ANATOMICAL PATHOLOGY

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Eighteen laboratories participated in the histological staining program and fourteen laboratories joined the immunohistochemical staining program. The laboratories came from various institutes including Hospital Authority, government institutes/clinics, university laboratories as well as private hospitals. Survey reports were issued quarterly to the participating laboratories documenting the performance, whereas late and nil returns were marked on the individual reports.

I. Survey Format

Table 1a, 1b and 2 summarises the various staining methods, cytopathology and antibodies assessed in 2011. A questionnaire was included in each survey asking details of the staining procedures done. These details allow the assessors to identify any cue that may cause suboptimal staining results. The staining procedure of the laboratory having top score was compiled in the survey report for reference.

Table 1a. Histological Staining Program

Survey	Code Number	Staining Methods
One	HC1102	Giemsa's Method
Two	HC1108	Elastic Van Gieson EVG Method
Three	HC1114	Masson’s Trichrome Method
Four	HC1120	Grocott’s Methenamine Silver Method

Table 1b. Cytopathology Program

Survey	Code Number	Targeted Diagnosis
One	HC1103	No evidence of malignancy
Two	HC1109	Atypical cells seen, cell block with IHC favoured adenocarcinoma
Three	HC1115	Adenocarcinoma
Four	HC1121	Malignant cells present, favoured adenocarcinoma

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Table 2. Immunohistochemical Staining Program

Survey	Code Number	Staining Methods
One	HC1104	CD10
	HC1105	CD10 (in house)
	HC1106	Ber-EP4
Two	HC1110	CD30
	HC1111	CD30(in house)
	HC1112	Ber-EP4
Three	HC1116	CDX2
	HC1117	CDX2 (in house)
	HC1118	Ber-EP4
Four	HC1122	PAcP
	HC1123	PAcP (in house)
	HC1124	Ber-EP4

II. Method of Analysis

The staining performance was assessed with the following criteria depicted in 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

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

To ensure objectivity in the 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	7.5
Y	6	4	5	7	5.5

III. Slide Return Summary

The slide return pattern of each survey was illustrated in Figures 1- 2.

