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 2012. 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	HC1202	Perls' Prussian Blue Method
Two	HC1208	Ziehl-Neelsen method Method
Three	HC1214	Alkaline Congo Red Stain
Four	HC1220	Periodic Acid Schiff/Periodic Acid Schiff Digestion Method

Table 1b. Cytopathology Program

Survey	Code Number	Targeted Diagnosis/Sample Type
One	HC1203	No evidence of malignancy / pleural sample
Two	HC1209	Malignant cells seen, favour Adenocarcinoma / sputum sample
Three	HC1215	Neg, LSIL, HSIL and LGSIL / Liquid base gynaecytology samples
Four	HC1221	HGSIL or Squamous Cell Carcinoma / Liquid base gynaecytology samples

Table 2. Immunohistochemical Staining Program

Survey	Code Number	Staining Methods
One	HC1204	CD10
	HC1205	CD10 (in house)
	HC1206	CK20
Two	HC1210	CD3
	HC1211	CD3 (in house)
	HC1212	CK20
Three	HC1216	Calretinin
	HC1217	Calretinin (in house)
	HC1218	CK20
Four	HC1222	P53
	HC1223	P53 (in house)
	HC1224	CK20

II. Method of Analysis

The staining performance was assessed with the following criteria depicted in Table 3.

Table 3. Scoring System

Staining	**Scores**
Fail	1-4
Satisfactory	5-6
Good	7-8
Excellent	9-10

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 fail.

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. Example for Calculation of the Average Score

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

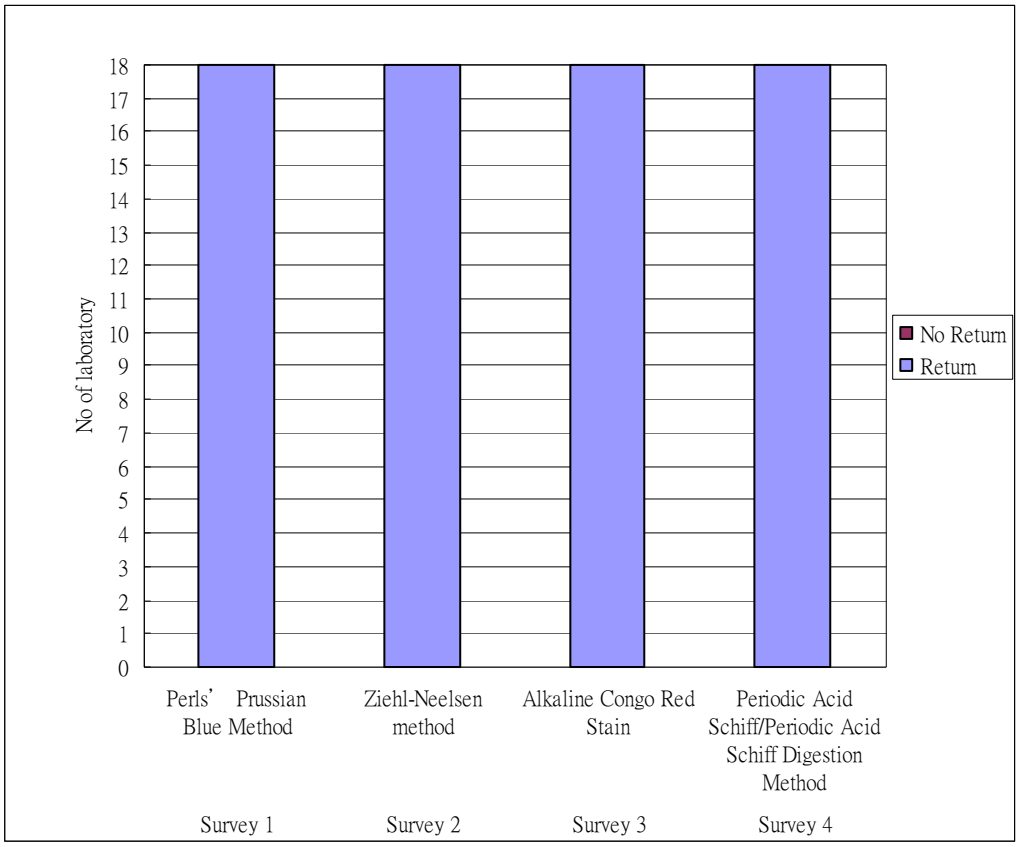


Figure 1b. Cytopathology

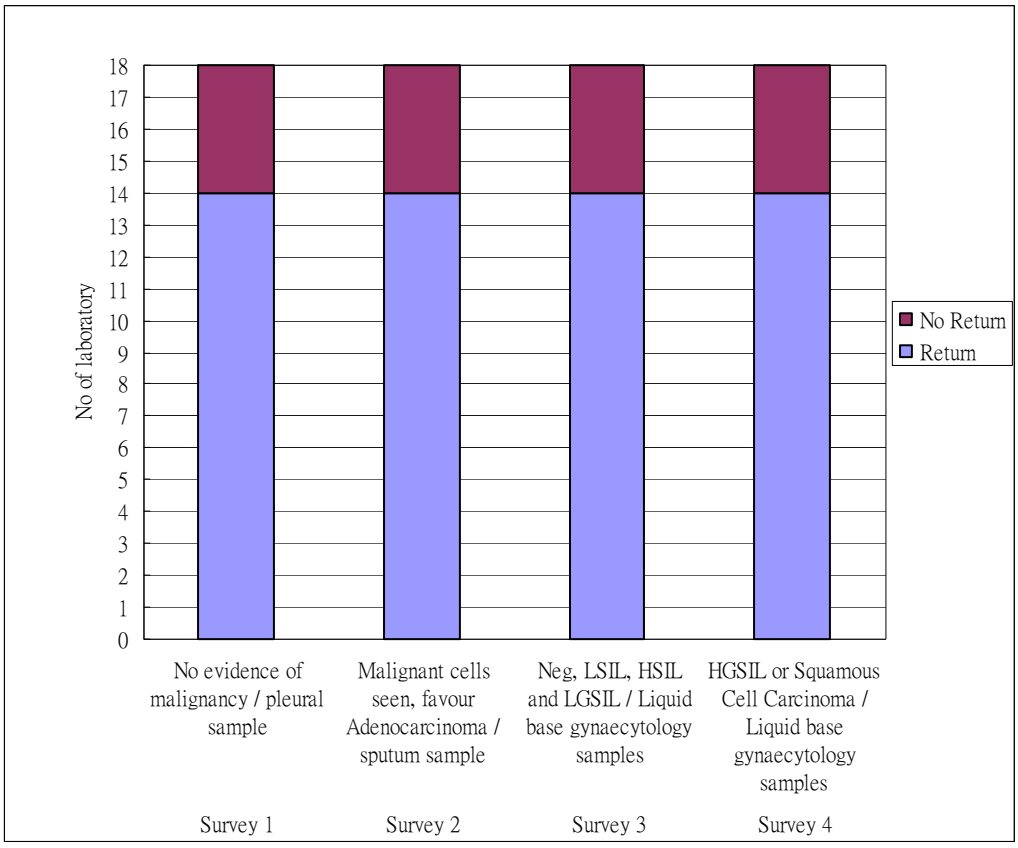
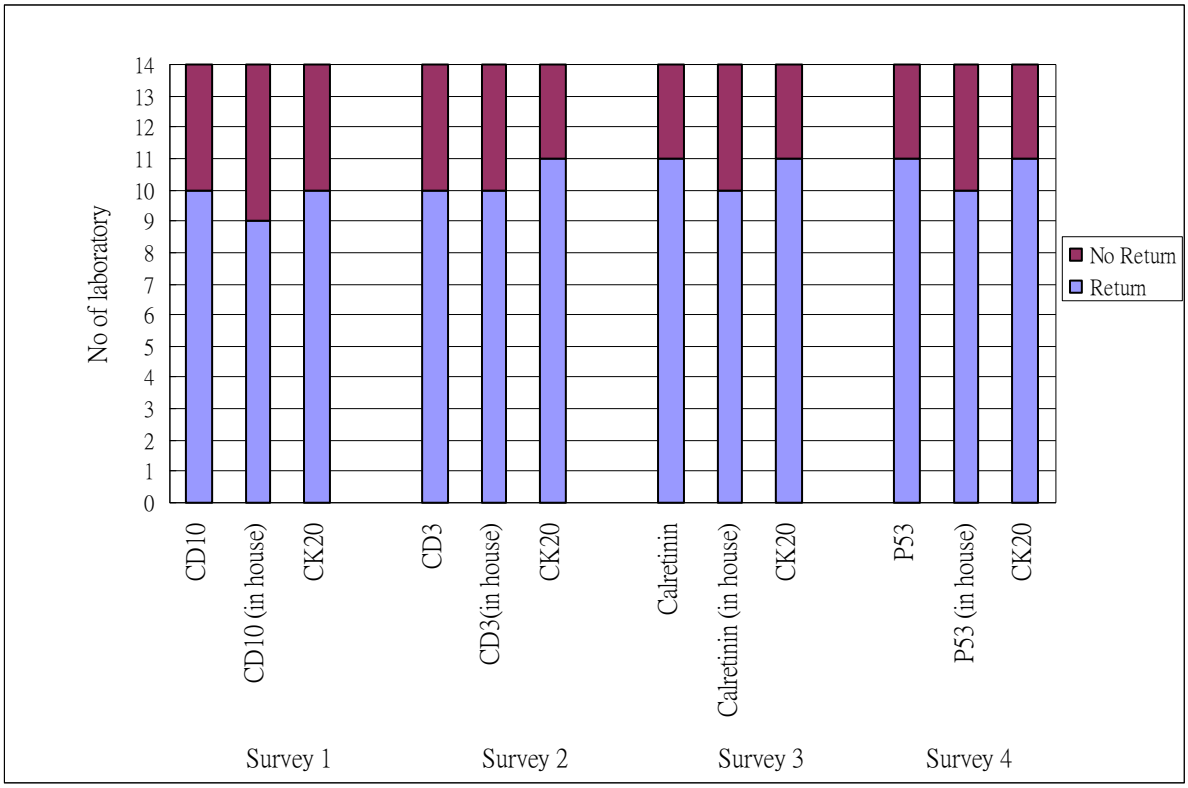


