D1
Three	HC0816	GFAP
	HC0817	GFAP (in house)
	HC0818	Cyclin D1
Four	HC0820	p53
	HC0821	p53 (in house)
	HC0822	Cyclin D1

II. Method of Analysis

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

Table 3. Scoring System

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: crisp and intense positive staining with minimal or no background (good staining contrast), there was no uneven or patchy staining and other unnecessary deposit and 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. Illustration of the Scoring system

	Scores given by Panel				Final
Participant	Member A	Member B	Member C	Member D	Score
X	9	7	8	10	7.5
Y	6	4	5	7	5.5

III. Slide Return Summary

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

Figure 1. Histological Staining

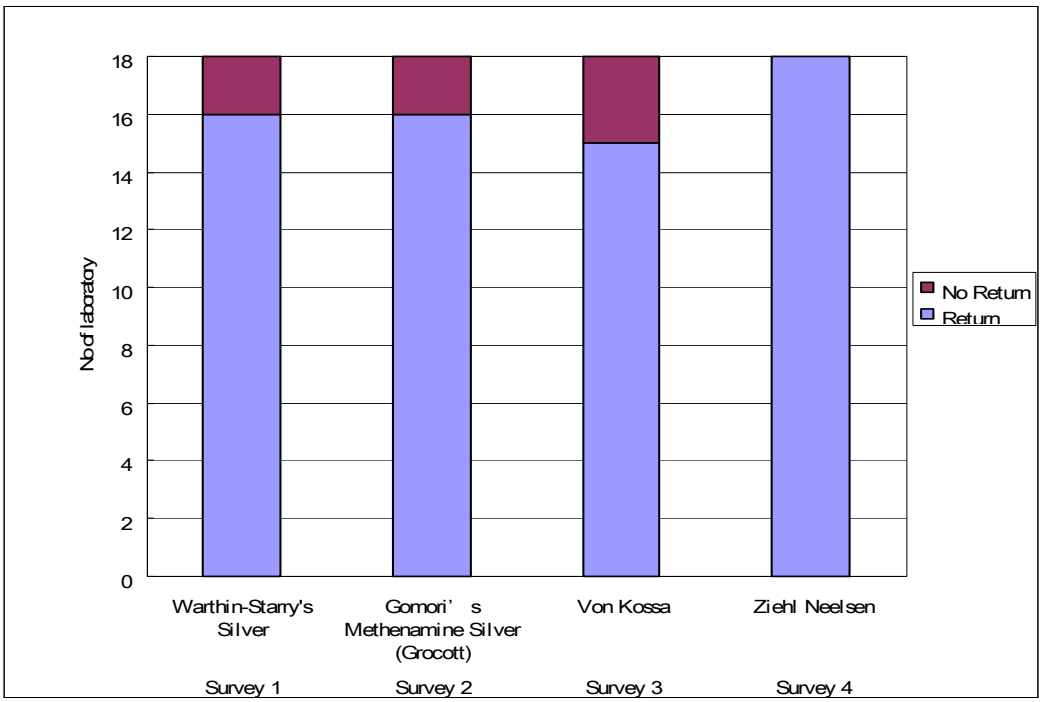
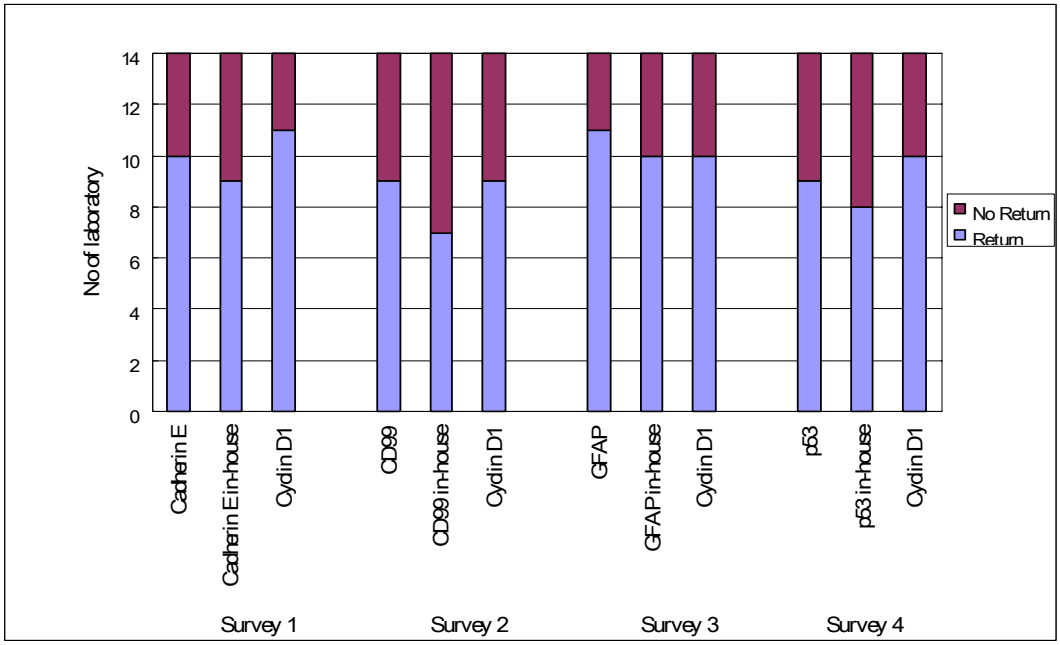


Figure 2. Immnohistochemical Staining



IV. Survey Analysis

i. Histological Staining Programme

Survey One material was a paraffin section with helicobacter associated chronic gastritis. H&E staining was fairly good. To stain the target helicobacter, laboratories could use Warthin-Starry's Silver, Giemsa, Dieterle, Toluidine blue and also immunostaining. For Warthin Starry, the preparation of working silver nitrate was the most prominent factor for demonstrating sharply the helicobacter and the keeping of the working solution. It might be the cause of the weak positive helicobacter staining for the two laboratories.