Figure 1a. Histological Staining

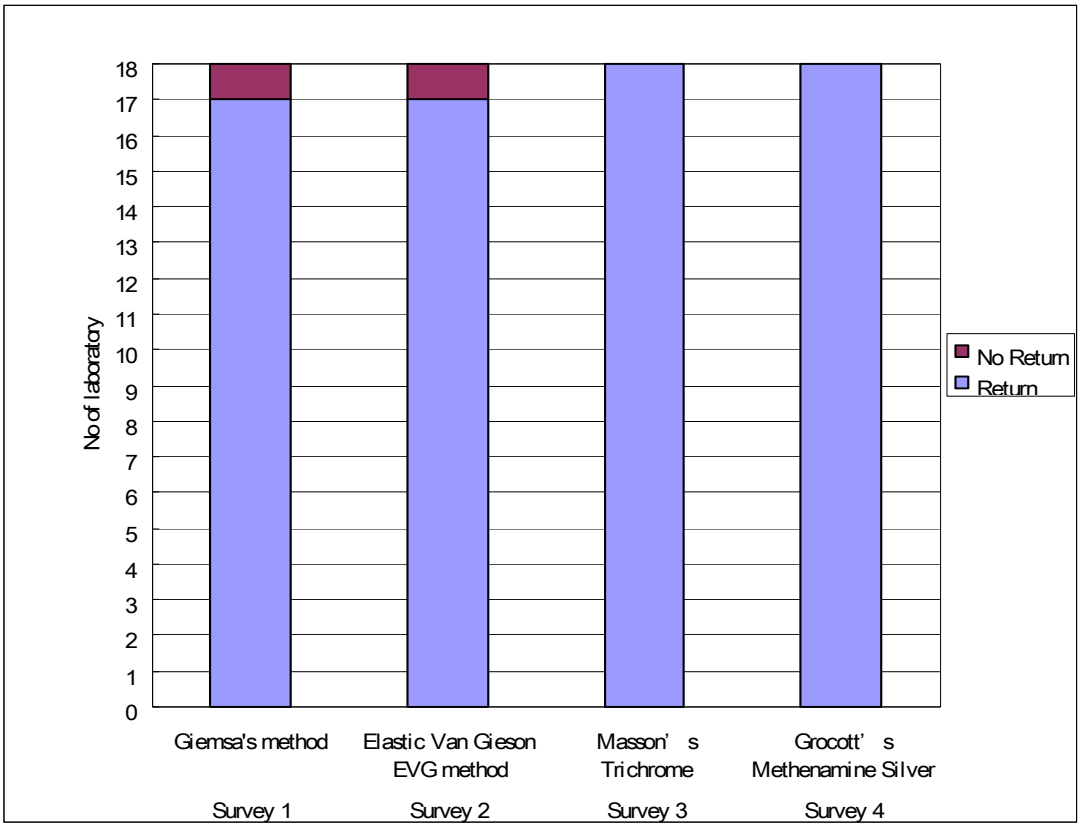


Figure 1b. Cytopathology

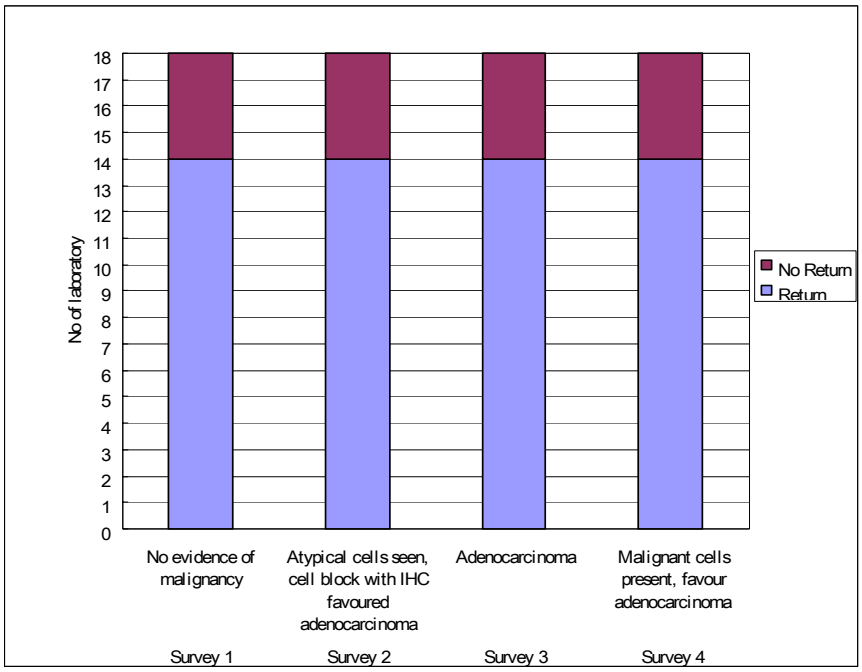
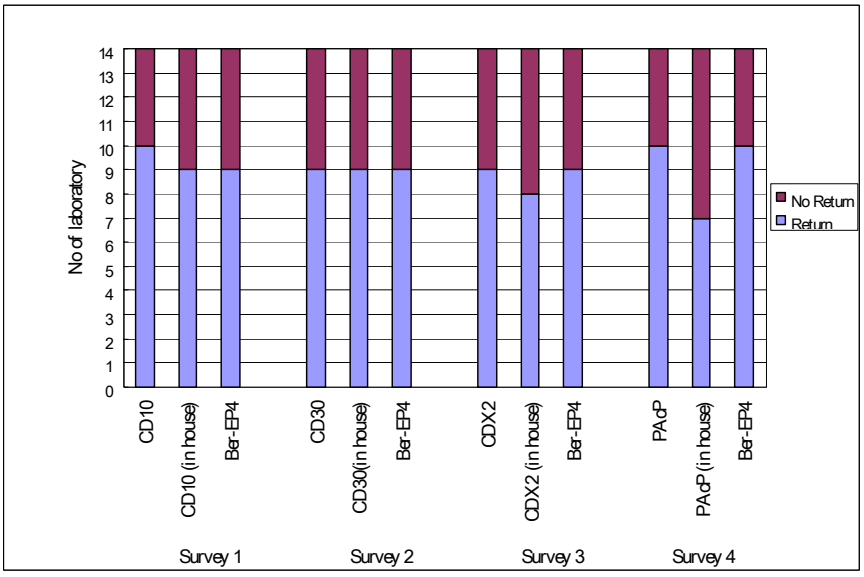


Figure 2. Immunohistochemical Staining



IV. Survey Analysis

i. Histological Staining Programme

1. Survey One
   H&E
   - Survey One material was a stomach biopsy with helicobacter-associated reactive chronic gastritis. All participants produced acceptable H&E staining (Figure 3).

   Giemsa's Method
   - Only one participant produced suboptimal result (Figure 4). The range of score was from 4.5 to 7.5 and the median score was 6.5.
   - All participants used 10% buffered formalin for the demonstration of helicobacter. The thickness of tissue sections for the demonstration of helicobacter of 10 (56%) laboratories was 4 μm. Four (22%) laboratories used 3 μm and four (22%) laboratories used 5 μm.
   - Among the 18 participants, seven (39%) laboratories used Modified Giemsa, six (33%) laboratories used Warthin-Starry, three (17%) laboratories used both modified Giemsa and Warthin-Starry, one laboratory used Giemsa and one used both modified Giemsa and IHC as their routine staining for the demonstration of helicobacter.
   - Ten out of 18 (56%) participants used commercial Giemsa solution for demonstrating helicobacter.
   - Sixteen (89%) participants diluted the stock Giemsa solution before staining.
   - Fifteen (83%) participants freshly prepared their working Giemsa solution.
   - Twelve (67%) participants differentiated their slides after Giemsa staining while the rest did not differentiate slides after staining. The differentiating agent used varied from 0.001% to 1 % acetic acid and 95% alcohol.

Figure 3. Survey One HC1101 H&E

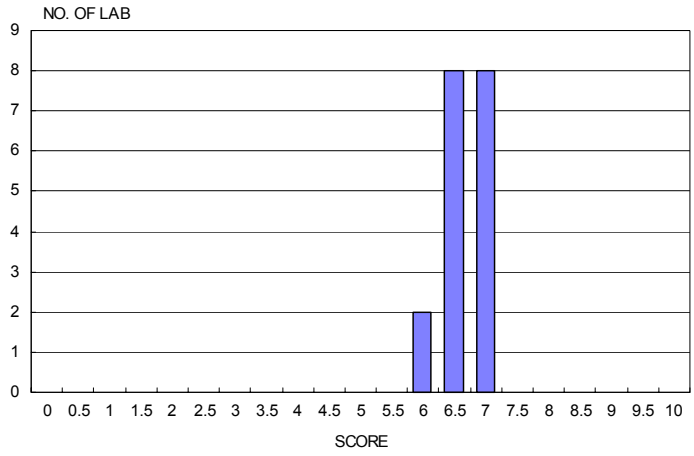
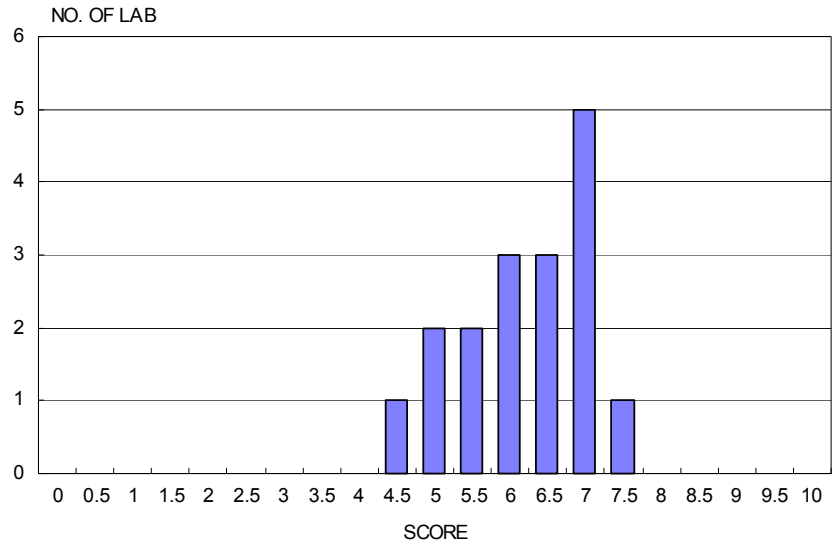


Figure 4. Survey One HC1102 Giemsa's Method



2. Survey Two

H&E

- Survey Two material was a heart tissue section of 4 μm in thickness. All participants produced acceptable H&E staining (Figure 5)

Elastic Van Gieson EVG Method

- All 17 participants produced acceptable staining results. The range of score was from 5.0 to 9.0 and the median score was 7.0 (Figure 6).
- Fourteen out of 17 (82%) participants used self-prepared Victoria Blue solution for the demonstration of elastic fibers. One (5.8%) used commercial staining solution and two (11.7%) used Verhoeff's Haematoxylin method in their routine staining of elastic fibers.
- Thirteen out of 14 (93%) participants prepared their Victoria Blue solution with similar method, except one participant did not add phenol.
- Overnight incubation of Victoria Blue was shown to generate the best staining and the duration of Van Gieson counterstaining varied from three to eight minutes.

