Anatomical Pathology

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In 2010, nineteen laboratories participated in the histological staining program and fourteen laboratories joined the immunohistochemical staining program. The laboratories come from various sectors including Hospital Authority, government institutes/clinics, university laboratories as well as private hospitals.

I. Survey Format

Table 1a, Table 1b and Table 2 show the survey summary of staining methods, cytopathology and immunohistochemistry, respectively, conducted in 2010. A questionnaire was included in each survey covering details of the staining procedures. The information helps the panel identify any process that might attribute to imperfect staining results. The staining procedure of the laboratory with the highest score was made available to all participants for reference.

Table 1a. Histological Staining Program

Survey	**Code Number**	**Staining Methods**
One	HC1002	Gordon & Sweets'
Two	HC1008	Southgate's Mucicarmine
Three	HC1014	Gram
Four	HC1020	Schmorl ± Bleach

Table 1b. Cytopathology

Survey	**Code Number**	**Diagnostic results**
One	HC1003	Malignant cells present, favour adenocarcinoma
Two	HC1009	Malignant cells present
Three	HC1015	Suspicious of adenocarcinoma
Four	HC1021	Malignant cells present, favour adenocarcinoma

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Date: 28th July, 2011

Table 2. Immunohistochemical Staining Program

Survey	Code Number	Staining Methods
One	HC1004	Bcl2
	HC1005	Bcl2 (in house)
	HC1006	CD4
Two	HC1010	CD3
	HC1011	CD3(in house)
	HC1012	CD4
Three	HC1016	CD 57
	HC1017	CD 57 (in house)
	HC1018	CD4
Four	HC1022	Ki67
	HC1023	Ki67 (in house)
	HC1024	CD4

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

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

To ensure the objectivity in assessment, the highest and the lowest marks from the panel of assessors were not taken into compilation of the scores to the participants (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 Members				Final Score
	Member A	Member B	Member C	Member D	
X	9	7	8	10	8.5
Y	6	4	5	7	5.5

III. Slide Return Summary

Figure 1a, Figure 1b and Figure 2 show the statistics of survey slides returned for assessment.