Figure 3: Survey one: HC0801, H&E

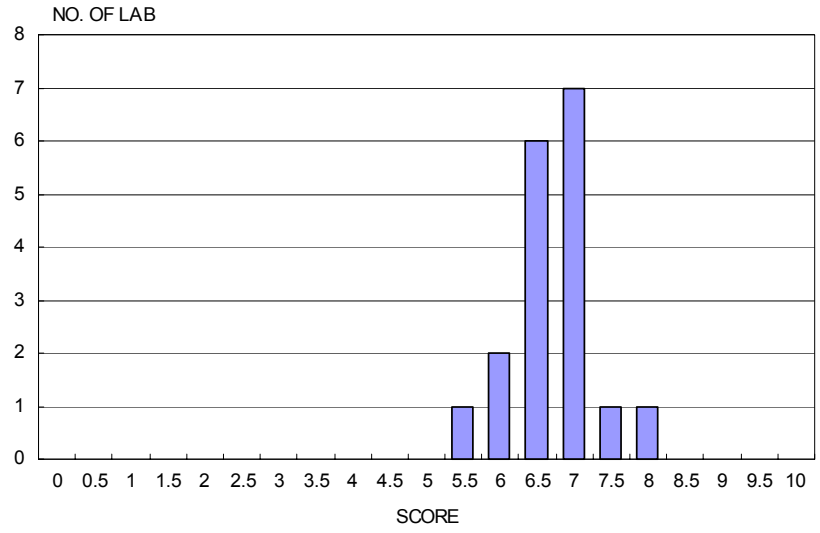
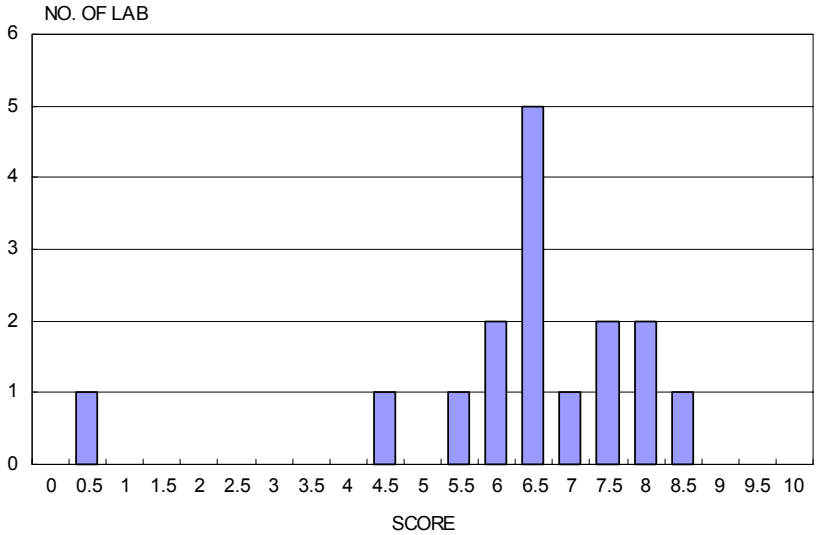


Figure 4: Survey one: HC0802, Warthin Starry



Survey-Two material was a paraffin section of infected lung tissue associated with fungi organisms. H&E staining was acceptable. The oxidizing step, the incubation temperature and the time of incubation would affect the result of the Gomori's Methenamine Silver (Grocott) stain. By trial and error we could standardize our method to get successful results. Two laboratories failed due to poor contrast of the fungi with the dark background.

Figure 5: Survey two: HC0807, H&E

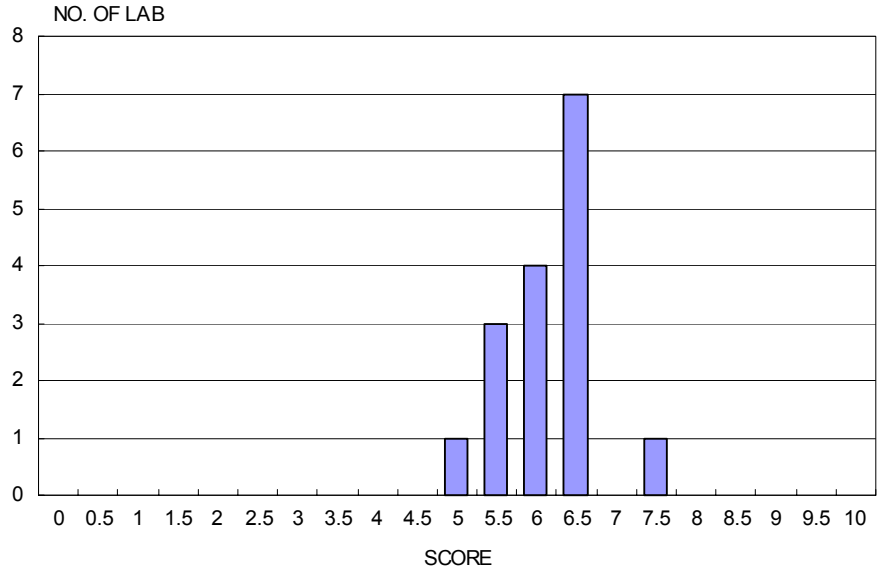
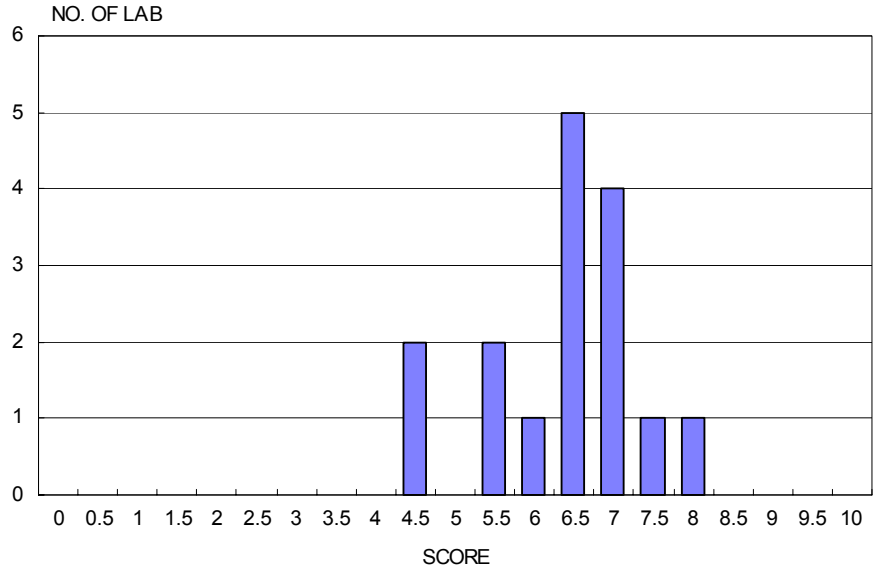