Figure 5. Survey Two HC1107 H&E

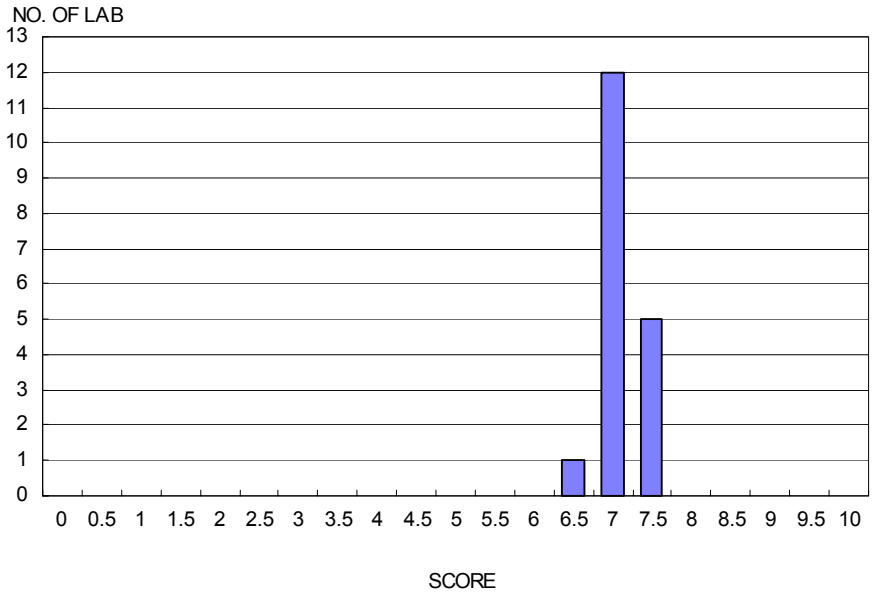
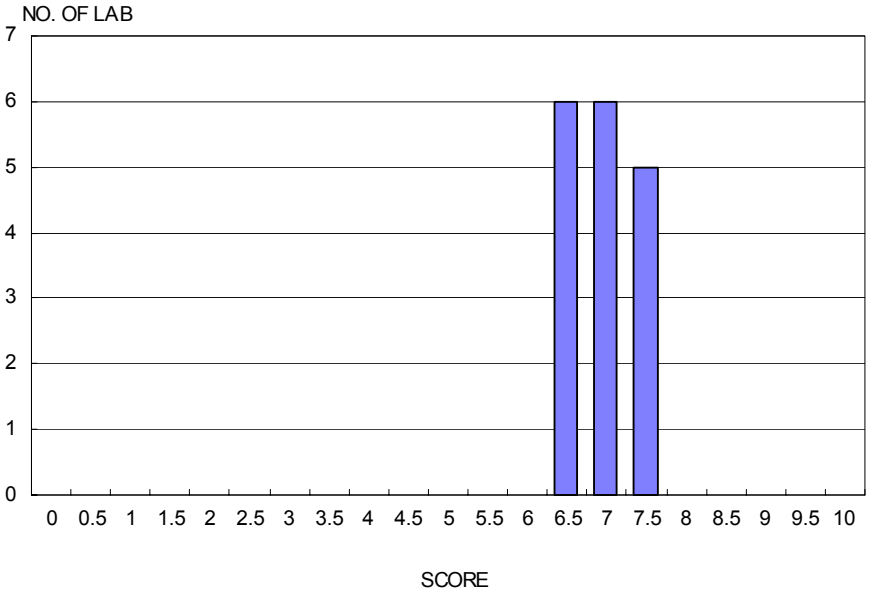


Figure 6. Survey Two HC1108 Elastic Van Gieson EVG Method



3. Survey Three

H&E

– Survey Three material was a kidney section of 2 μm in thickness. All laboratories produced acceptable H&E staining (Figure 7).

Masson's Trichrome Method

– All laboratories had acceptable Masson's Trichome staining. The range of score was from 5.0 – 7.0 and the median score was 6.5 (Figure 8).
– Among the 18 participants, nine (50%) laboratories did not use mordant before staining. For those used mordant before staining, five (27%) employed potassium dichromate and four (23%) utilized Bouin's solution.
– For nuclei staining, ten (55%) laboratories used Celestine blue-haematoxylin, five (28%) laboratories used Iron-alum haematoxylin, two (11%) used Weigert's haematoxylin and one (6%) used Harris's haematoxylin.
– For the demonstration of connective tissue, 14 (78%) laboratories used Masson's Trichome staining, three (16%) laboratories used Masson's Trichome staining and Van Gieson staining, one (6%) used both Masson's Trichome staining, Van Gieson staining and Gomori's one-step trichrome.