Figure 1a. Histological Staining

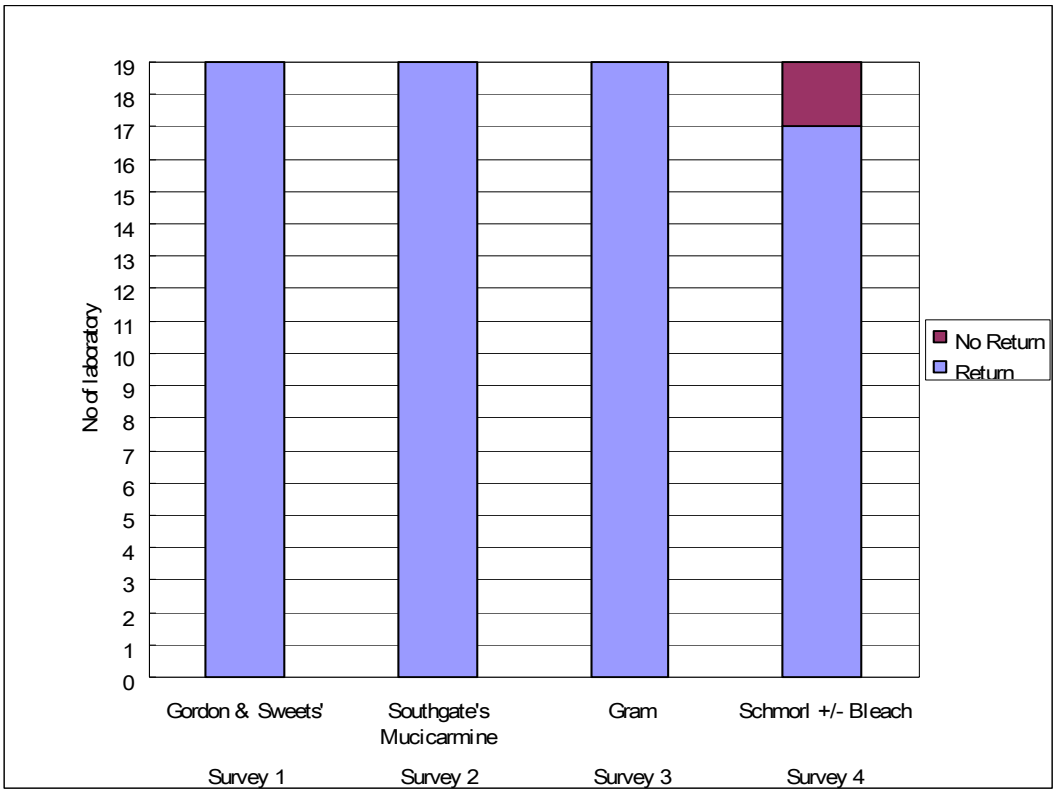


Figure 1b. Cytopathology

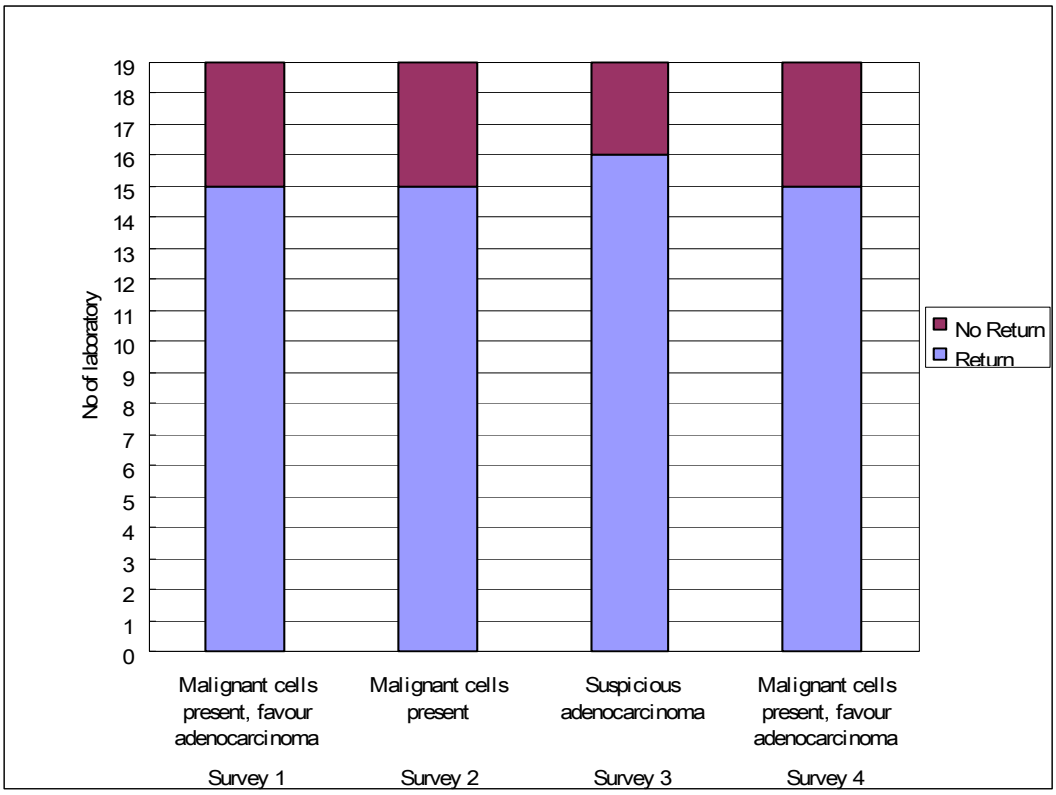
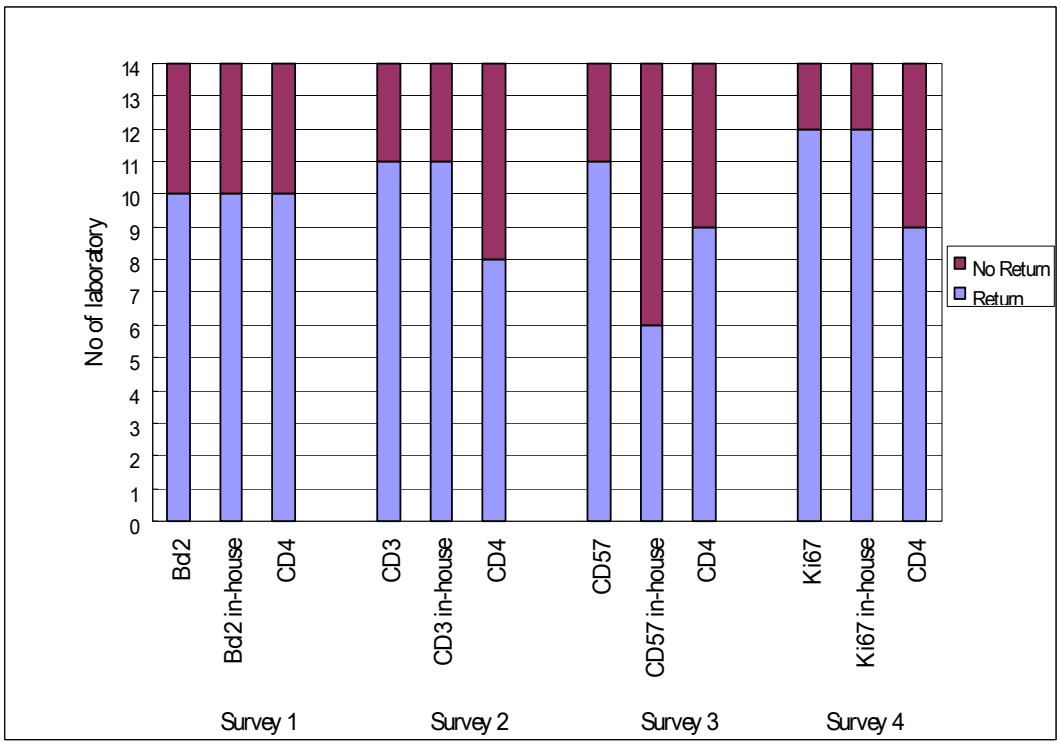


Figure 2. Immunohistochemical Staining



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IV. Survey Analysis

i. Histological Staining Programme

The survey material of Survey One was a trephine specimen after decalcification.

Figure 3: Survey one: HC1001, H&E

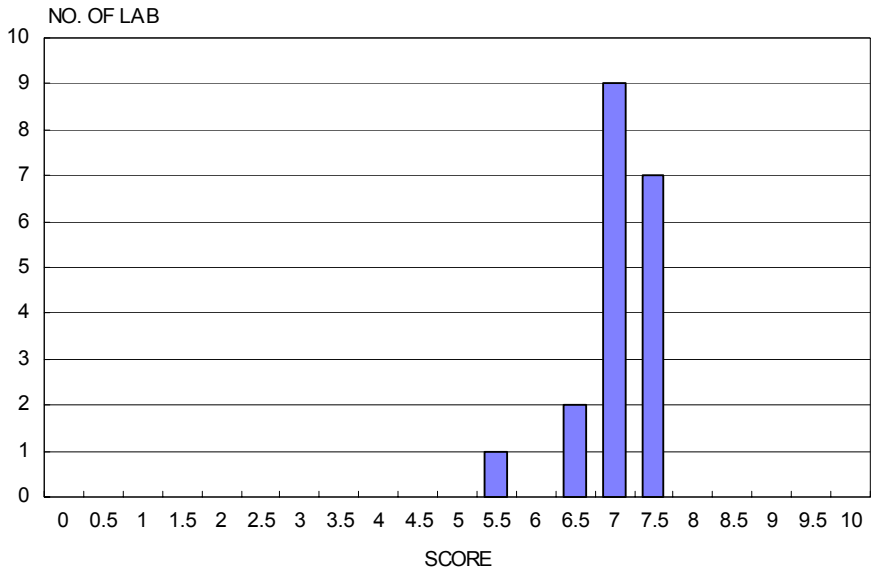
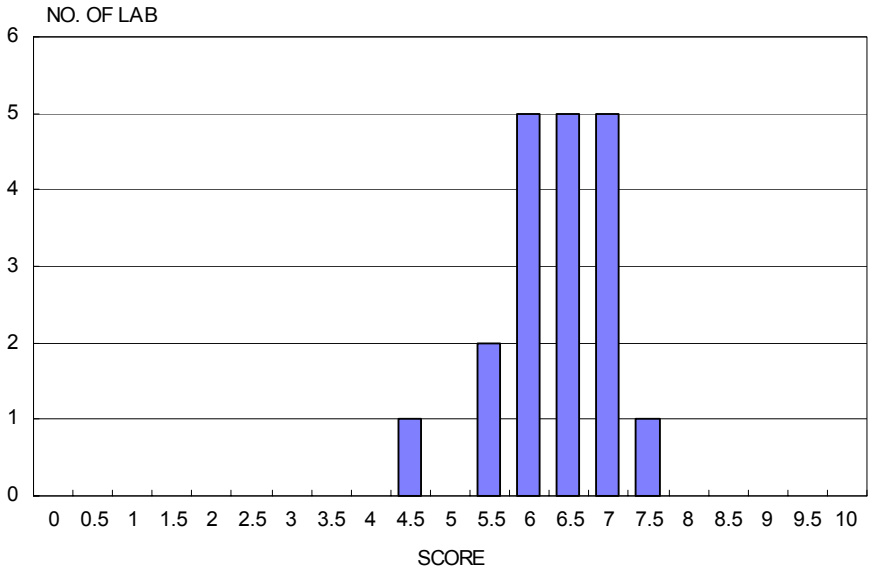


Figure 4: Survey one: HC1002, Gordon & Sweets'



The survey material of Survey Two was a section of colon tissue diagnosed of adenocarcinoma.