Figure 2. Immunohistochemical Staining



IV. Survey Analysis

i. Histological Staining Programme

1. Survey One
   H&E
   - The supplied survey material was a 4 microns thick spleen tissue section. All participants produced acceptable H&E staining (Figure 3).

   Perls' Prussian Blue Method

   - All participants returned the slides for assessment and produced acceptable staining results (Figure 4). The range of score was from 6.5 to 7.5 and the median score was 7.0.
   - All participants used 10% buffered formalin for the demonstration of hemosiderin.
   - The section thickness for the demonstration of hemosiderin of five (28%) laboratories was 3 μm, eleven (61%) was 4 μm and two (11%) was 5 μm.
   - All participants used freshly prepared Perls' solution.
   - Fifteen (83%) laboratories used AnalaR grade hydrochloric acid and three (17%) laboratories used general grade hydrochloric acid.
   - Seventeen (94%) participants used iron-free distilled water for the staining.
   - One third of the laboratories perform Perls' stain on decalcified specimens, which could lead to false negative staining as hemosiderin would be removed by acidic decalcification solution or acid fixatives.
   - Sixteen participants used neutral red and two participants used nuclear fast red for the nuclei counterstain.

Figure 3. Survey One HC1201 H&E

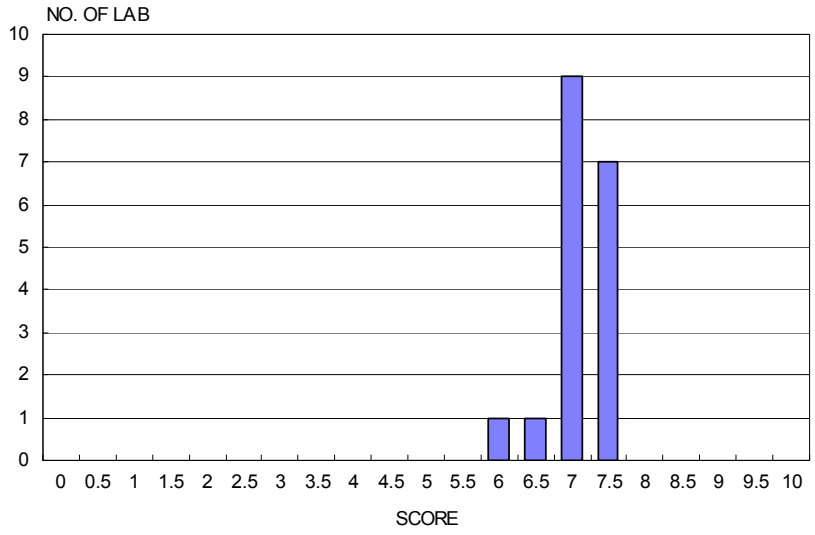
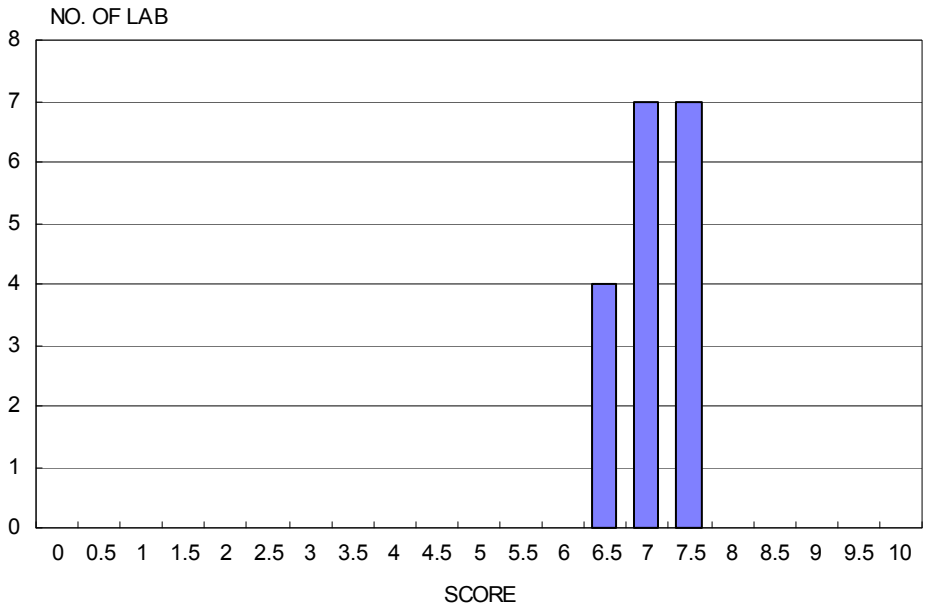


Figure 4. Survey One HC1202 Perls' Prussian Blue Method



2. Survey Two

H&E

- The supplied survey material was a 4 microns thick lung tissue section. All participants produced acceptable H&E staining (Figure 5)

Ziehl-Neelsen Method

- 16 participants produced acceptable staining results and one participant did not return the slide for assessment. The range of score was from 5.5 to 8.0 and the median score was 7.0 (Figure 6).
- The section thickness for the demonstration of tubercle bacilli of four laboratories (22%) was 3 microns, three (17%) was 4 microns, four (22%) was 5 microns, and seven (39%) was 6 or above 6 microns.
- Ten (55%) participants used self-prepared carbol fuchsin staining solution and the other 45% of them used commercial solution for the demonstration of tubercle bacilli.
- Thirteen (72%) participants stained the slides at room temperature for 30 to 75 minutes, four (22%) stained the slides with hot carbol fuchsin solution and one participant stained the sections in 56^{o}C staining solution for 15 mins.
- Five (28%) participants had included a bleaching step to remove background after the acid alcohol differentiation.