Figure 7. Survey Three HC1113 H&E

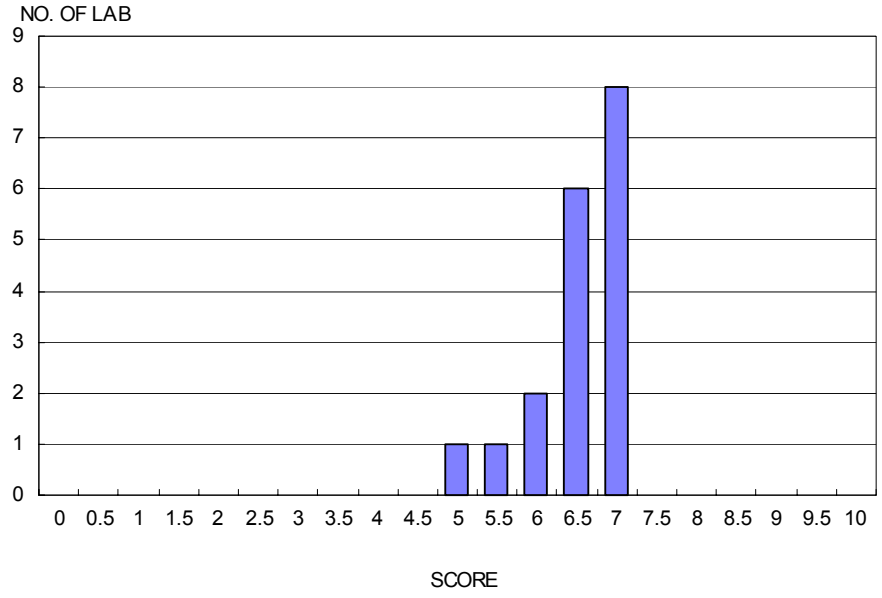
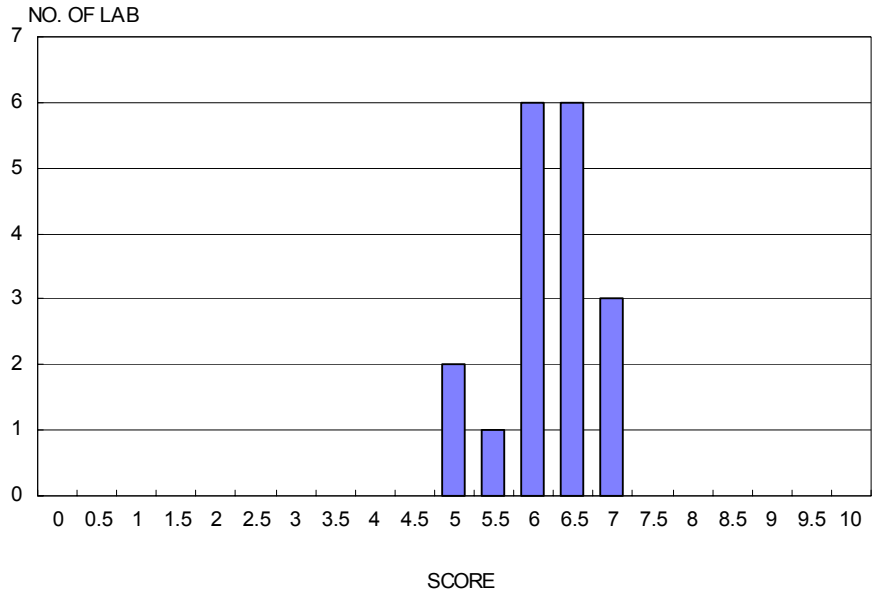


Figure 8. Survey Three HC1114 Masson's Trichrome Method



4. Survey Four

   H&E

   – Survey Four material was a skin tissue section of 4 μm in thickness. All participants produced acceptable H&E staining (Figure 9).

   Grocott's Methenamines Silver Method

   – All participants produced acceptable (Figure 10). The ranges of score for Schmorl without Bleach and Schmorl plus Bleach were 5.5 - 7.5 and 5.0 - 7.5, respectively. The median scores for both staining methods were 6.5.
   – Eleven out of 17 (65%) participants used freshly prepared ferric ferricyanide solution and five (29%) participants prepared fresh staining solution in 5 - 30 minutes before use. The staining time in ferric ferricyanide varied from two to ten minutes and 12 (71%) of participants checked the endpoint microscopically.
   – Four (24%) participants used acetic acid as rinse in the staining process.
   – For counterstaining, nine (53%) used van Gieson or modified van Gienson, five (29%) employed neutral red and two (12%) utilized nuclear fast red or aqueous red.

Figure 9. Survey Four HC1119 H&E

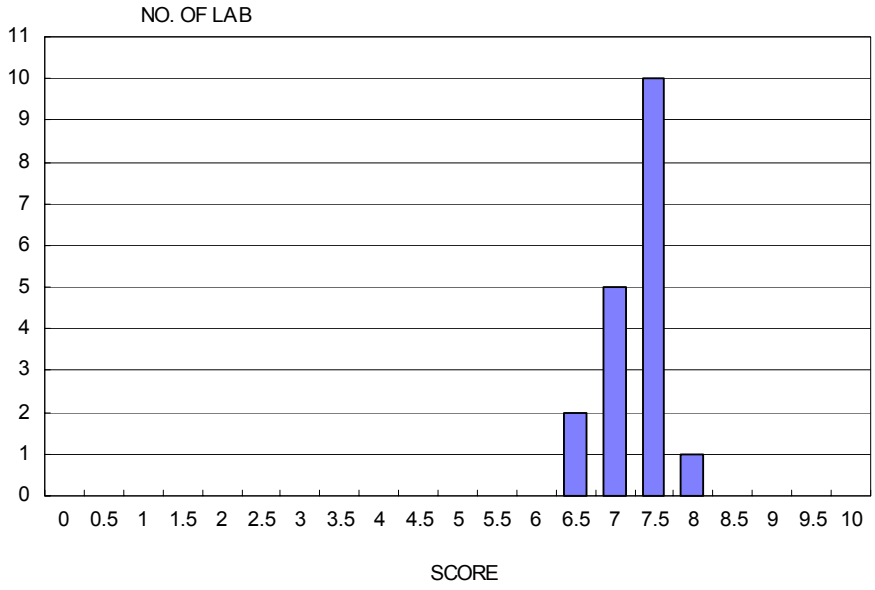
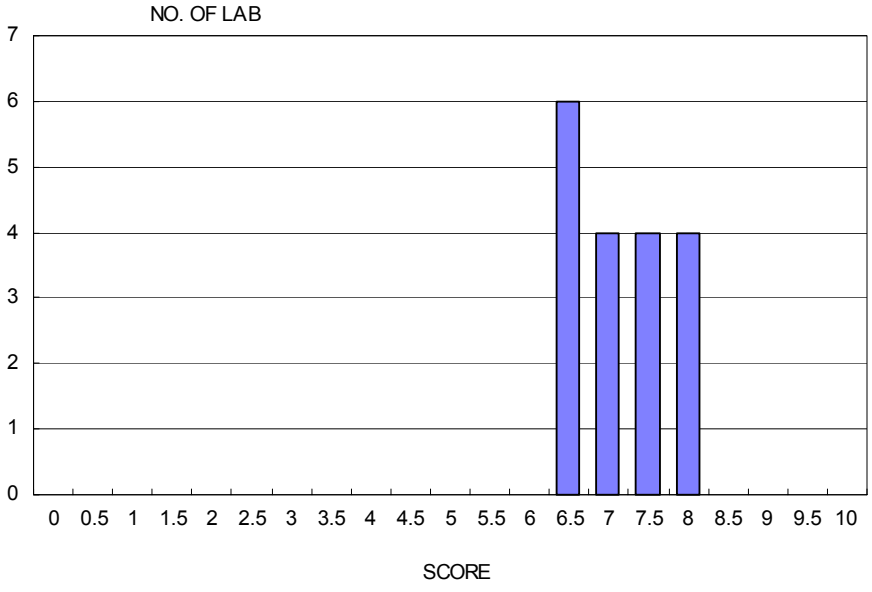