Figure 5: Survey two: HC1007, H&E

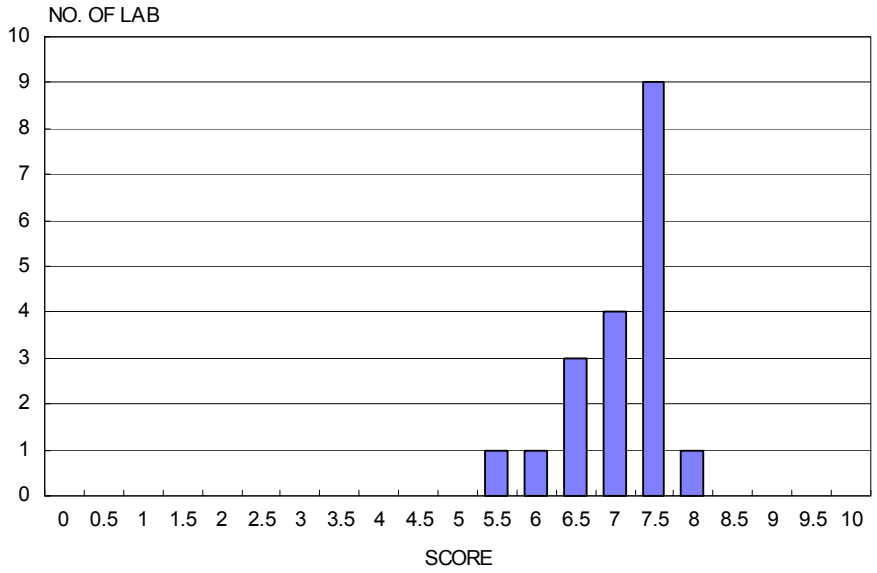
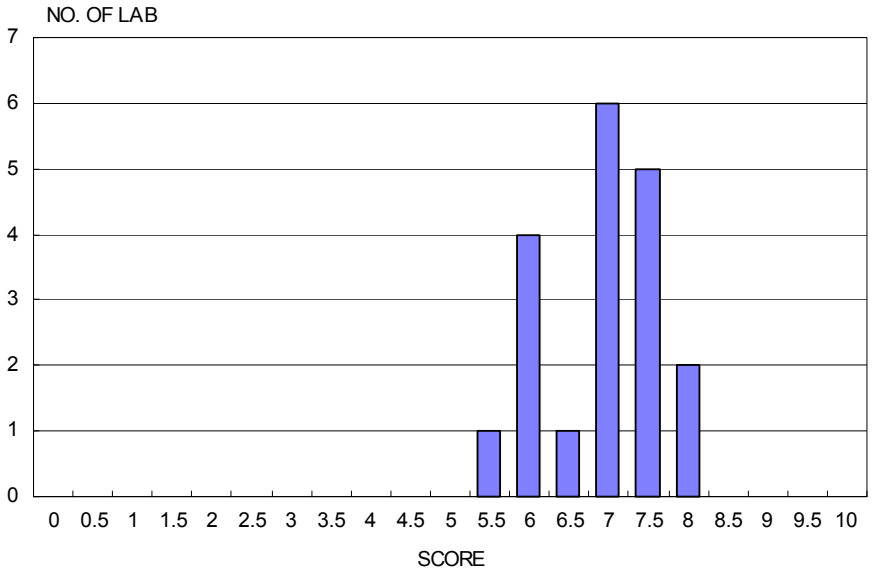


Figure 6: Survey two: HC1008, Southgate's Mucicarmine



The survey material of Survey Three was a necrotic tissue with Gram-positive cocci and Gram-negative bacilli.

Figure 7: Survey three: HC1013, H&E

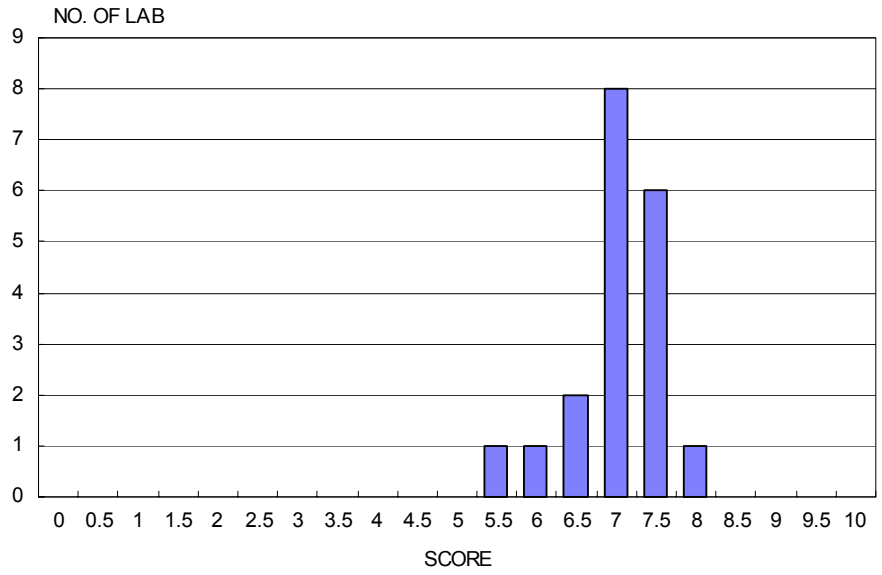
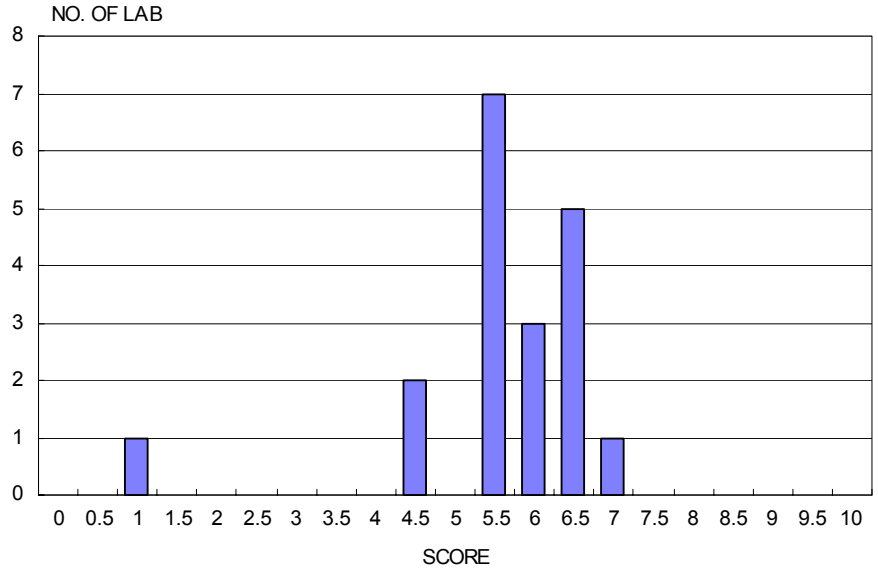


Figure 8: Survey three: HC1014, Gram's Stain



The survey material of Survey Four was a paraffin section of skin tissue.