Figure 6: Survey two: HC0808, Gomori's Methenamine Silver (Grocott)



Survey Three material was a heart with foci of calcium deposition. H&E staining was acceptable. For demonstrating calcium deposition, laboratories could use Alizarin Red S and Von Kossa together or Von Kossa only. The result would be different due to the keeping of the working silver solution and the use of reducing agents such as tungsten filament, quartz halogen lamp, sunlight and both sunlight and tungsten lamp. All the laboratories produced acceptable results.

Figure 7: Survey three: HC0813, H&E

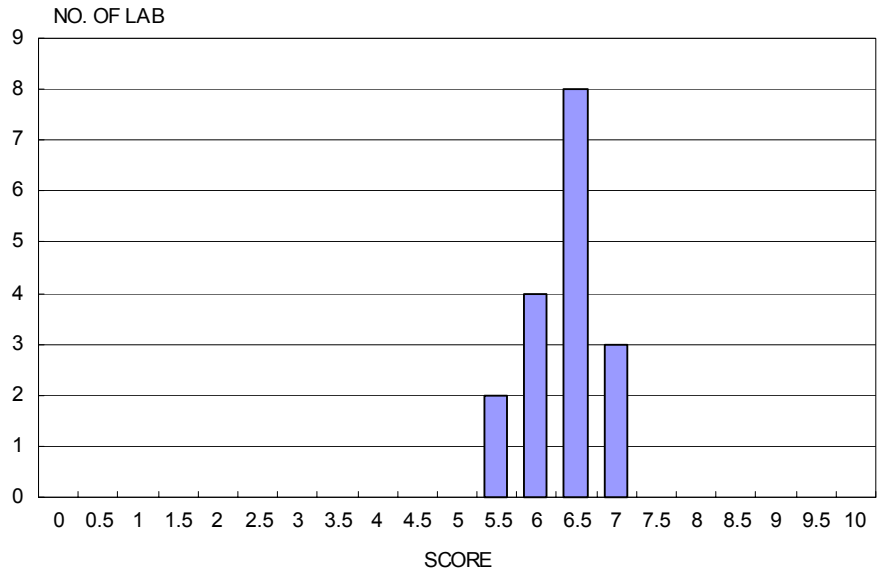
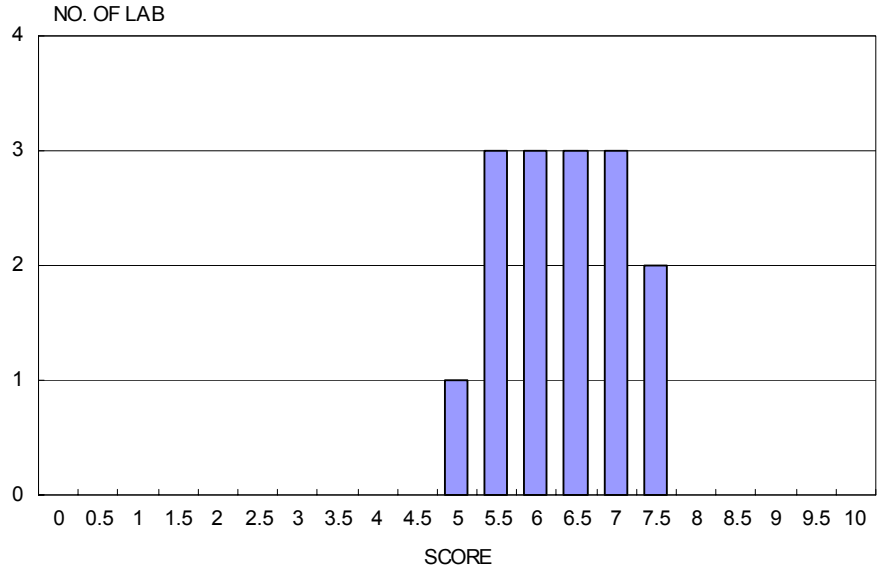


Figure 8: Survey three: HC0814, Von Kossa



Survey-Four material was a paraffin section of infected lung tissue associated with tuberculosis bacilli. All the laboratories produced acceptable H&E. All participants stained the targeted material with Ziehl Neelsen and produced acceptable results. The targeted material could also be demonstrated by Thioflavine T and examined under fluorescence microscope. The staining intensity and the amount of the targeted material demonstrated would be affected by the temperature of the staining solution and the time of incubation. Some laboratories had taken additional safety measures, e.g. use of gloves (varies from plastic, heat resistant to latex), goggles, surgical masks and fume absorbers during preparation of the Carbol Fuchsin staining solution.