Figure 5. Survey Two HC1207 H&E

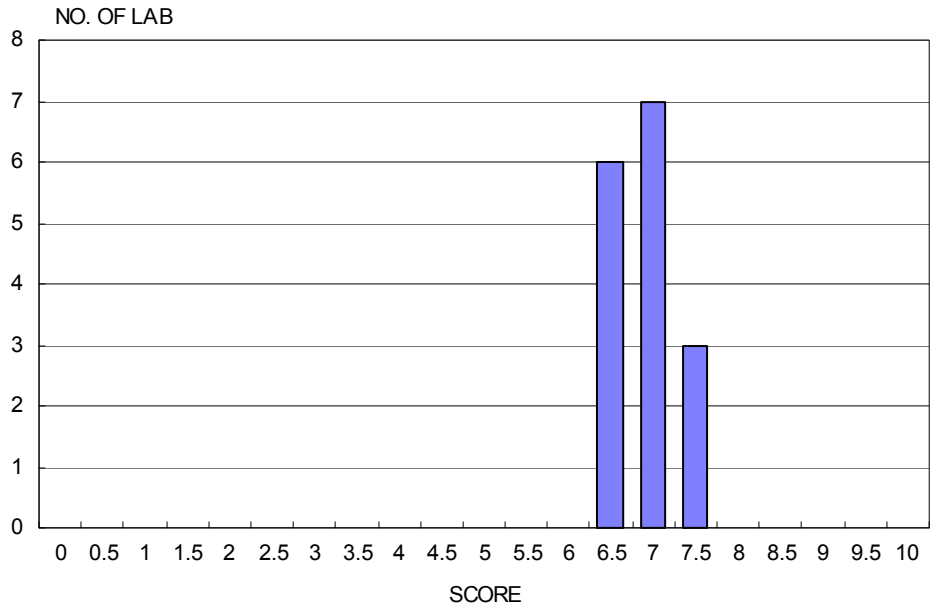
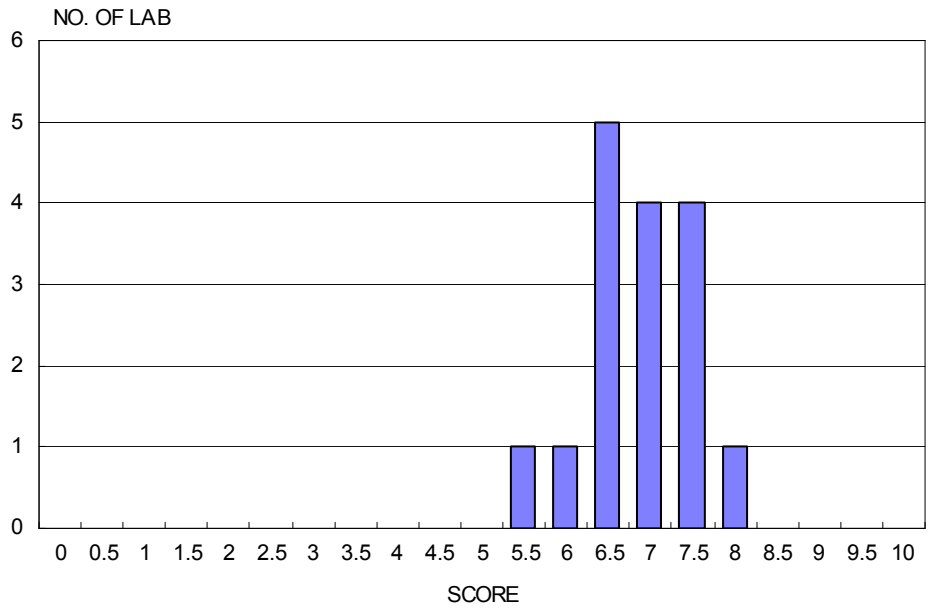


Figure 6. Survey Two HC1208 Ziehl-Neelsen Method



3. Survey Three

H&E

- The supplied survey three material was a lung and skin section with amyloidosis. All laboratories produced acceptable H&E staining (Figure 7).

Alkaline Congo Red Stain Method

- All laboratories had acceptable Alkaline Congo Red staining. The range of score was from 5.5 – 7.0 and the median score was 6.5 (Figure 8).
- The section thickness for the demonstration of amyloid of 5 (28%) laboratories was 8μm, 4 (22%) laboratories was 6 μm , 3 (17%) laboratories was 5 μm , 4 (22%) laboratories was 4 μm and 2 (11%) laboratories was 3μm.
- For the alkaline solution, majority of the participants freshly prepared it for use, while two laboratories (12%) kept the working solution for a week; one at room temp. and the other one in the refrigerator.
- For the Congo Red stock solutions and the Alkaline stock solution, 11 (60%) laboratories freshly prepared them for use, 5 (28%) laboratories kept them for 1 month and 2 (12%) laboratories kept them for 1 week.
- For the dehydrating agent after Congo Red stain, 9 (50%) laboratories used absolute alcohol, 6 (33%) laboratories used 70% alcohol and 3(17%) used 95% alcohol.
- All of laboratories filtered the working alkaline before use.

Figure 7. Survey Three HC1213 H&E

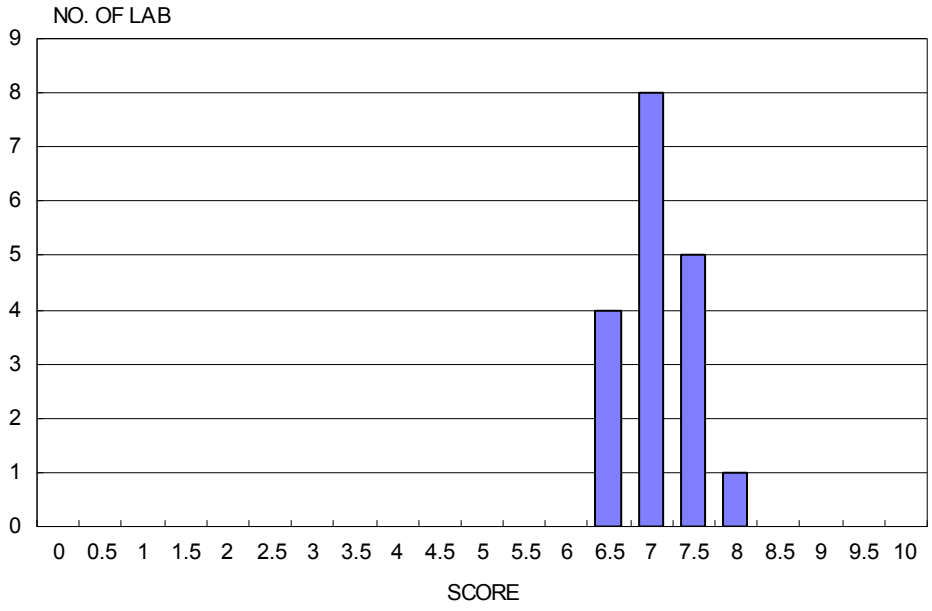
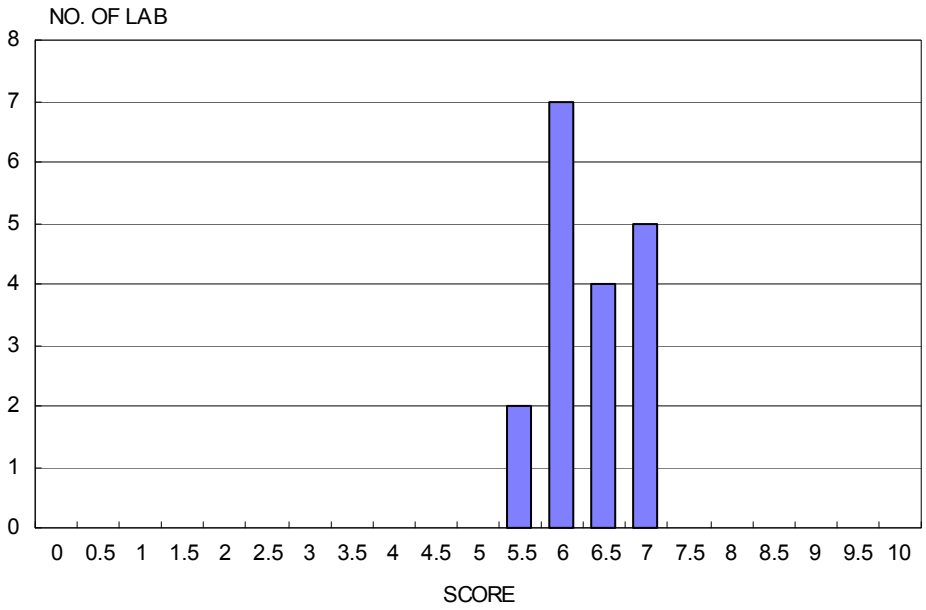