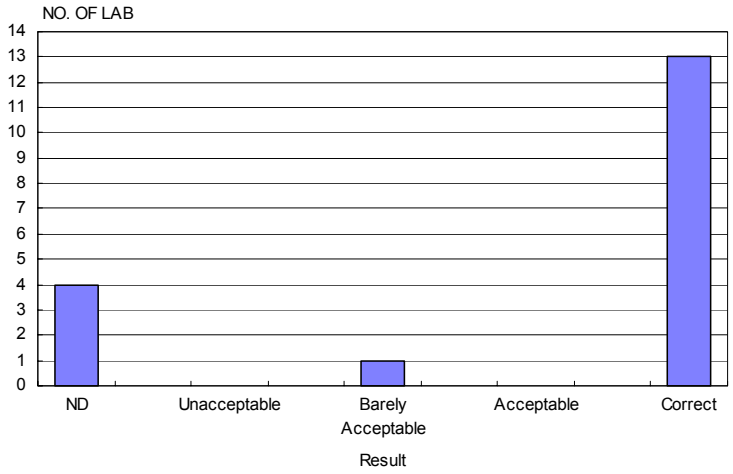
Figure 10. Survey Four HC1120 Grocott's Methenamine Silver Method



ii. Cytopathology

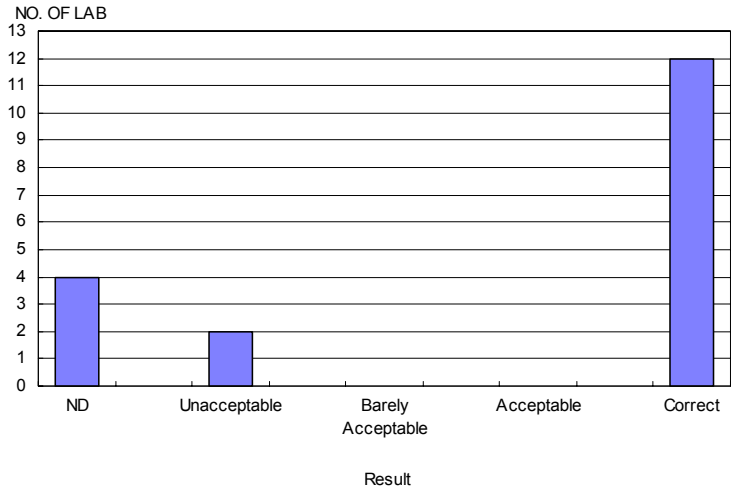
The survey material in Survey One was prepared from pleural fluid. The target answer was "No evidence of malignancy". Fourteen (78%) out of 18 participants returned their results for assessment. All gave correct diagnosis (Figure 11).

Figure 11. Survey One HC1103



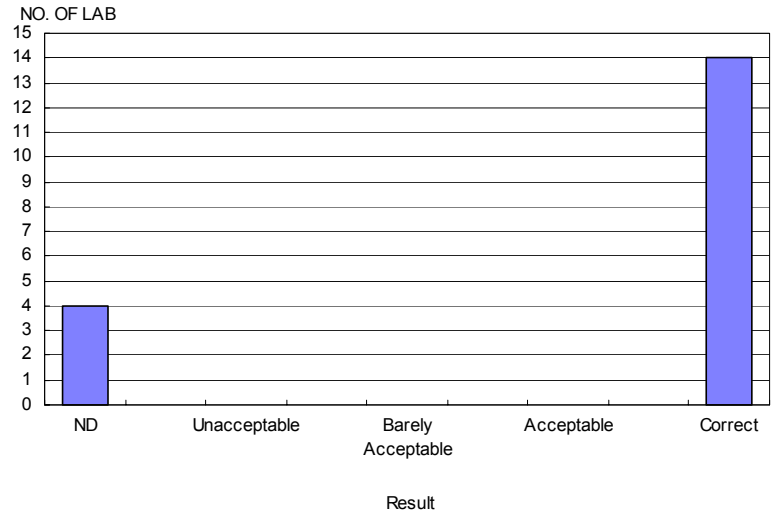
In Survey Two, the survey material was also prepared from pleural fluid. The target answer was "Atypical cells seen". Fourteen out of 18 (78%) participants returned their results for assessment. Twelve (86%) gave correct diagnosis, while two (14%) made unacceptable diagnosis (Figure 12).

Figure 12. Survey Two HC1109



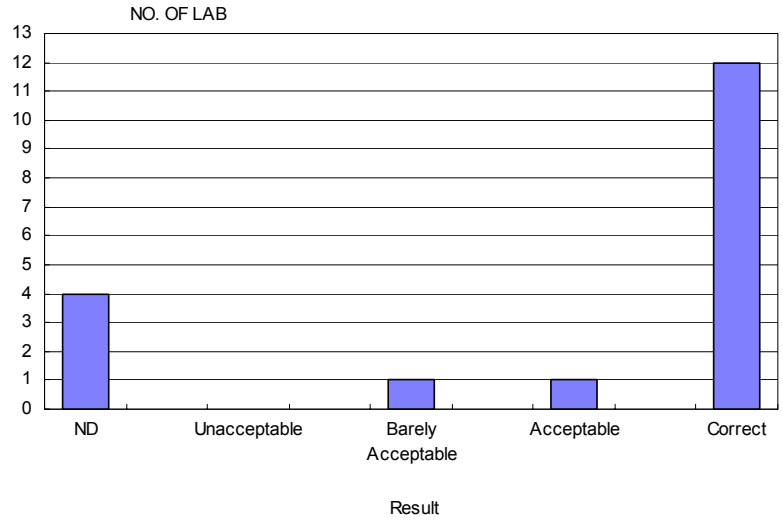
Sixteen (84%) participants returned their readouts in the Survey Three. The expected result was "Adenocarcinoma". Fourteen participants were correct (Figure 13).

Figure 13. Survey Three HC1115



In Survey Four, the survey material was prepared from sputum. The expected readout was "Malignant cells present, favouring adenocarcinoma". Fifteen out of 19 (79%) participants returned their results and all were correct (Figure 14).

Figure 14. Survey Four HC1121



iii. Immunohistochemical Staining Programme

a. Survey One CD10

The survey material for the CD10 demonstration was a case of follicular lymphoma. Sections show effaced nodal architecture with separated follicles occupying the whole node and extra-capsular lymphoid infiltrate is seen. The follicles consist of predominantly centrocytes with scattered centroblastic cells and follicular dendritic cells. Immunohistochemical staining shows strongly positive CD10 in the follicle centres.