Figure 9: Survey four: HC1019, H&E

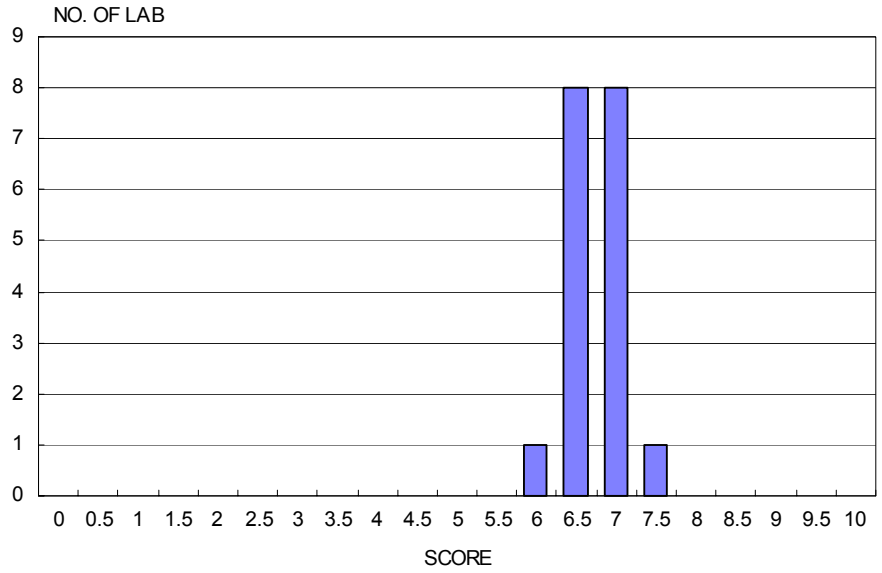


Figure 10a: Survey four: HC1020a, Schmorl without Bleach

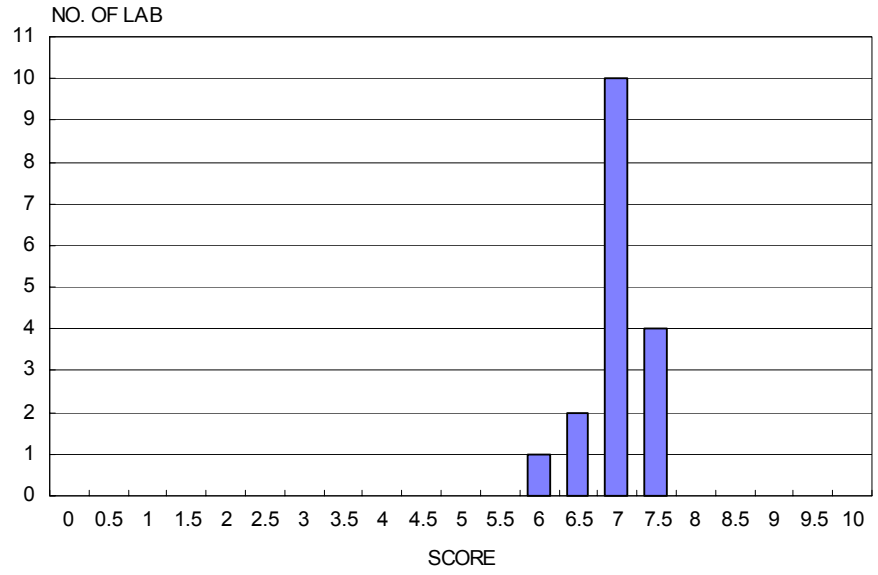
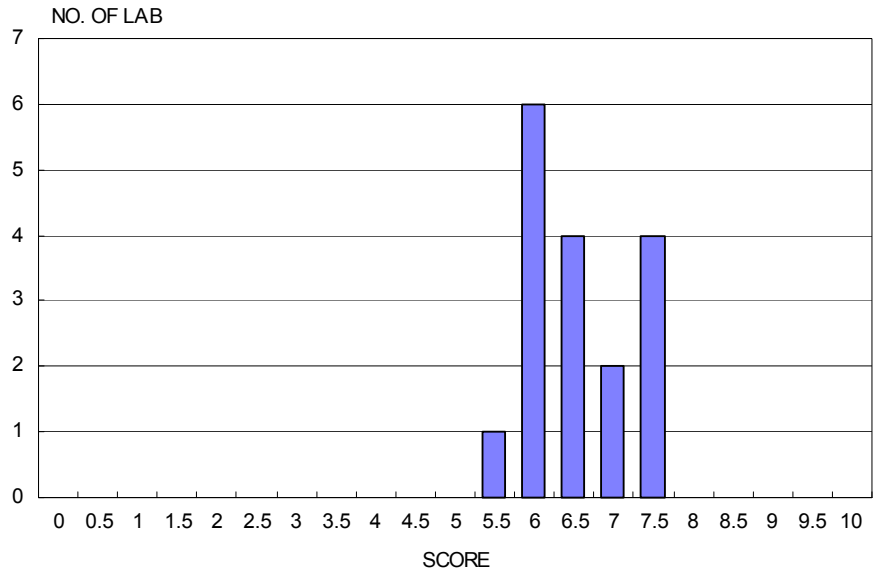


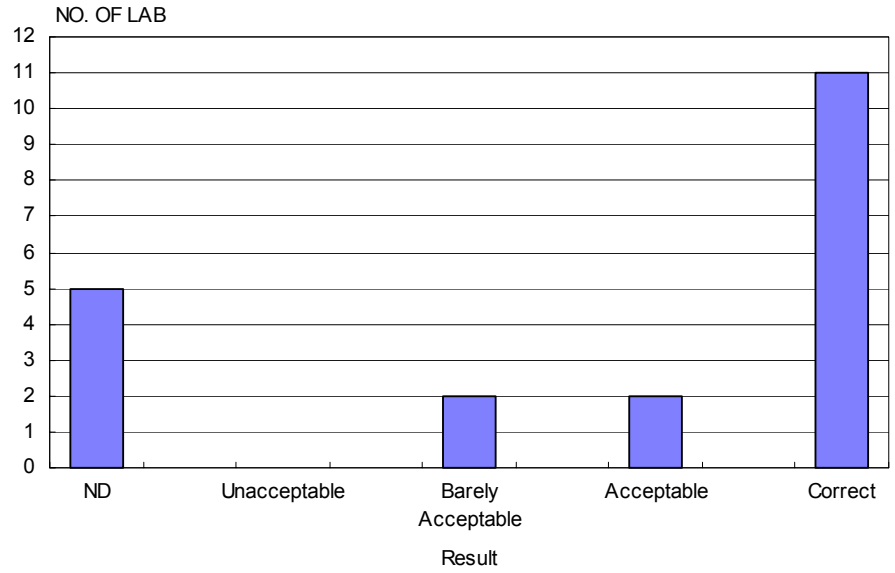
Figure 10b: Survey four: HC1020b, Schmorl plus Bleach