Figure 8. Survey Three HC1214 Alkaline Congo Red Stain Method



4. Survey Four

H&E

- The supplied survey four material was a liver tissue section with glycogen. All participants produced acceptable H&E staining (Figure 9).

Periodic Acid Schiff/Periodic Acid Schiff Digestion Method

- All except one participants produced acceptable (Figure 10) PAS/PASD staining. The range of score was 4.5 - 8.0 and the median score was 7.0.
- The section thickness for routine PAS/PASD stain of 5 (28%) laboratories was 3 μm, 10 (61%) laboratories was 4μm, , 2 (11%) laboratories was 5 μm.
- For the Schiff's reagent 8 (44%) laboratories self-prepared it for use, while 10 (56%) laboratories used commercial reagent. All laboratories kept their working Schiff's reagent in refrigerator.
- For the α-amylase digestion, 10 (56%) laboratories used 1%, 3 (17%) laboratories used 0.5 %, 2 (11%) laboratories used 0.3% and the remaining three laboratories (6%) each used 0.1%, 0.66% and 2% respectively.
- There was great variation in the staining time of Periodic acid and Schiff's reagent among the participants, these indicate that the staining time and the time for digestion are very much depend on the batch of chemicals used and the method of preparation.

Figure 9. Survey Four HC1219 H&E

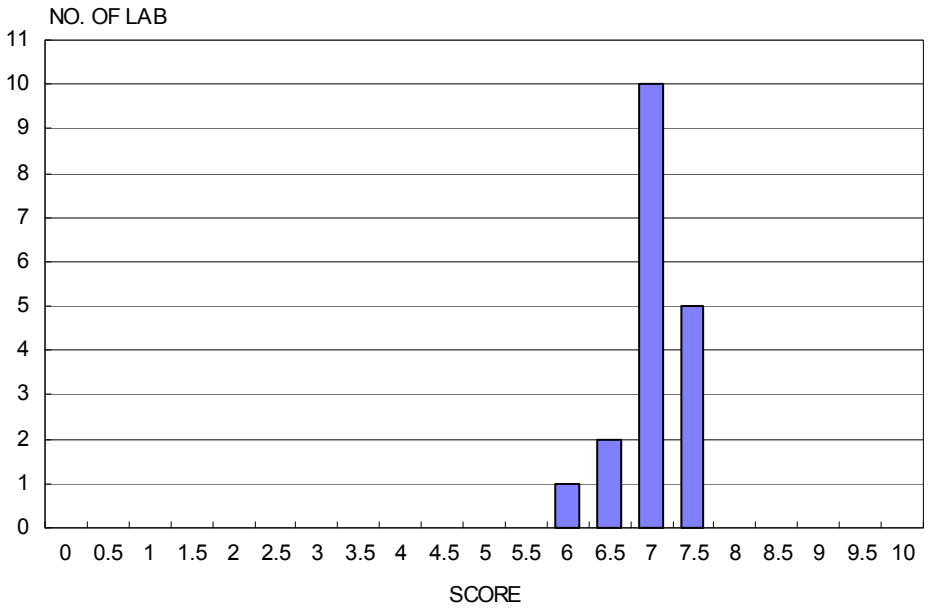
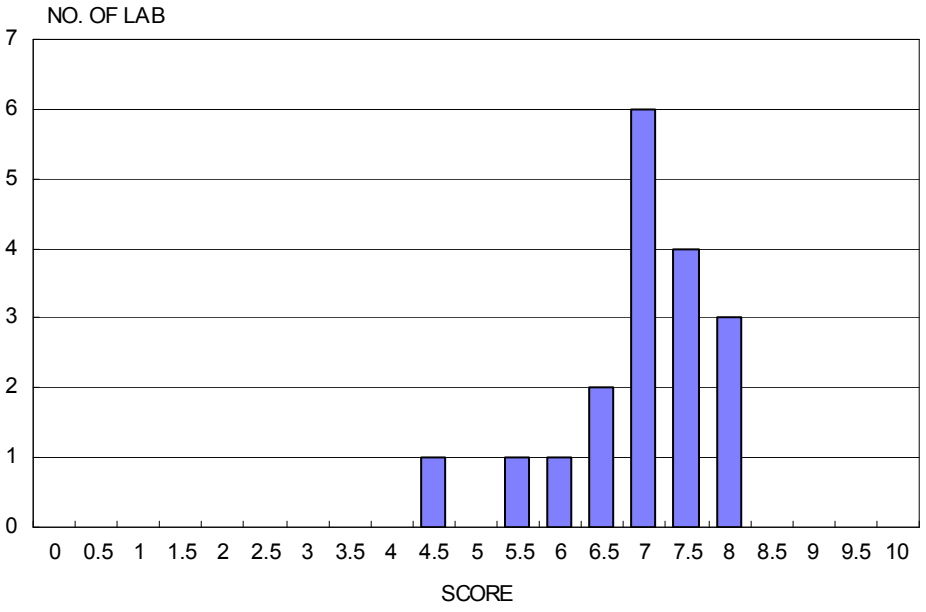


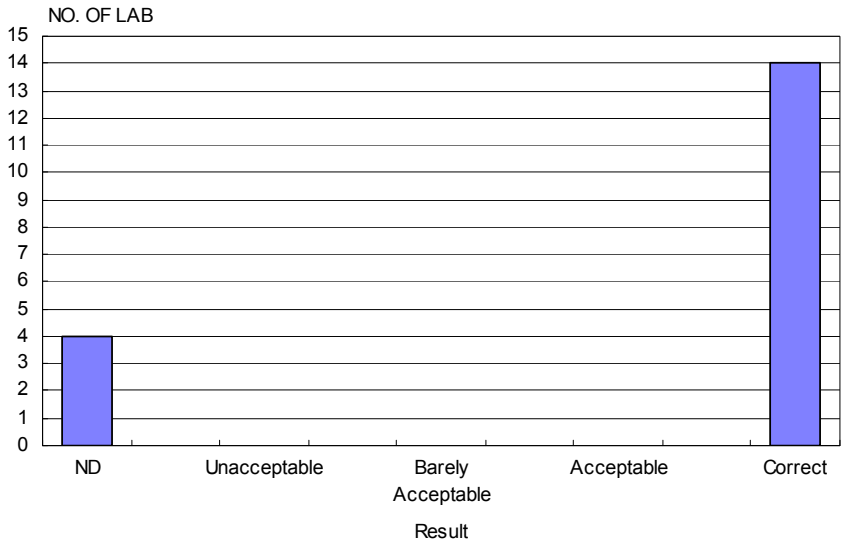
Figure 10. Survey Four HC1220 Periodic Acid Schiff ± Digestion Method