Figure 9: Survey four: HC0819, H&E

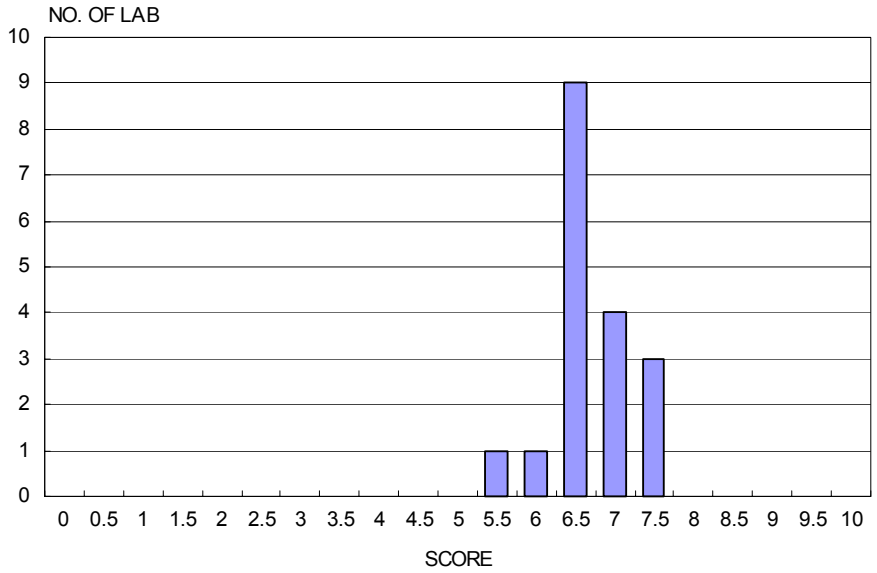
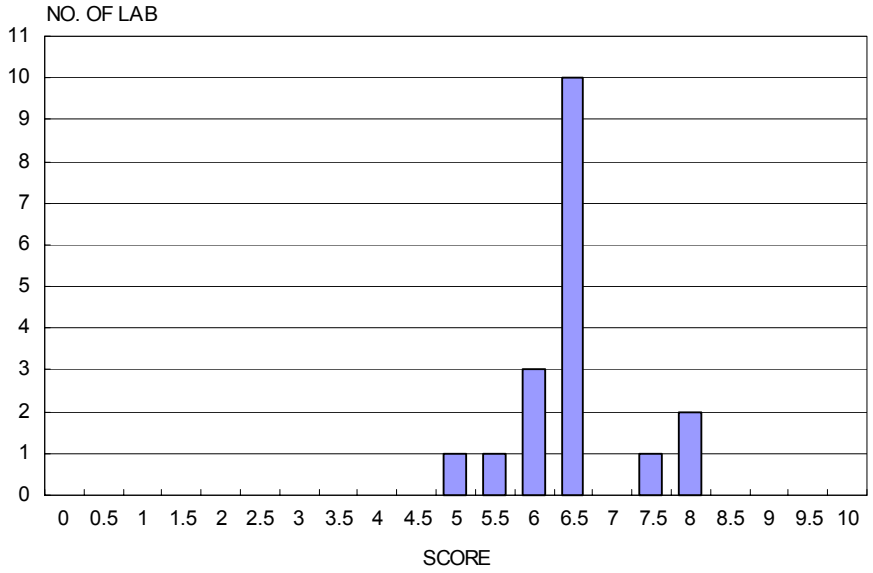


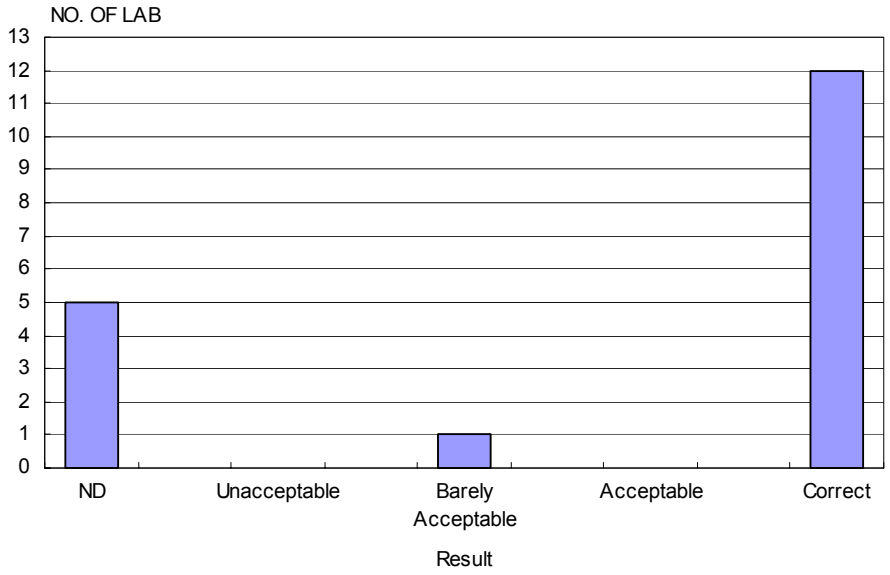
Figure 10: Survey four: HC0820, Ziehl Neelsen