ii. Cytopathology

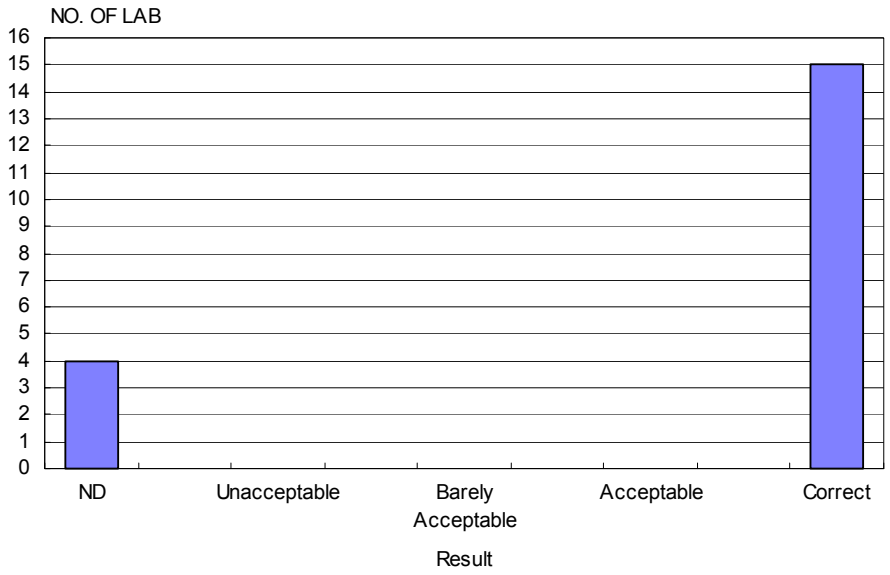
The material of Survey One was prepared from peritoneal fluid. Fifteen (79%) out of 19 participants returned results for assessment. Only 11 participants made the correct diagnosis; two provided acceptable diagnosis and another two gave barely acceptable diagnosis. The expected answer is malignant cells present; favour adenocarcinoma.

Figure 11: Survey one: HC1003



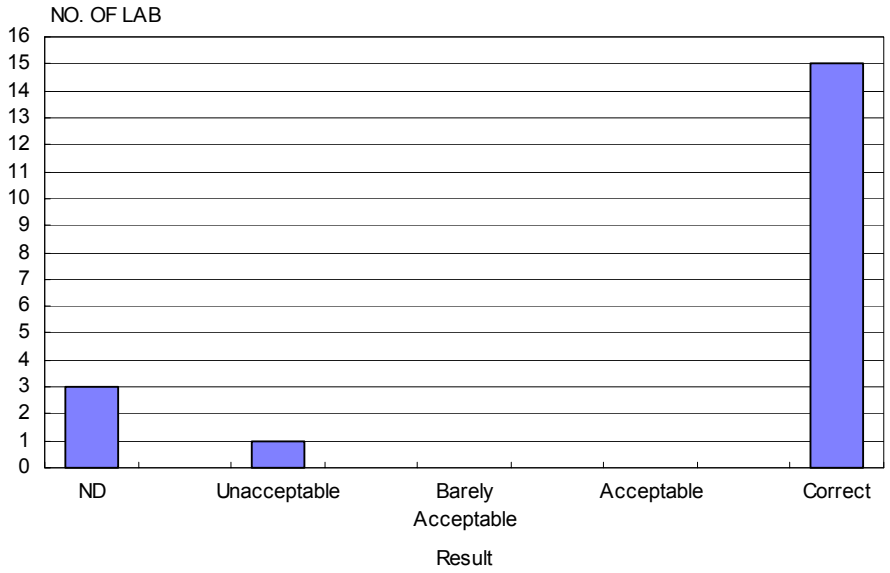
The material of Survey Two was prepared from sputum. Fifteen out of 19 (79%) participants returned results for assessment and all of them provided the correct diagnosis. The expected answer is malignant cells present.

Figure 12: Survey two: HC1009



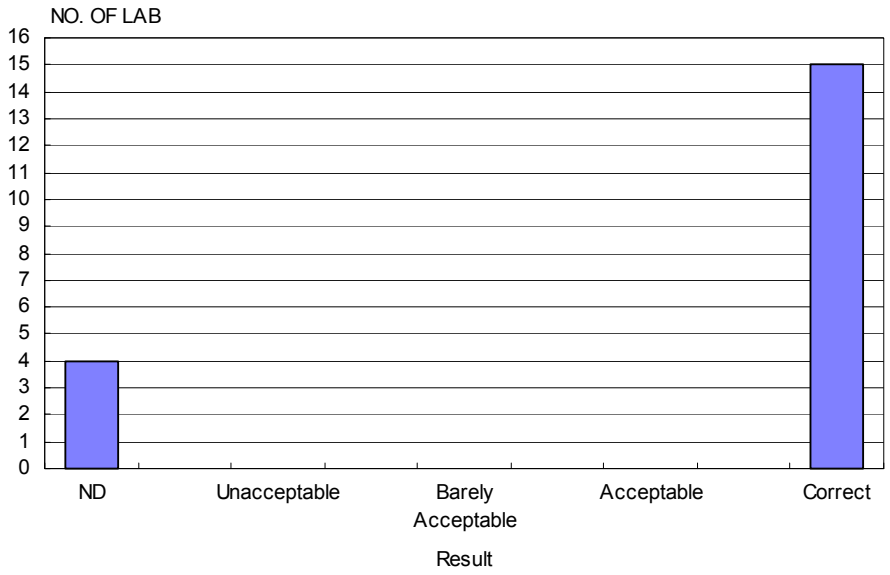
For Survey Three, 16 (84%) laboratories returned results. Fifteen (79%) laboratories provided the correct answer, whereas one (5%) laboratory gave an incorrect result. The expected answer is auspicious of adenocarcinoma.

Figure 13: Survey three: HC1015



In Survey Four, the material was prepared from sputum. Fifteen out of 19 (79%) participants returned results for assessment. All provided the correct readout. The expected answer is malignant cells seen.

Figure 14: Survey Four: HC1021



iii. Immunohistochemical Staining Programme

a. Survey One: Bcl2

The survey material was a case of reactive lymphoid follicular hyperplasia. The section shows the tonsillar tissue with focal neutrophilic infiltrate and neutrophilic exocytosis in the overlying squamous epithelium. Reactive lymphoid follicles are noted. Immunohistochemical study showed that the lymphoid cells were strong positive for Bcl2 stain.

One out of ten laboratories failed in HC1004 (supplied antibody) and no laboratory failed in HC1005 (in-house antibody) assessment. The median score of HC1004 and HC1005 were found to be both 7.0 in score. Figure 15 and Figure 16 illustrated the statistics. Table 5 details the staining procedure to best demonstrate bcl2.