ii. Cytopathology

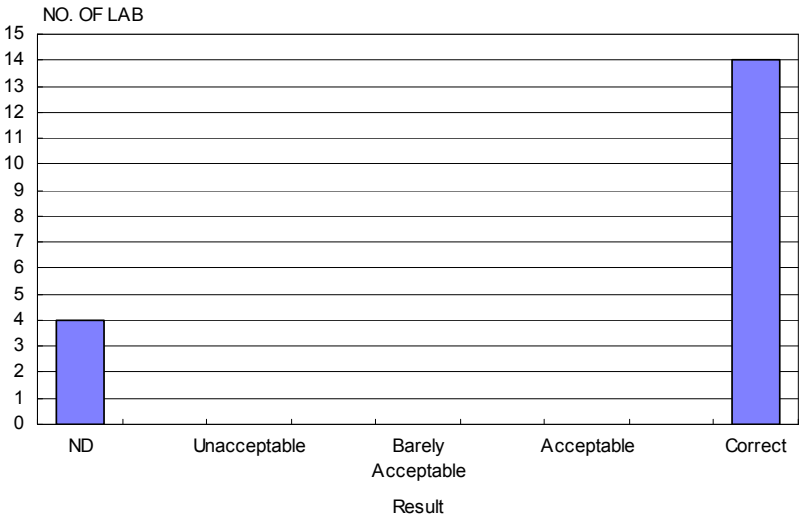
The survey material in Survey One was prepared from pleural samples. The target answer was "No evidence of malignancy". 14 (78%) participants returned their results for assessment and all of them gave the correct diagnosis (Figure 11).

Figure 11. Survey One HC1203



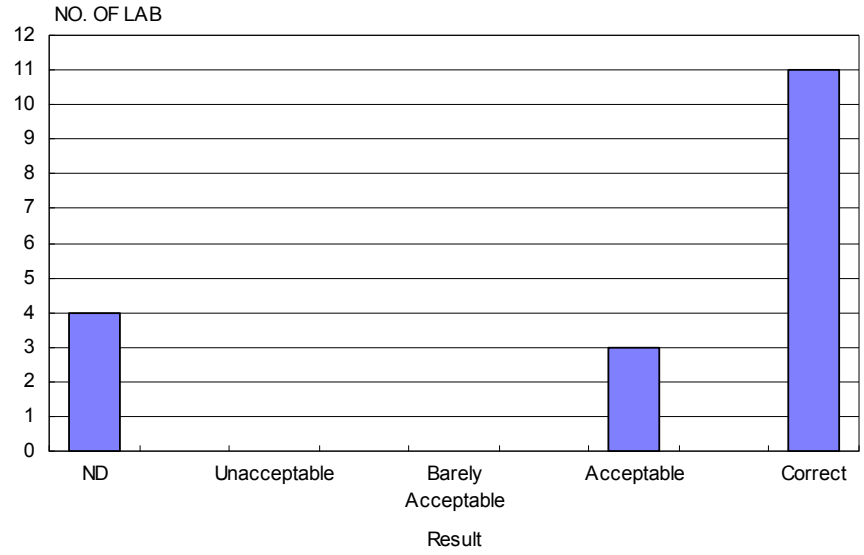
In Survey Two, the survey material was also prepared from sputum samples. The target answer was "Malignant cells seen, favour Adenocarcinoma". 14 (78%) participants returned their results for assessment and all of them gave the correct answer (Figure 12).

Figure 12. Survey Two HC1209



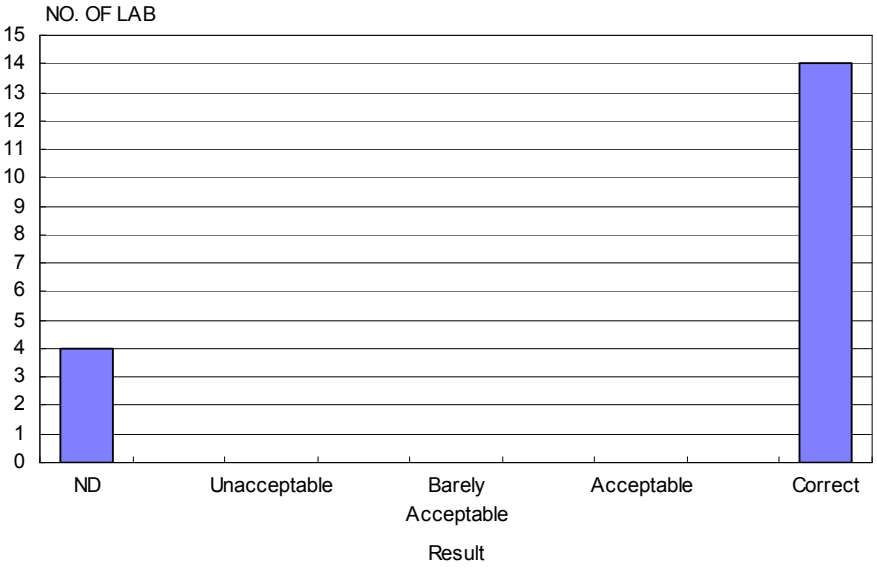
The supplied survey material of survey three was prepared from liquid base gynaecytology samples. 14 (78%) participants returned their results for assessment. Eleven laboratories gave correct answers and the remaining three laboratories gave acceptable answers (Figure 13).

Figure 13. Survey Three HC1215



The supplied survey material of survey four was prepared from liquid base gynaecytology samples. 14 (78%) participants returned their results for assessment and all of them gave correct answers (Figure 14).

Figure 14. Survey Four HC1221



iii. Immunohistochemical Staining Programme

a. Survey One CD10

The survey material for the CD10 demonstration was a case of follicular lymphoma. The lymph nodes of the sections show complete effacement of nodal architecture by neoplastic follicles with very focal diffuse area. Both follicular and diffuse area shows admixed centrocyte-like cells and large nucleolated centroblasts. Immunostudy shows that the follicle centers are strongly positive for CD10.

For the supplied CD10 antibody (HC1204), no laboratory failed in the assessment. For the in-house CD10 antibody (HC1205), one out of laboratories failed in the assessment. The median score of HC1204 and HC1205 were found both to be 6.5 respectively. The laboratories with sub-optimal results were due to weak signal to noise ratio. The distributions of scores were shown in Figures 15 – 16.

Figure 15. Survey One HC1204 CD10 (supplied antibody)