ii. Cytopathology

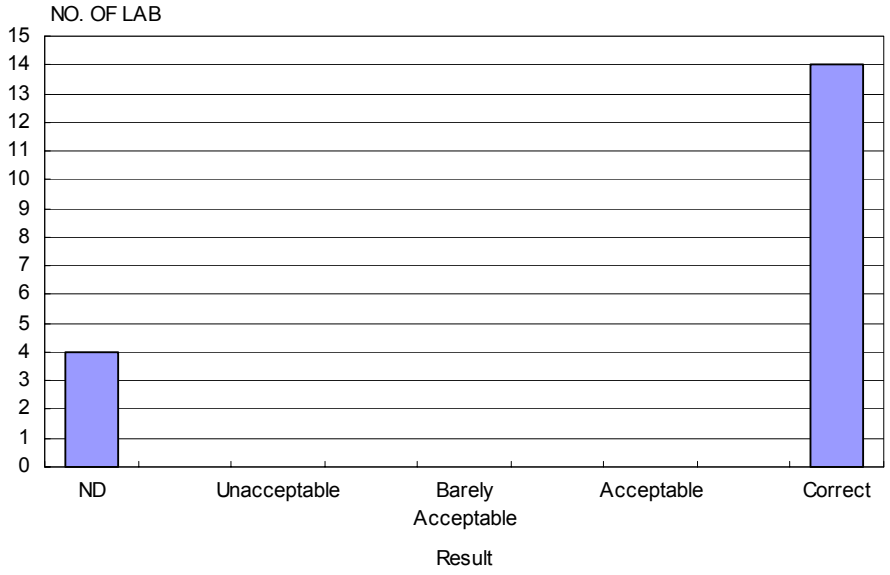
For Survey One only 72% participants returned their results for assessment. All except one with correct results.

Figure 11: Survey one: HC0803, Target Answer: Malignant Cells Seen favour Adenocarcinoma



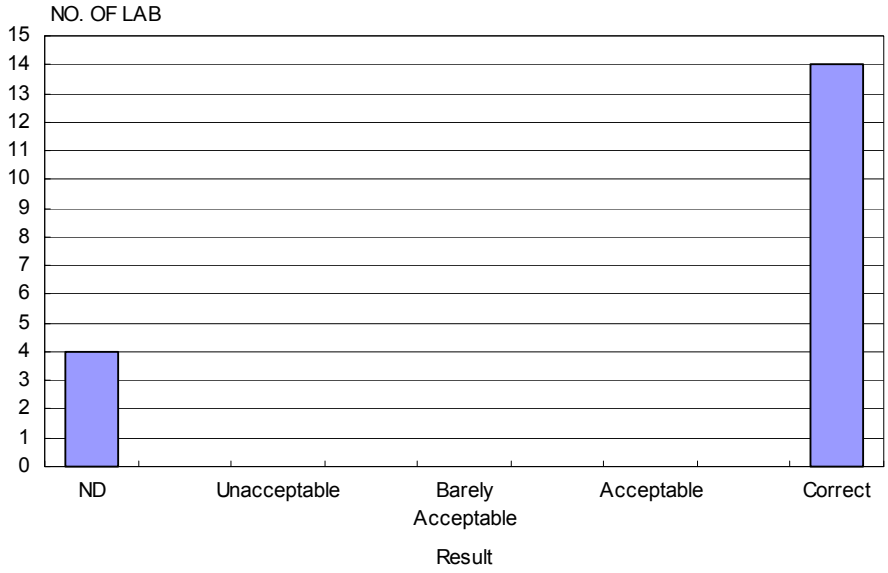
For Survey Two about 78% participants returned their results. All got correct answers.

Figure 12: Survey two: HC0809, Target Answer: Adenocarcinoma



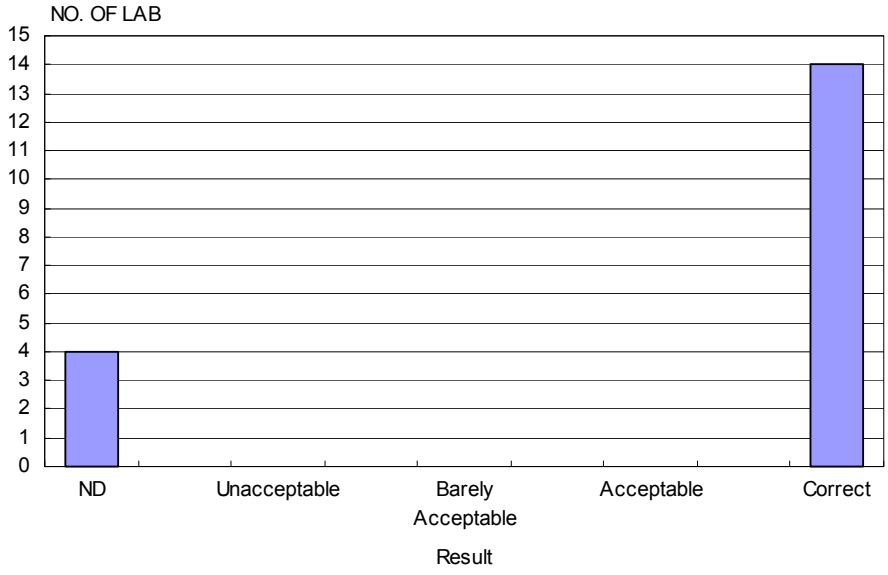
With Survey Three 78% laboratories returned their results. All got correct results.

Figure 13: Survey three: HC0815, Target Answer: No Evidence of Malignancy



In Survey Four 78% participants returned their results and all gave correct answers.

Figure 14: Survey Four: HC0821, Target Answer: Squamous Cell Carcinoma



iii.. Immunohistochemical Staining Programme

a. Survey One

Cadherin E

The Cadherin E belongs to the Cadherin family. Members of the family are transmembrane glycoproteins involving in Ca^{2+} dependent cell adhesion of the epithelial cells and have important function in the regulation of tissue morphology and structure.