Figure 15: Survey one: HC1004, Bcl2 (supplied antibody)

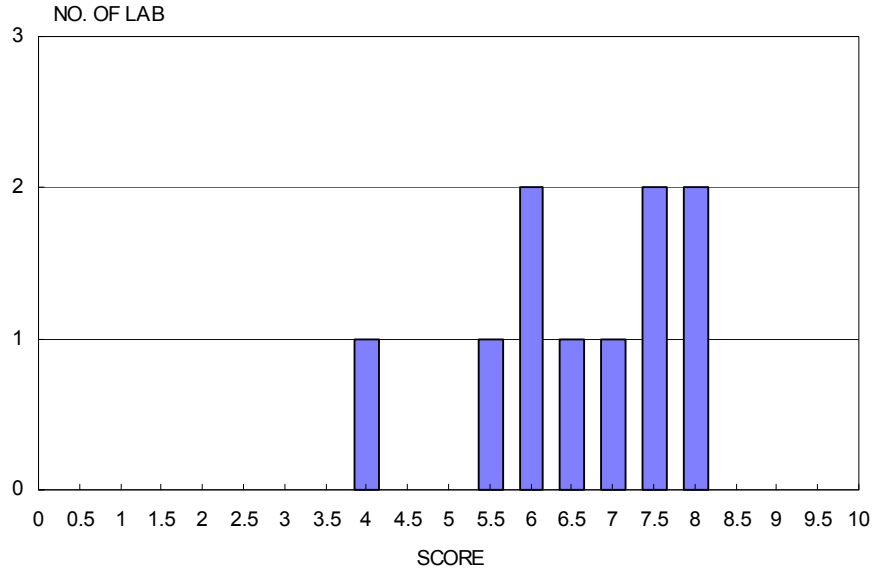


Figure 16: Survey one: HC1005, Bcl2 (in-house)

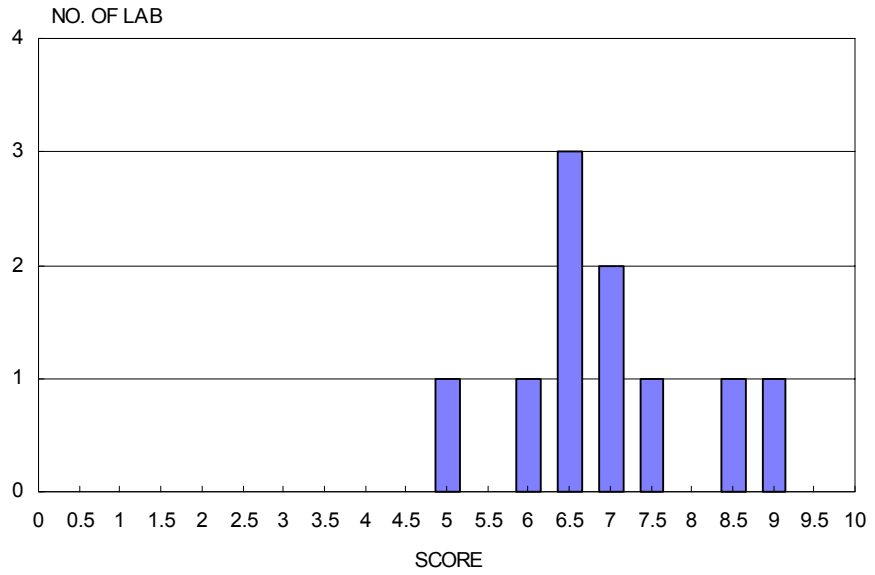


Table 5. The staining procedure to best reveal bcl2.

STEP	**HC1004 (Bcl2 Supplied)**	**HC1005 (Bcl2 in-house)**
Supplier	Novocastra	Zymed
Dilution	1:100	1:150
Peroxidase Blocking	10 min	10 min
Antigen Retrieval by Pressure Cooking Pre-treatment	3.5 min	3.5 min
Detection System	Envision	Envision
Duration of Colour Development	DAB 10 min.	DAB 10 min.

b. Survey Two: CD3

The survey material for the demonstration of CD3 was a case of enteropathy associated T cell lymphoma. The section shows a diffuse infiltration of the small bowel by a monomorphic population of small to medium size lymphoid cells with round dark staining nuclei. Immunohistochemical study displayed that the lymphoid cells were strong positive for CD3 stain. One out of eleven laboratories failed in HC1010 (supplied antibody) and no laboratory failed in HC1011 (in-house antibody) assessment. The median score of HC1010 and HC1011 were found to be 7.5 and 7.0, respectively. The statistics were shown in Figure 17 and Figure 18: Table 6 details the staining procedure to best demonstrate CD3.

Figure 17: Survey two: HC1010, CD3 (supplied antibody)

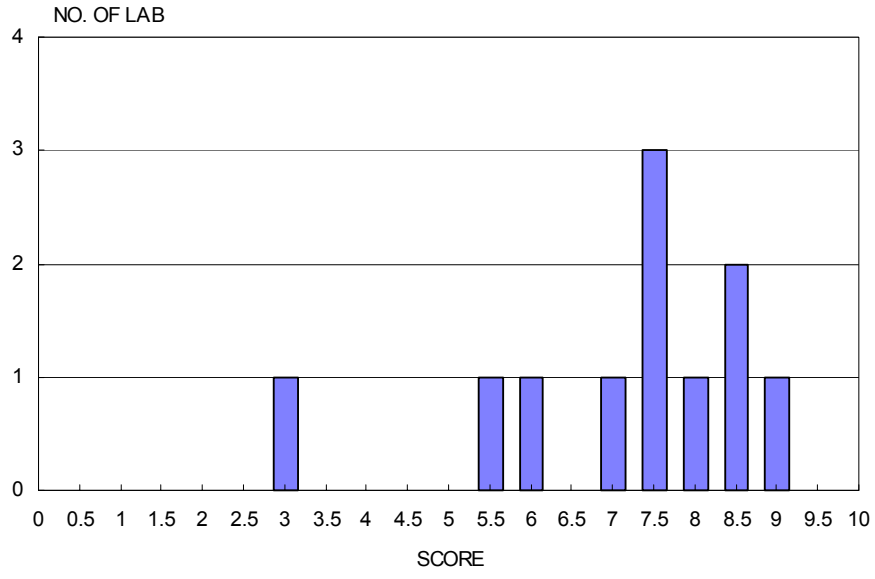


Figure 18: Survey two: HC1011, CD3 (in-house)