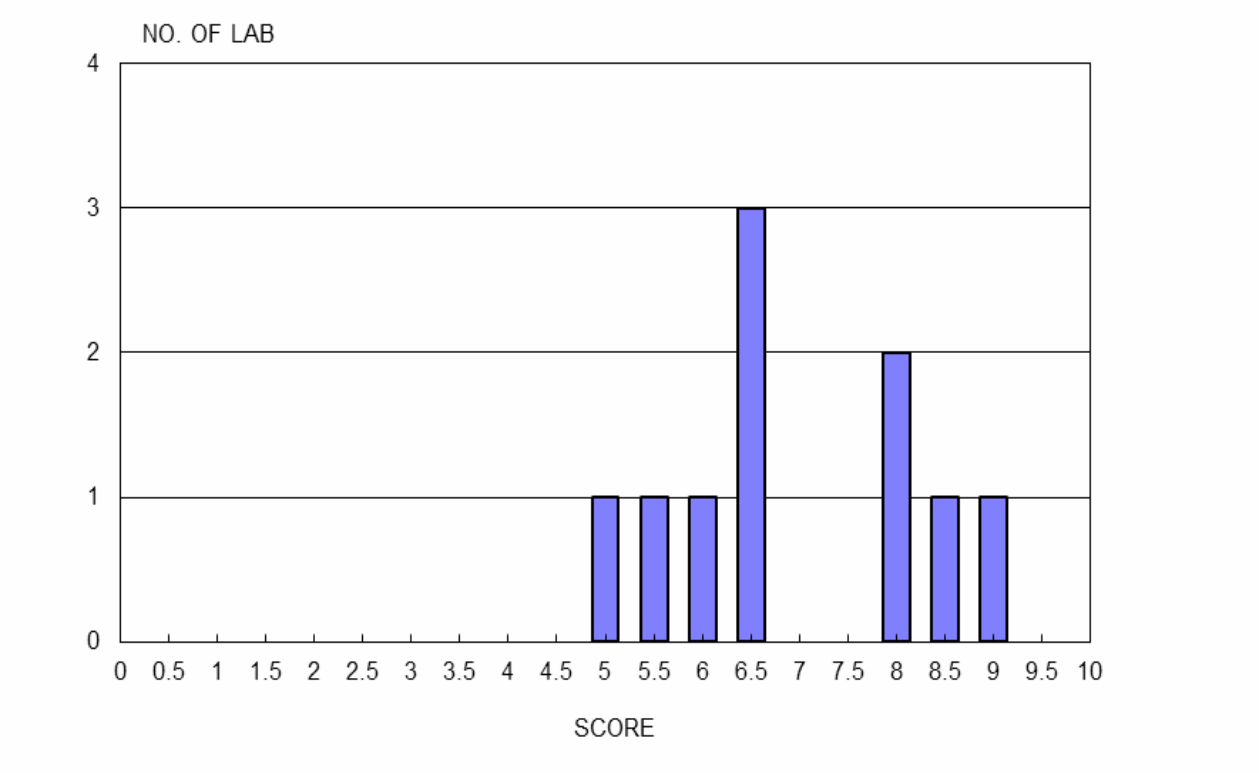


Figure 16. Survey One HC1205 CD10 (in-house)

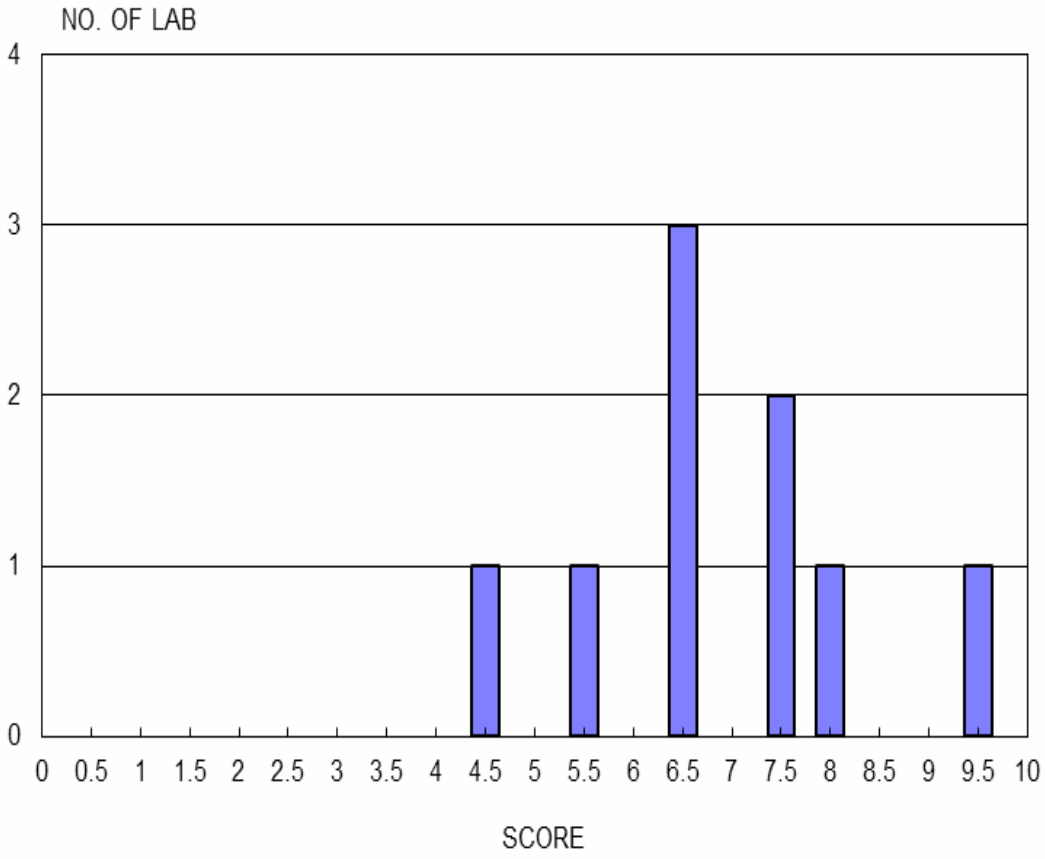


Table 5. The Best Method

STEP	**HC1204 (CD10 Supplied)**	**HC1205 (CD10 in-house)**
Supplier	Dako	Dako
Dilution	1:60	1:60
Peroxidase Blocking	10 min	10 min
Antigen retrieval	PT module Pretreatment 20 min	PT module Pretreatment 20 min
Detection System	Envision Flex	Envision Flex
Duration of Colour Development	DAB 10 min.	DAB 10 min.

b. Survey Two CD3

The survey material was a case of enteropathy associated T cell lymphoma. Sections show diffuse infiltration of the small bowel by monomorphic population of small to medium size lymphoid cells with round dark staining nuclei. Immunohistochemical study showed that the lymphoid cells were strong positive for CD3 stain.

For the supplied CD3 antibody (HC1210) and the in-house CD3 antibody (HC1211), no laboratory failed in both assessments. The median score of HC1210 and HC1211 were found to be both to be 7.0. The distributions of scores were shown in Figures 17 – 18.

Figure 17. Survey Two HC1210 CD3 (supplied antibody)

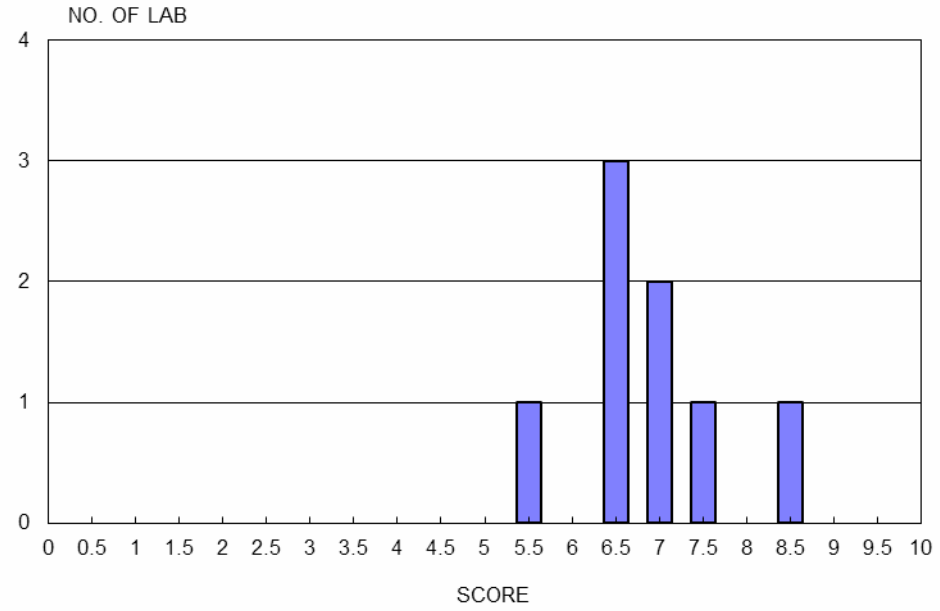


Figure 18. Survey Two HC1211 CD3 (in-house)