The anti-cadherin E antibody is used to detect cadherin E protein in formalin fixed, paraffin embedded sections. Clinically, it may be useful in the differentiation of pleural mesotheliomas from lung adenocarcinomas (cadherin E positive)

One laboratory failed because of weak demonstration of cadherin E with the supplied antibody. The median score of the supplied antibody and the in-house were 6.5 and 7.5 respectively. The distributions of score were shown in Figure 15 and 16:

Figure 15: Survey one: HC0804, Cadherin E

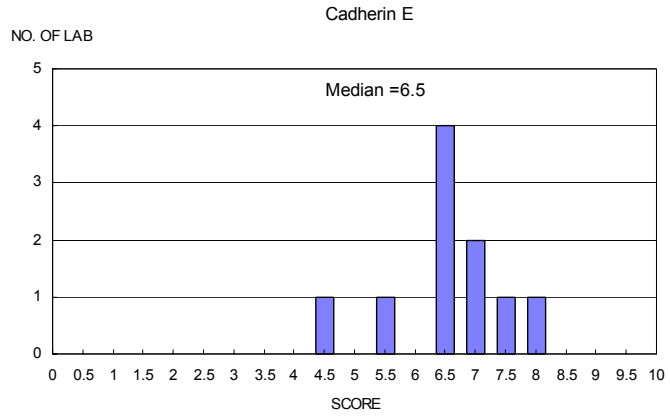


Figure 16: Survey one: HC0805, Cadherin E in-house

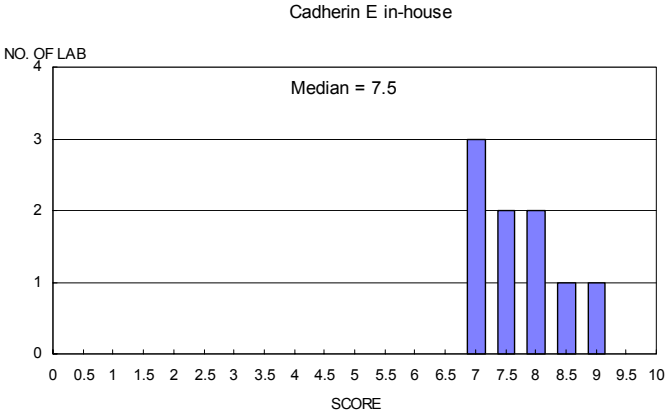


Table 5. The Best Method

STEP	HC0804 (Cadherin E Supplied)	HC0805 (Cadherin E in-house)
Supplier	NeoMarkers	Zymed
Dilution	1:100	1:50
Peroxidase Blocking	10 min	(Unspecified)
Antigen retrieval:	Pressure cooking pretreatment	Pressure cooking pretreatment
	7 min	15 min
Detection System	Dako Envision HRP	Ventana i View
Duration of Colour Development	DAB	DAB
	10 min.	5 min.

b. Survey Two

CD99

CD99 is a 30kD transmembrane glycoprotein, which is useful in the differentiation of Ewing's sarcoma (ES) and primitive neuroectodermal tumours (PNET). The anti-CD99 antibody stains the membrane and cytoplasm of the ES and PNET strongly. Normal cells including: pancreatic islet cells, Sertoli's cells, granulosa cells and thymocytes have been reported to be stained by CD99.

All laboratories stained the CD99 satisfactorily with the supplied and their in-house antibodies, the median scores were 7.5 for both antibodies.