One of the ten participants failed in HC1104 by using the provided antibody and none failed in HC1105 by using in-house antibody. The median score of HC1104 and HC1105 were 7.5 and 6.5, respectively, suggesting that the commonly used antibody did better than the in-house antibodies of participants. The distributions of scores were shown in Figures 15 - 16.

Figure 15. Survey One HC1104 CD10 (supplied antibody)

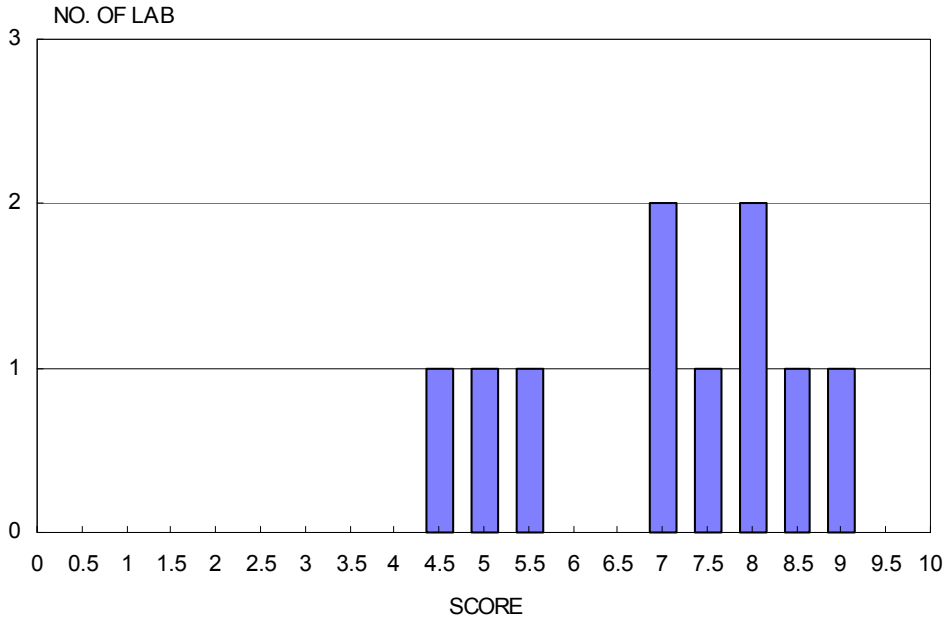


Figure 16. Survey One HC1105 CD10 (in-house)

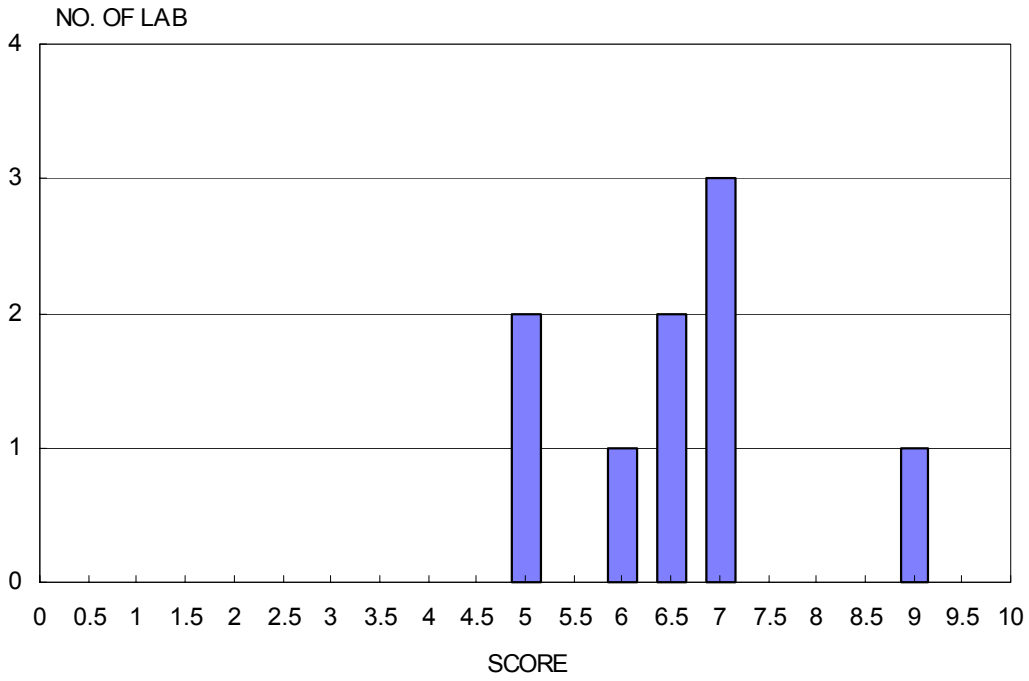


Table 5. The Best Method

STEP	**HC1104 (CD10 Supplied)**	**HC1105 (CD10 in-house)**
Supplier	Dako	Novocastra
Dilution	1:40	1:30
Peroxidase Blocking	10 min	5 min
Antigen retrieval	Pressure cooking pre-treatment 3.5 min	Microwave pre-treatment 12 + 8 min
Detection System	Dako Envision	Dako Flex+
Duration of Colour Development	DAB 10 min.	DAB 8 min.
End product Colour enhancement (if any)	5 min	2 min

b. Survey Two CD30

The survey material was derived from a case of Hodgkin's lymphoma. The tissue section displays a mixture of small and large lymphoid cells compatible with lacunar cells and classical Reed-Sternberg cells. Immunohistochemical staining shows that the large lymphoid cells are strongly positive for CD30.

One of the nine laboratories failed in HC1110 by using the provided antibody. Three laboratories failed in HC1111 with their in-house antibodies. The median scores of HC1110 and HC1111 were both 5.5, suggesting that the quality of the commonly used and in-house antibodies were comparable. The distributions of scores were shown in Figures 17 – 18.