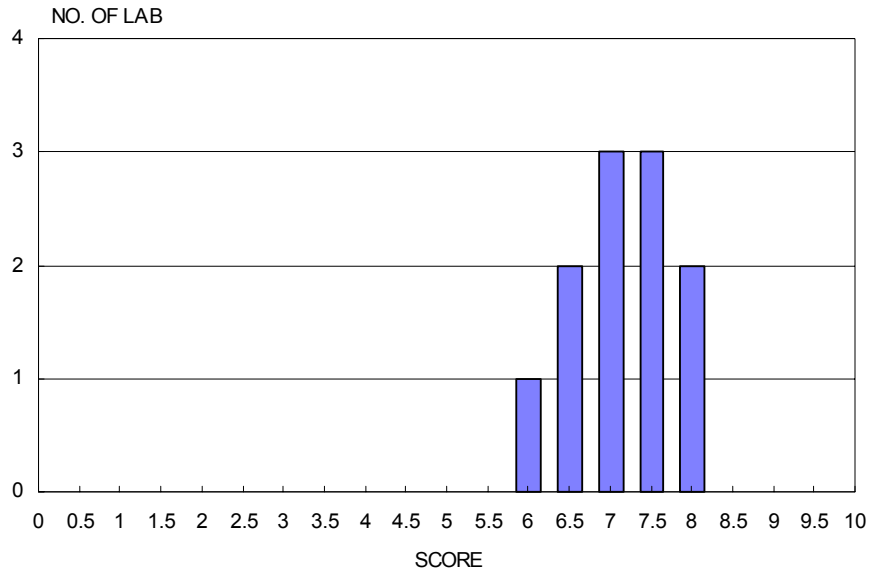


Table 6. The staining procedure to best reveal CD3

STEP	**HC1010 (CD3 Supplied)**	**HC1011 (CD3 in-house)**
Supplier	Novocastra	Dako
Dilution	1:100	1:120
Peroxidase Blocking	10 min	10 min
Antigen Retrieval by Pressure Cooking Pre-treatment	3 min 20 sec	3 min 20 sec
Detection System	Ventana I-view	Ventana I-view
Duration of Colour Development	DAB 8 min.	DAB 8 min.

c. Survey Three: CD57

The survey material for the demonstration of CD57 was a normal left tonsil from a patient with salivary gland tumour. The section shows tonsillar tissue with reactive lymphoid germinal centres. Immunohistochemical study demonstrated that the lymphoid cells were strong positive for CD57 stain. One out of 11 laboratories failed in HC1016 (supplied antibody) and one out of seven laboratories failed in HC1017 (in-house antibody). The median score of HC1016 and HC1017 were found to be 7.5 and 6.0, respectively. Those laboratories with sub-optimal demonstration were mainly due to weak signal to noise ratio. Figure 19 and Figure 20 depict the statistics. Table 7 details the staining procedure to best demonstrate CD57.

Figure 19: Survey three: HC1016, CD57 (supplied antibody)

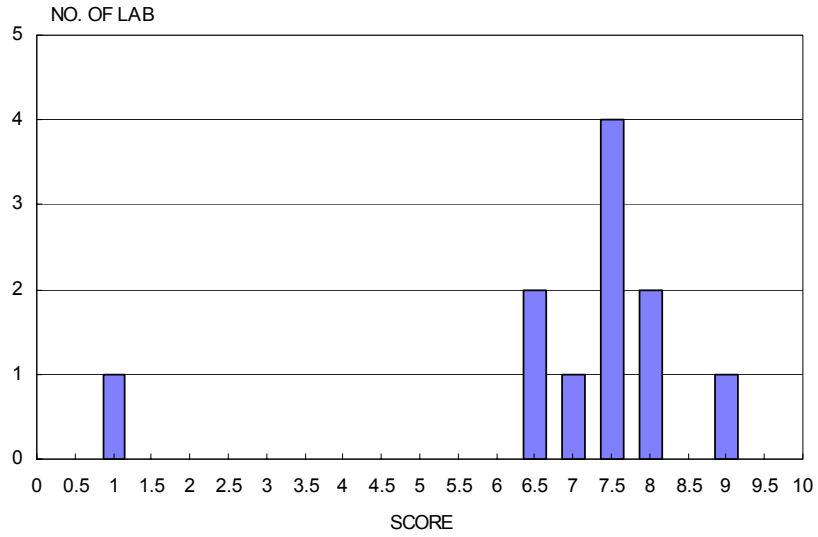


Figure 20: Survey three: HC1017, CD57 (in-house)

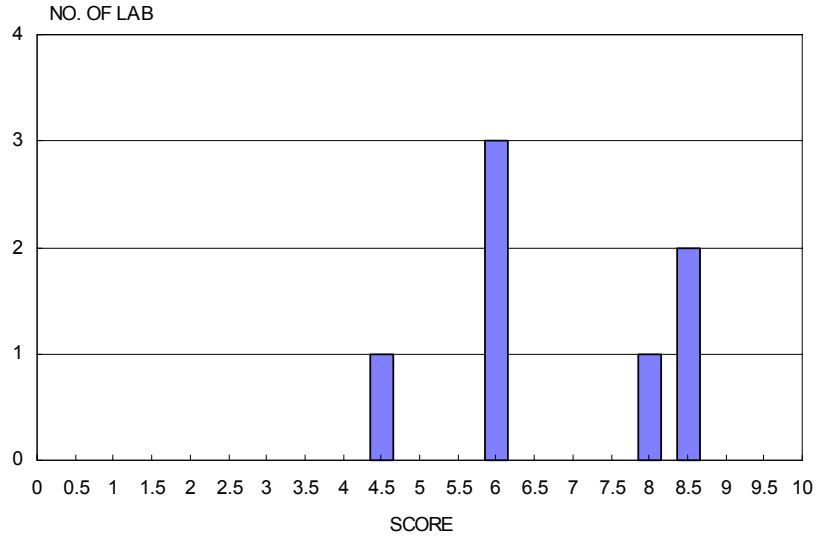


Table 7. The staining procedure to best reveal CD57.

STEP	**HC1016 (CD57 Supplied)**	**HC1017 (CD57 in-house)**
Supplier	Novocastra	Biogenex
Dilution	1:50	1:20
Peroxidase Blocking	10 min	10 min
Antigen Retrieval by Pressure Cooking Pre-treatment	3.5 min	3.5 min
Detection System	Dako Envision	Dako Envision
Duration of Colour Development	DAB 10 min.	DAB 10 min.

d. Survey Four: Ki67

The material for the demonstration of Ki67 was a diffuse large B cell lymphoma. The section shows the lymph node with partial effacement of nodal architecture by sheets of atypical lymphoid cells. Most of the atypical lymphoid cells are large sized with one to several nucleoli that are focally membrane-bounded. About 80% of the atypical lymphoid cells express Ki67. No laboratory failed in HC1022 (supplied antibody) and HC1023 (in-house antibody). The median score of HC1022 and HC1023 were found to be 7.0 and 8.0, respectively. Figures 21 and Figure 22 show the statistics. Table 8 details the staining procedure to best demonstrate Ki67.