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

Figure 17: Survey two: HC0810, CD99

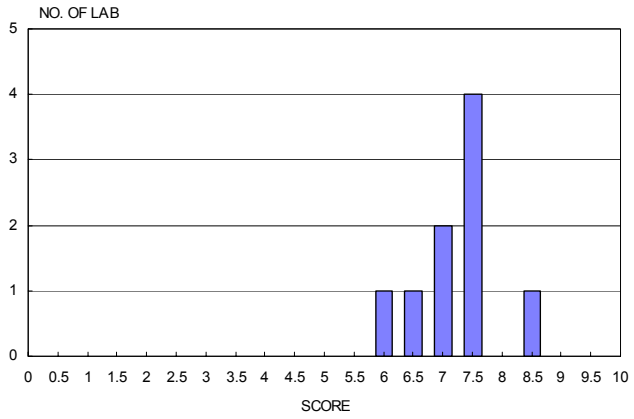


Figure 18: Survey two: HC0811, CD99 in-house

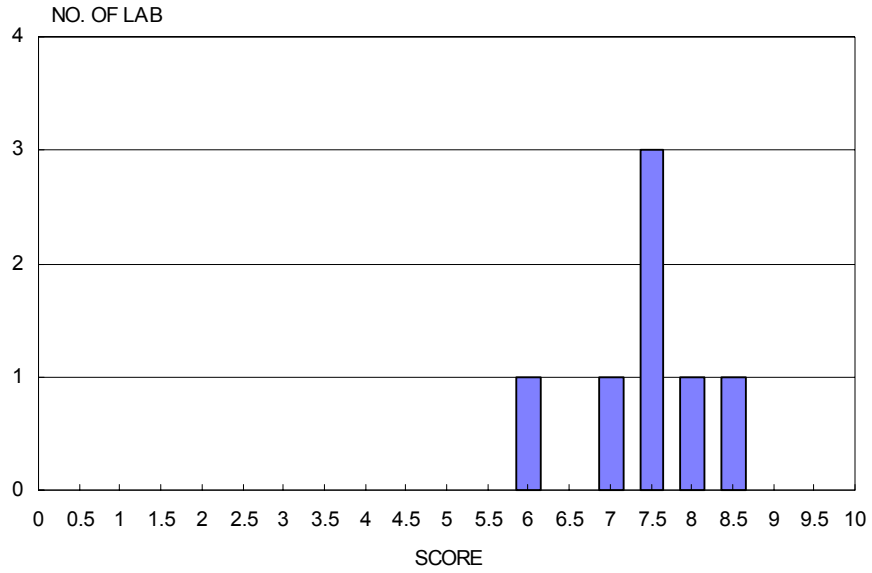


Table 6. The Best Method

STEP	HC0810 (CD 99 Supplied)	HC0811 (CD99 in-house)
Supplier	NeoMarkers	Signet
Dilution	1:100	1:40
Peroxidase Blocking	(Unspecified)	(Unspecified)
Antigen retrieval:	Pressure cooking pretreatment	Pressure cooking pretreatment
	15 min.	15 min.
Detection System	Ventana i View	Ventana i View
Duration of Colour Development	DAB	DAB
	5 min.	5 min.

c. Survey Three

GFAP

Glial Fibrillary Acidic Protein (GFAP) is an intermediate filament protein of 50kD of astroglia. The anti-GFAP antibody stains normal and neoplastic astrocytic cells. Schwann cells and enteric glia are also stained.

Two laboratories failed in the staining of GFAP with the supplied antibody, the median score was 7.5. All laboratories passed in the staining of GFAP with their in-house antibodies, the median score was also 7.5.

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

Figure 19: Survey three: HC0816, GFAP

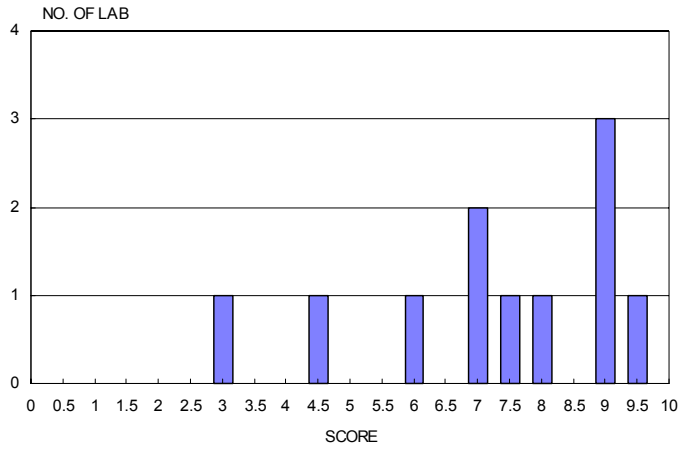


Figure 20: Survey three: HC0817, GFAP in-house

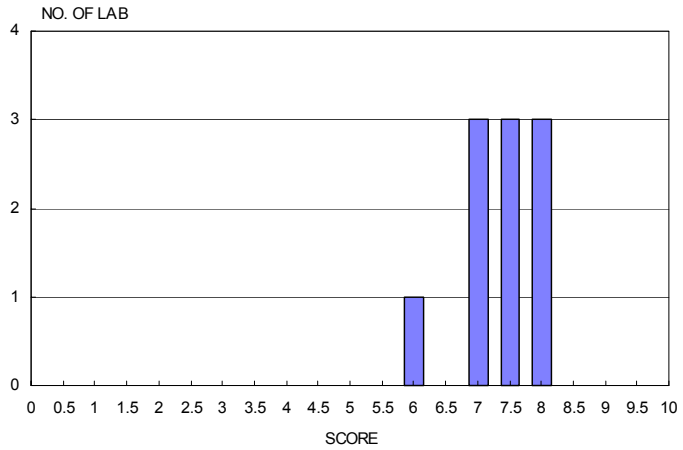


Table 7. The Best Method

STEP	HC0816 (GFAP Supplied)	HC0817 (GFAP in-house)
Supplier	NeoMarkers	Dako (clone 6F2)
Dilution	1:50	1:600
Peroxidase Blocking	7 min.	(Unspecified)
Antigen retrieval:	Unspecified 22 min.	Pressure cooking pretreatment 3 ⅓ min
Detection System	Dako Envision Goat anti-mouse Polymer	Ventana i View
Duration of Colour Development	DAB 8 min.	DAB 8 min.

d. Survey Four

p53

p53 is a nuclear phosphoprotein of 53kD. The anti-p53 stains wild type and mutant type p53 protein, but as the wild type p53 protein has a very short half life and thus the amount present is generally below the detection limit of IHC. On the contrary, the mutant type p53 protein is much more stable and accumulates to a high level in human neoplasias. Clinically, it is useful in the differentiation of malignant mesothelioma from reactive mesothelial cells in effusion. It is also a marker for poor prognosis if found to be over-expressed concurrently with c-erbB-2 in node negative breast carcinoma.

All laboratories failed except one in the staining of p53 with the supplied antibody and the median score was 1. The poor performance with this antibody was properly due to prolong exposure to high temperature during transportation.