Figure 17. Survey Two HC1110 CD30 (supplied antibody)

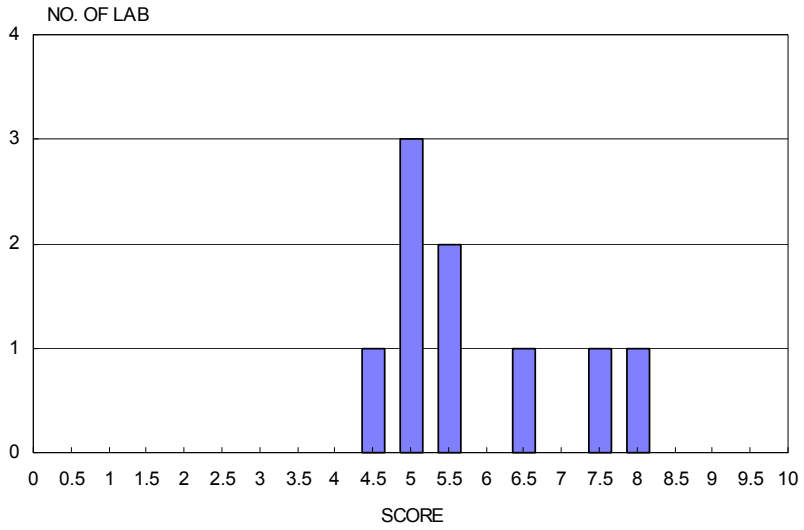


Figure 18. Survey Two HC1111 CD30 (in-house)

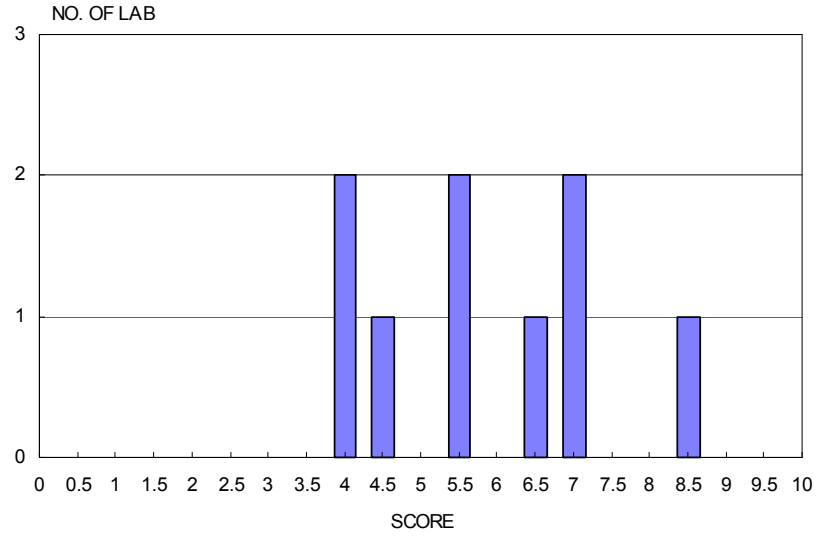


Table 6. The Best Method

STEP	**HC1110 (CD30 Supplied)**	**HC1111 (CD30 in-house)**
Supplier	Dako	Dako
Dilution	1:40	1:40
Peroxidase Blocking	10 min	5 min
Antigen retrieval	PT module pre-treatment 17 min	PT module pre-treatment 17 min
Detection System	Envision Flex kit	Envision Flex kit
Duration of Colour Development	DAB 10 min.	DAB 10 min.
End product Colour enhancement (if any)	5 min	5 min

c. Survey Three CDX2

The survey material is a case of carcinoid tumour. The tissue section of the appendix shows carcinoid tumour with 10mm in the greatest dimension. Immunohistochemistry demonstrates that tumour cells are negative for CDX2, while the normal epithelium is positive.

No participant failed in HC1116 and HC1117 by using either common or in-house antibodies. The median scores of HC1116 and HC1117 were both 7.5, suggesting comparable quality of commonly used and in-house antibodies. The distributions of scores were shown in Figures 19 - 20.

Figure 19. Survey Three HC1116 CDX2 (supplied antibody)

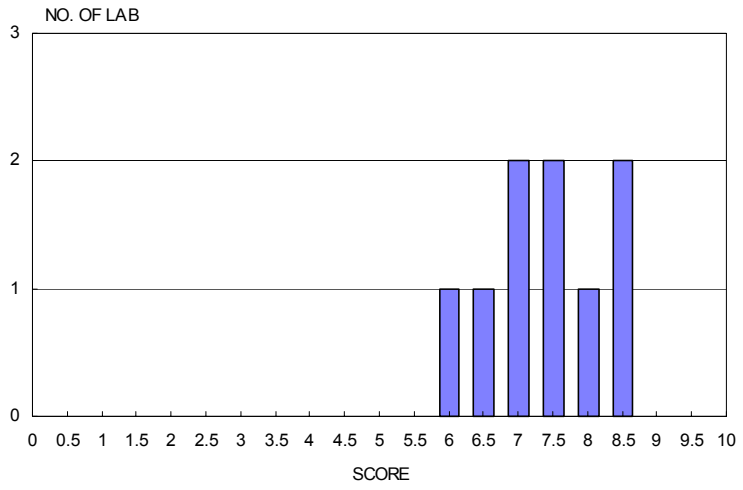


Figure 20. Survey Three HC1117 CDX2 (in-house)

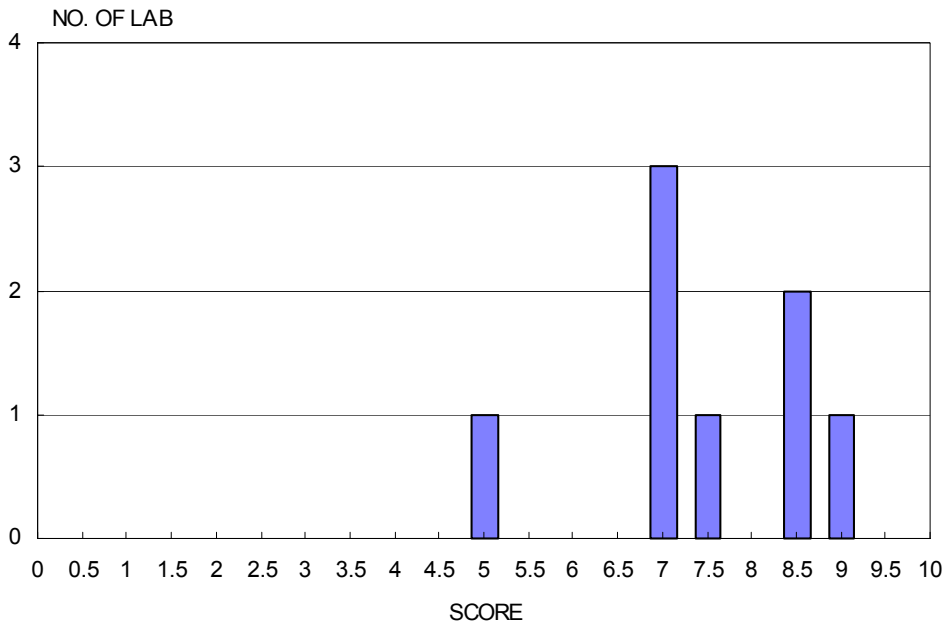


Table 7. The Best Method

STEP	**HC1116 (CDX2 Supplied)**	**HC1117 (CDX2 in-house)**
Supplier	Dako	Novocastra
Dilution	1:40	1:20
Peroxidase Blocking	4 min	5 min
Antigen retrieval	Microwave Tris-EDTA 12 + 10 min	PT module pre-treatment 20 min
Detection System	Ventana Ultraview	Ventana i-view
Duration of Colour Development	DAB 8 min	DAB 10 min
End product Colour enhancement (if any)	8 min	0 min

d. Survey Four PAcP

The survey material is a case of rectal tumour displaying a metastatic adenocarcinoma invading from the adventitia/peri-rectal fat into the muscularis propria and submucosa. Sections show mainly the pattern of cribriform growth with luminal comedo necrosis. Tumour cells have prominent nucleoli and moderate amount of foamy cytoplasm. Immunohistochemical staining illustrates that the malignant cells are strongly positive for PAcP.