Figure 21: Survey four: HC1022, Ki67 (supplied antibody)

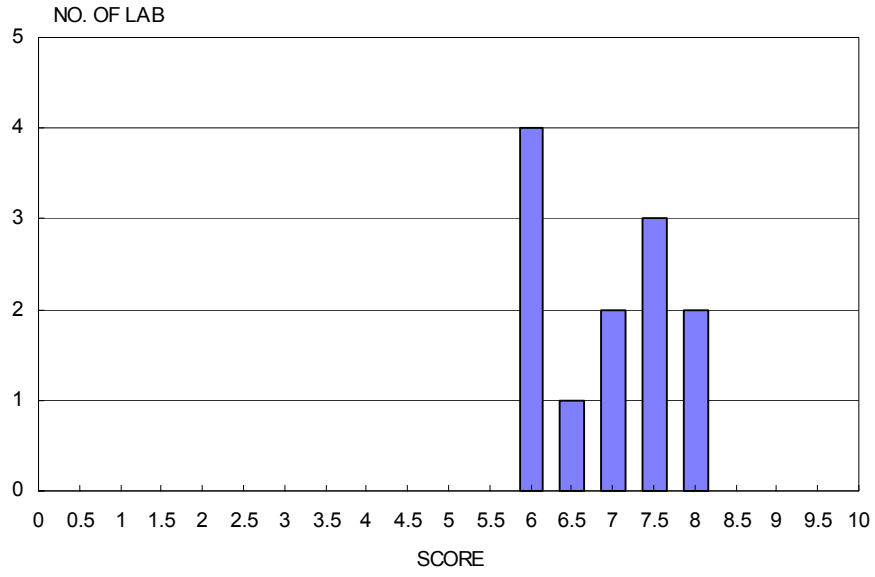


Figure 22: Survey four: HC1023, Ki67 (in-house)

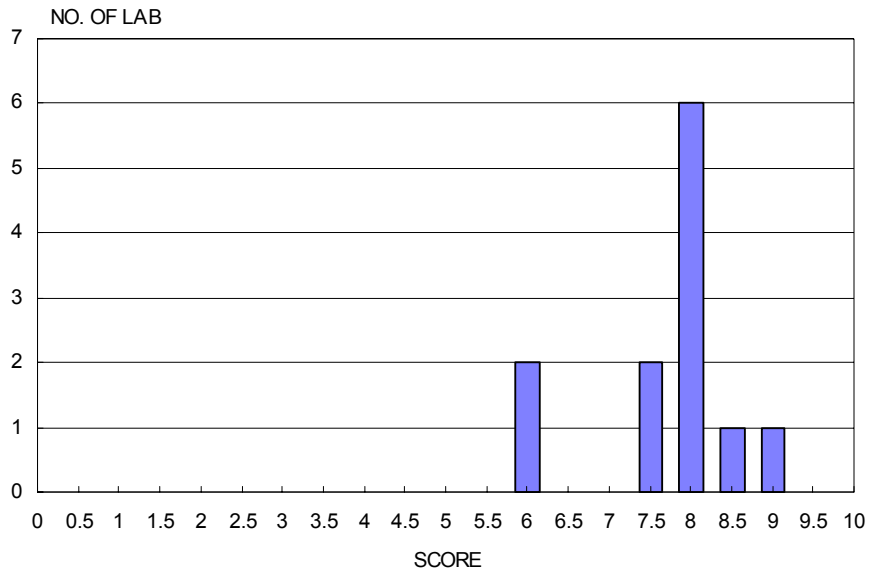


Table 8. The staining procedure to best reveal Ki67.

STEP	**HC1022 (Ki67 Supplied)**	**HC1023 (Ki67 in-house)**
Supplier	Novocastra	Ventana
Dilution	1:20	1:1.5
Peroxidase Blocking	5 min	5 min
Antigen Retrieval	PT Module Pre-treatment 20 min	Microwave Pre-treatment EDTA 12 + 8 min
Detection System	Dako Envision	Ventana Ultra View
Duration of Colour Development	DAB 10 min.	DAB 10 min.

e. Continuous Assessment of Laboratory Performance: CD4

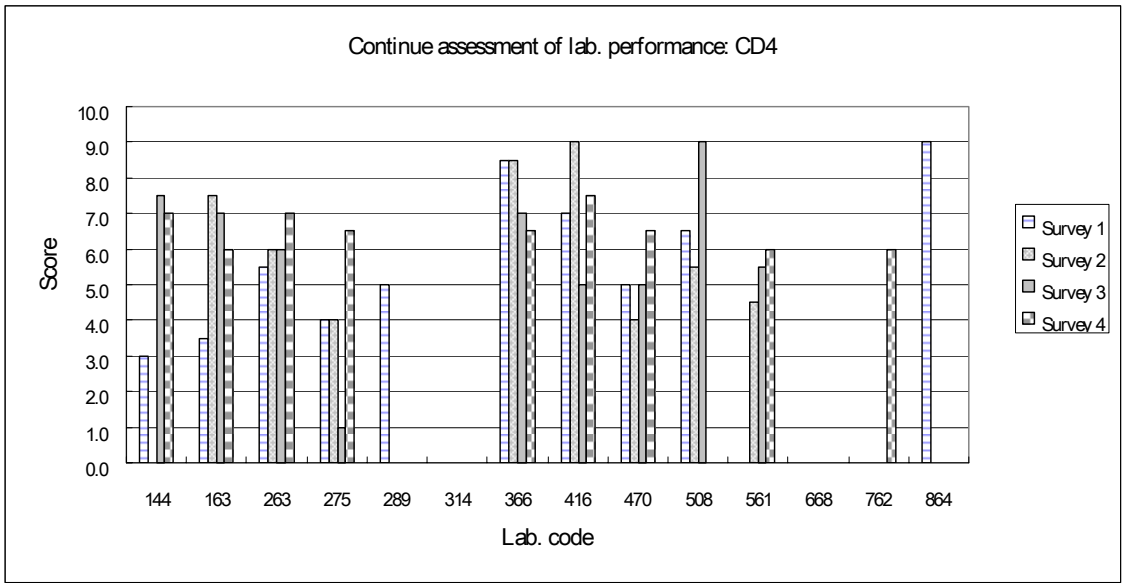
The material for the demonstration of CD4 was a case of atypical lymphoid proliferation. The section shows sheets of monotonous population of medium to large size lymphoid cells. There is no lympho-epithelial lesion. Those atypical lymphoid cells are of B-cell lineage. There are scattered reactive T-cells in the background, highlighted by CD4 staining.

To monitor the performance consistency, sections of the same tissue block were sent to participants in all four surveys. The returned slides were assessed as stated in Section II, Method of Analysis. The median scores of the four surveys were tabulated in Table 9. Figure 23 illustrates the statistics. The calculated mean of all the returns was 6.1 and the standard deviation was 1.78.

Table 9. Median Score Summary

CD4	Survey 1	Survey 2	Survey 3	Survey 4
Median	5.5	6	6	6.5

Figure 23. Distribution of Scores



Five laboratories which had not returned the survey slides for more than two survey exercises were excluded from the continuous assessment. The scores of performance of the participants range from 1.5 to 4.5.

References

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