Two laboratories failed in the staining of p53 with their in-house antibodies. The median score was 5. The distributions of score were shown in Figure 21 and 22:

Figure 21: Survey four: HC0822, p53

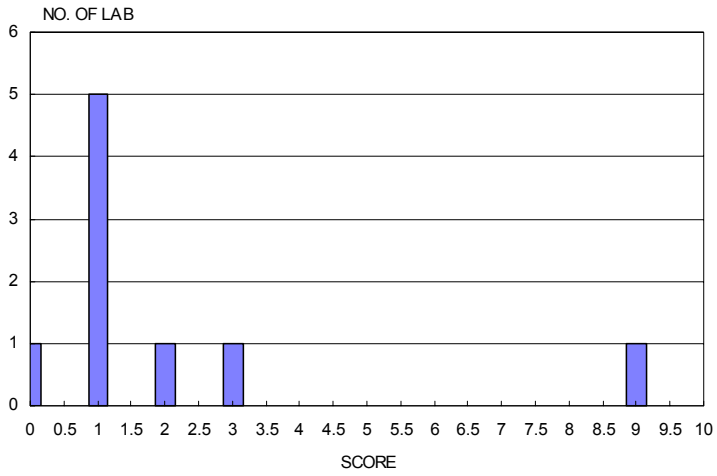


Figure 22: Survey four: HC0823, p53 in-house

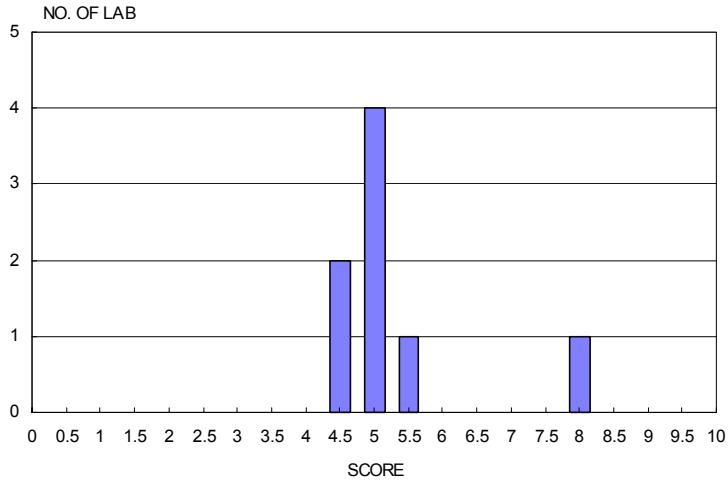


Table 8. The Best Method

STEP	HC0822 (p53 Supplied)	HC0823 (p53 in-house)
Supplier	NeoMarkers	Dako
Dilution	1:40	1:2000
Peroxidase Blocking	5 min.	5 min.
Antigen retrieval:	100°C water bath	100°C water bath
	EDTA	EDTA
	20 min.	20 min.
Detection System	Bond Polymer Refine	Bond Polymer Refine
Duration of Colour Development	DAB	DAB
	10 min.	10 min.

e. Continue Assessment of Laboratory Performance: Cyclin D1

Cyclin D1

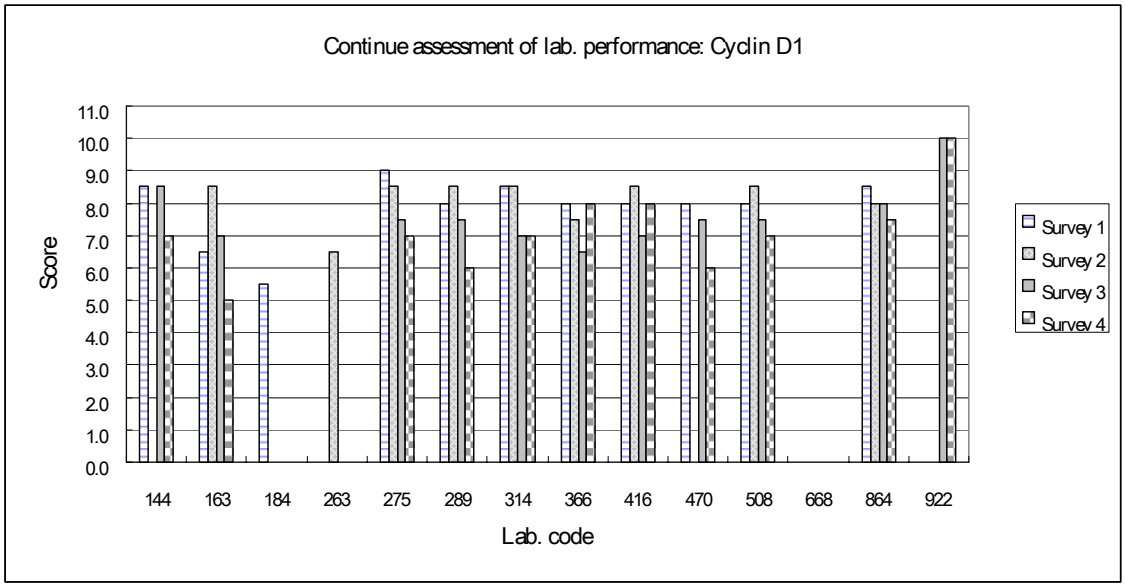
Cyclin D1 is one of the key regulators of the cell cycle acting by the phosphorylation of the retinoblastoma gene product. The specific staining is nucleus. Clinically, it is used to differentiate mantle cell lymphoma from other B cell lymphomas.

To monitor the performance consistency, the cyclin D1 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.

Table 9. Median Score Summary

Cyclin D1	Survey 1	Survey 2	Survey 3	Survey 4	Score Spread
Median	8	8.5	7.5	7	1.5

Figure 23. Distribution of Scores



Four laboratories; 184, 263, 668 and 992, had not return their cyclin D1 stained slides for 2 or more surveys, so they were excluded from the continue assessment.

To assess the performance consistency, the average score of the laboratories in the four surveys was compared to the median "score spread"; which was 1.5 marks, i.e. 8.5 minus 7 of the four surveys.

- Six laboratories; 144, 314, 366, 416, 508, and 864, with "score spread" of less than or equal to 1.5, were considered as consistence.
- Four laboratories; 163, 275, 289 and 470, with "score spread" of greater than 1.5, were considered as inconsistence.

References

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