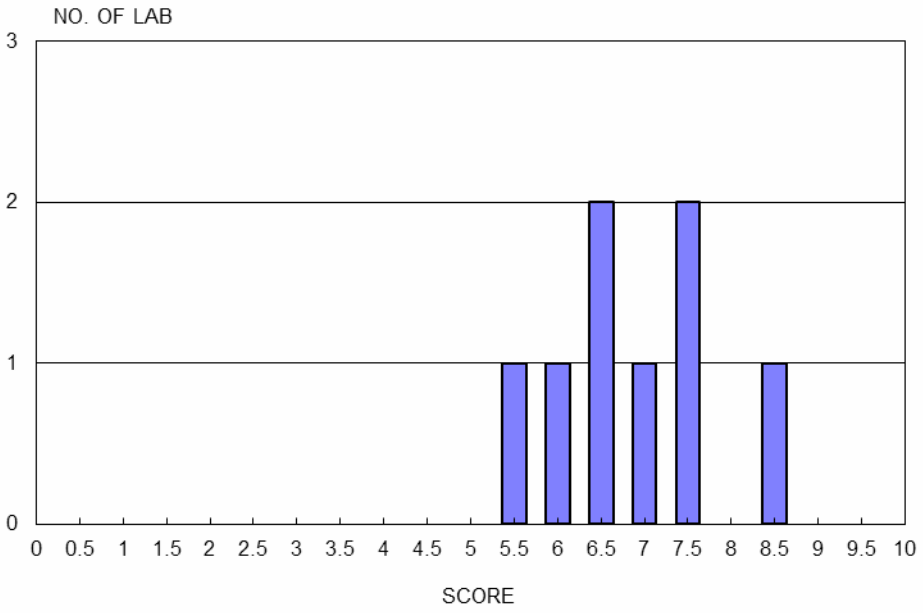


Table 6. The Best Method

STEP	**HC1210 (CD3 Supplied)**	**HC1211 (CD3 in-house)**
Supplier	Dako	Dako
Dilution	1:50	1:50
Peroxidase Blocking	5 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)	0.5 CuSO4 5 min	0.5 CuSO4 5 min

c. Survey Three Calretinin

The survey material for the Calretinin demonstration was a case of mesothelioma. Section showed three pieces of desmoplastic and fibrofatty tissue infiltrated by isolated and small nests of tumour cells arranged in papillae. They had enlarged oval nuclei, central prominent nucleoli and pinkish cytoplasm. Immunohistochemical stained strongly positive for calretinin.

One participant failed in the supplied Calretinin antibody (HC1216) and no participant failed in the in-house Calretinin (HC1217). The median scores of HC1216 and HC1217 were 6.5 and 8.0 respectively. The laboratory with sub-optimal demonstration was due to weak signal to noise ratio. The distributions of scores were shown in Figures 19 - 20.

Figure 19. Survey Three HC1216 Calretinin (supplied antibody)

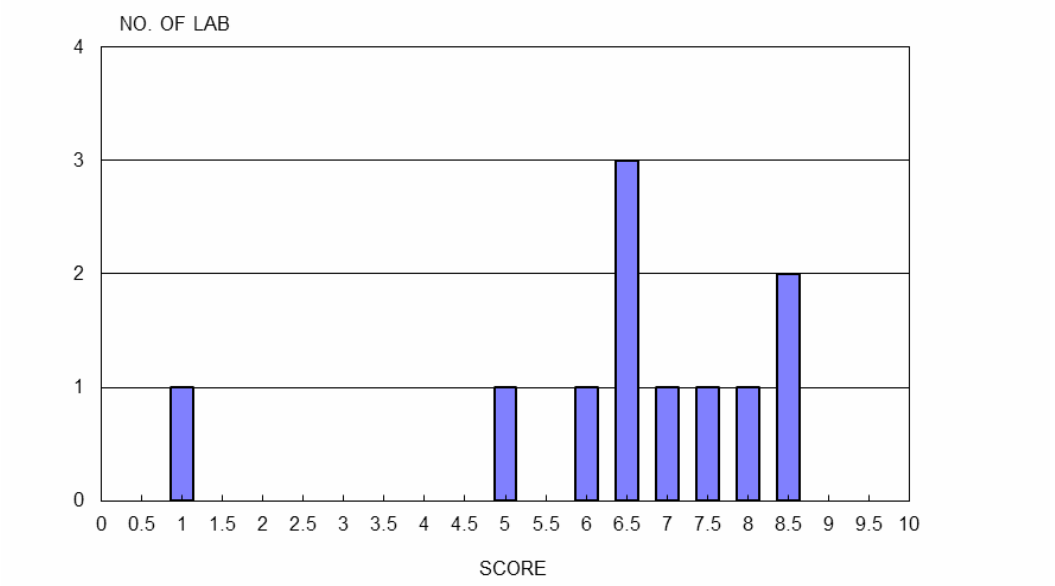


Figure 20. Survey Three HC1217 Calretinin (in-house)

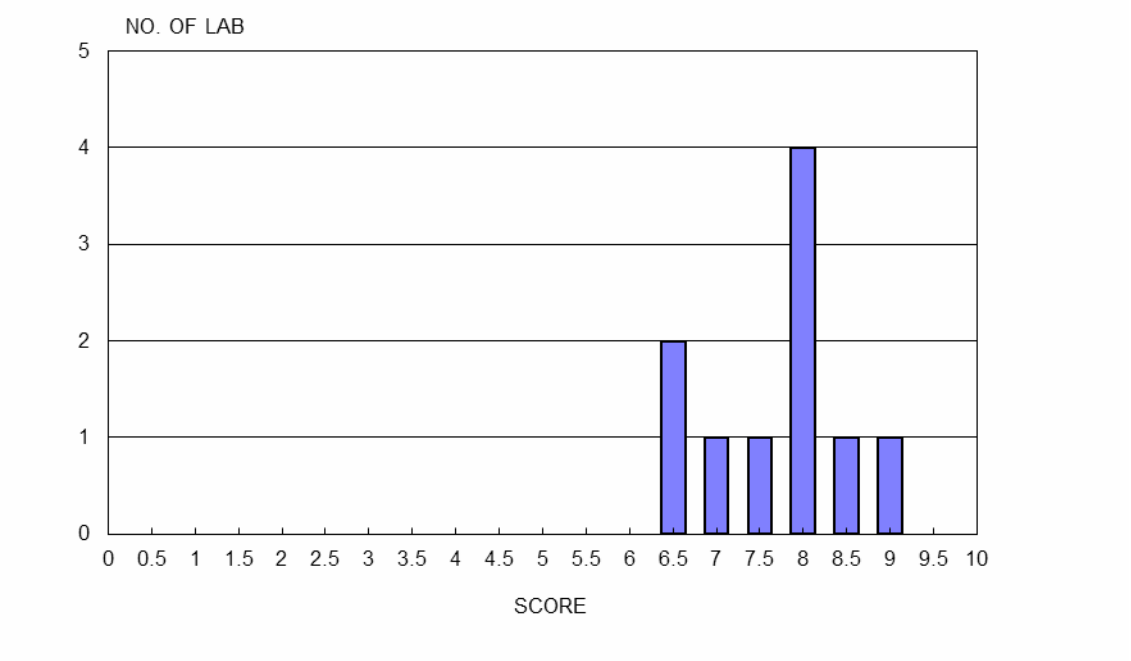


Table 7. The Best Method

STEP	**HC1216 (Calretinin Supplied)**	**HC1217 (Calretinin in-house)**
Supplier	Dako	Novocastra
Dilution	1:60	1:80
Peroxidase Blocking	10 min	5 min
Antigen retrieval	PT module pre-treatment 20 min	EDTA pH8 heating 100^{o}C 20 min
Detection System	Envision Flex kit	Bond Polymer Refine Detection Kit
Duration of Colour Development	DAB 10 min	DAB 7 min

d. Survey Four p53

The survey material for the p53 demonstration was a case of ovarian carcinoma. Sections show tumour cells are arranged in solid nests and thick papillae with focal cystic area. They have enlarged hyperchromatic and plemorphic nuclei, distinct nucleoli and with high mitotic rate and frequent atypical mitotic figures. Immunohistochemically the tumour cells show strong nuclear positivity for p53.