No laboratory failed in HC1122 and HC1123. The median scores of HC1122 and HC1123 were 7.0 and 8.0, respectively, implying a suboptimal quality of commonly used antibody. The distributions of scores were shown in Figure s 21 - 22.

Figure 21. Survey Four HC1122 PAcP (supplied antibody)

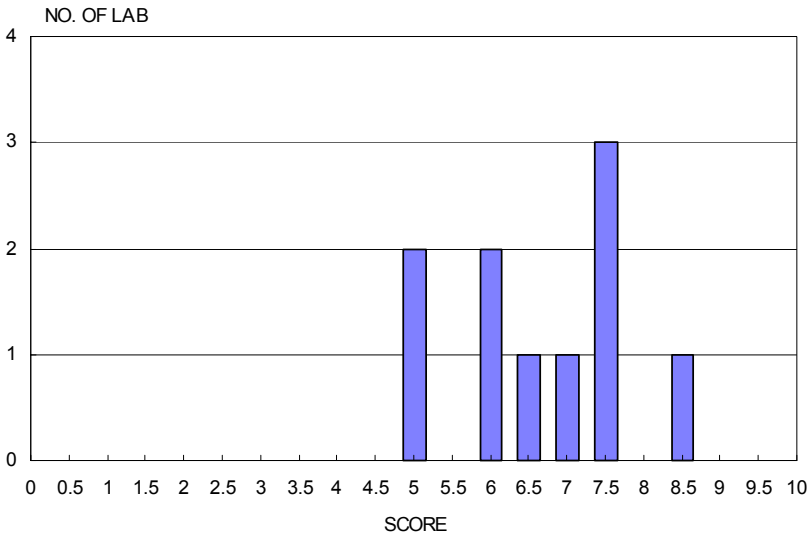


Figure 22. Survey Four HC1123 PAcP (in-house)

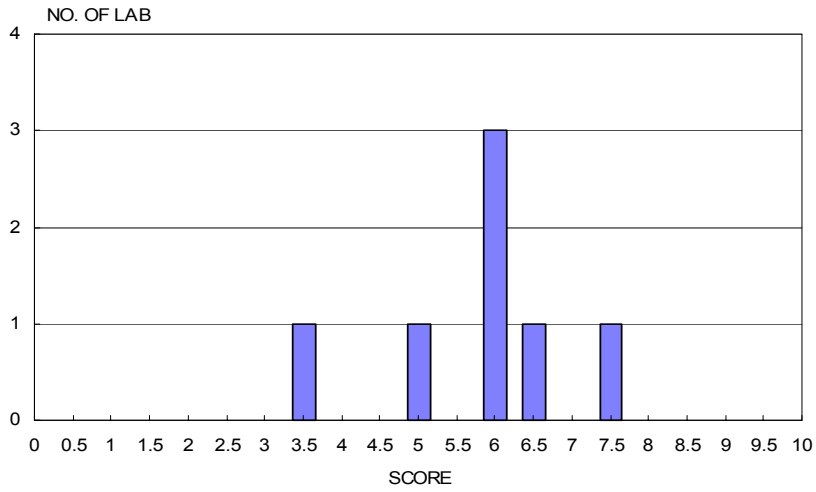


Table 8. The Best Method

STEP	**HC1122 (PAcP Supplied)**	**HC1123 (PAcP in-house)**
Supplier	Dako	Dako
Dilution	1:40	1:40
Peroxidase Blocking	10 min	5 min
Antigen Retrieval	PT module pre-treatment 17 min	PT module pre-treatment 17 min
Detection System	Envision Flex kit	Envision Flex kit
Duration of Colour Development	DAB 10 min	DAB 10 min

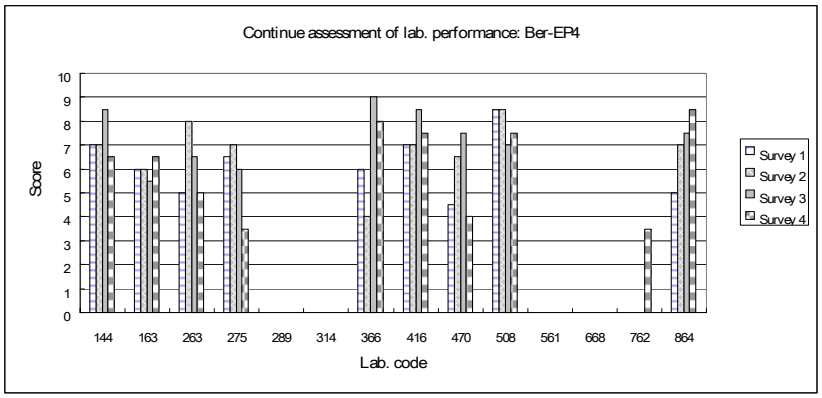
e. Continue Assessment of Laboratory Performance: Ber-EP4

The survey material was a case of adenocarcinoma in caecum. The epithelium is stained by Ber-EP4. To evaluate the consistency of continuous performance, sections of the same tissue block were sent to participants for assessment in four survey exercises. The returned slides were assessed as stated in the Section II, Method of Analysis. 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

Ber-EP4	Survey 1	Survey 2	Survey 3	Survey 4
Median	6	7	7.5	6.5

Figure 23. Distribution of Scores



Five laboratories did not return survey slides in more than two survey exercises. They were excluded from the continue assessment. Consistently good performances were noted in three laboratories, while the performance of one laboratory was consistently satisfactory. A trend of improvement of performance was noted in one participant, while another participant was noted to exhibit a decline of performance. Inconsistent performances were observed in three laboratories, which might be worth to investigate the root causes.

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