One laboratory failed in the supplied p53 antibody (HC1222) and no laboratory failed in the in-house p53 antibody (HC1223). The median scores of HC1222 and HC1223 were found to be both 7.5. The laboratory with sub-optimal demonstration was due to weak signal to noise ratio. The distributions of scores were shown in Figure s 21 - 22.

Figure 21. Survey Four HC1222 p53 (supplied antibody)

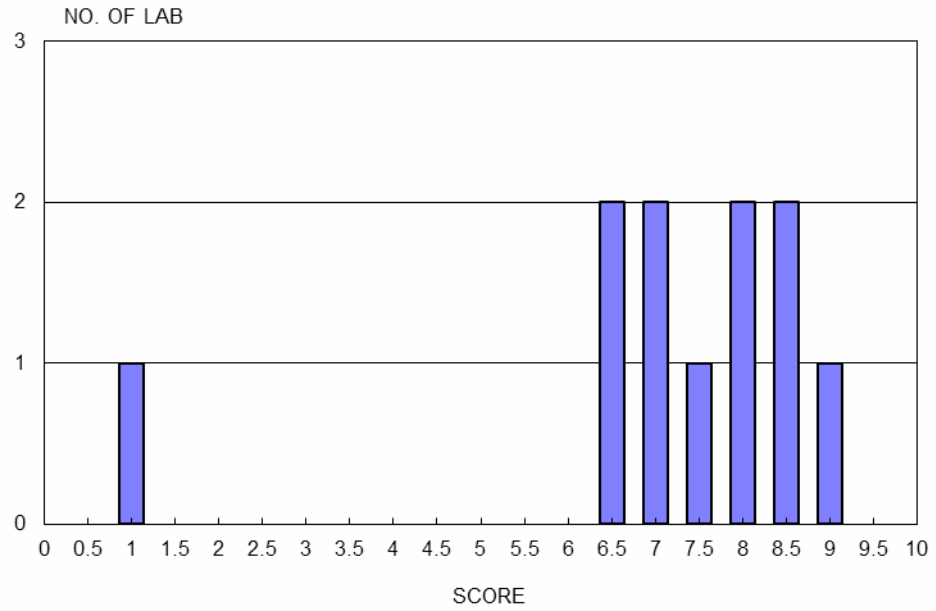


Figure 22. Survey Four HC1223 p53 (in-house)

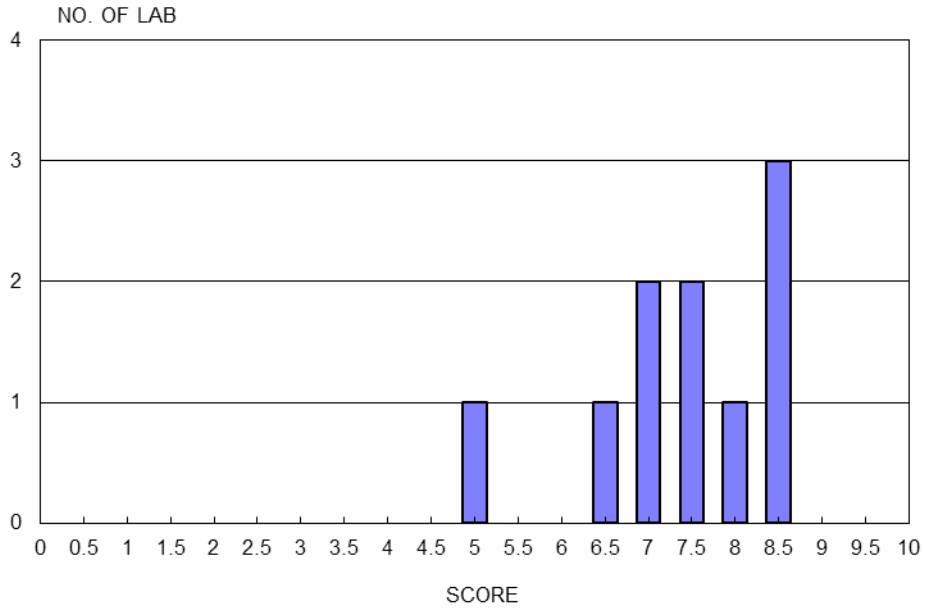


Table 8. The Best Method

STEP	**HC1222 (p53 Supplied)**	**HC1223 (p53 in-house)**
Supplier	Dako	Dako
Dilution	1:50	1:4000
Peroxidase Blocking	10 min	5 min
Antigen Retrieval	PT module pre-treatment 15 min	PT module pre-treatment 20 min
Detection System	Ultra View	Dako REAL Envision
Duration of Colour Development	DAB 5 min	DAB 10 min
End product Colour enhancement (if any)	5 min	Nil

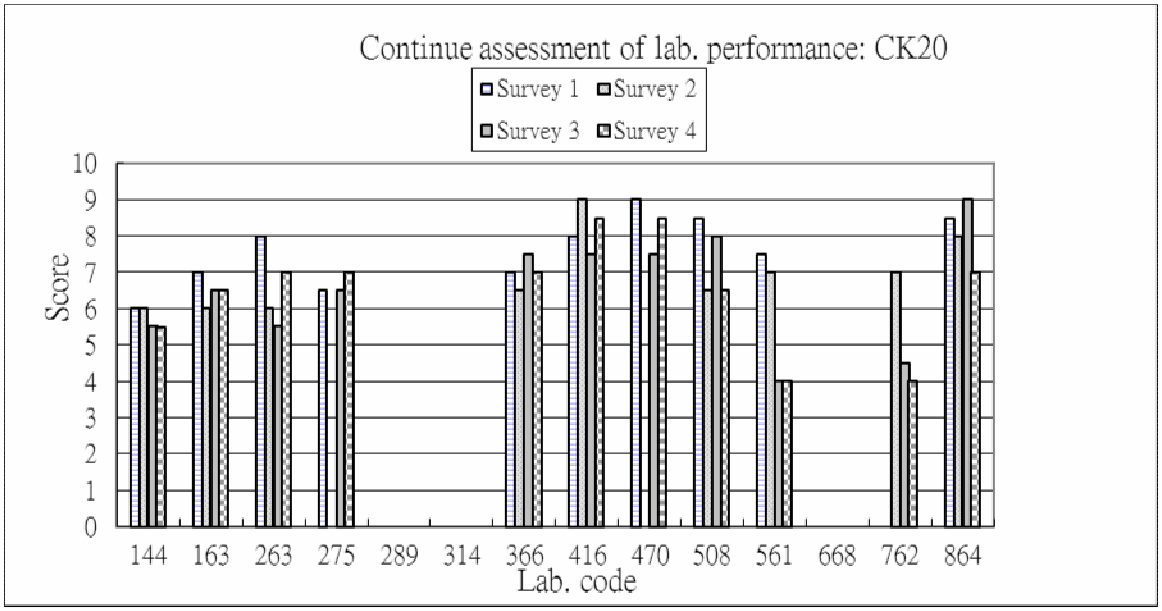
e. Continue Assessment of Laboratory Performance: CK20
The survey material was a case of moderately differentiated adenocarcinoma in sigmoid colon. The malignant tumour cells were highly pleomorphic with frequent mitosis. Immunostudy showed the tumour cells were positive for CK20.

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

CK20	Survey 1	Survey 2	Survey 3	Survey 4
Median	8	6.5	6.5	7

Figure 23. Distribution of Scores



Three laboratories; 289, 314 and 668, did not return any slides for the CK antibody, they were excluded from the continue assessment. Consistently good performance, range of score from 7 to 9, were noted in three laboratories; 416, 470 and 864. Two laboratories; 561 and 762 were noted to exhibit a declining trend of performance. The performance of the remaining laboratories was found to be consistently satisfactory.

References

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