HISTOPATHOLOGY & CYTOLOGY

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In 1997, twenty-three laboratories have participated in the external quality assurance programme (HKMTAQAP). Eighteen laboratories joined the immunohistochemical staining. In 1997, there was an increase from 22 to 23 laboratories participated in the programme.

The participating laboratories were from the Hospital Authority, government institutes / clinics, university laboratories as well as private hospitals (Table 1). The number of returns in each survey was recorded in Table 2.

Table 1. Types of Participating Laboratories

	Histopathological		Immunohistochemical	
	Number	(%)	Number	(%)
Hospital Authority	15	(65)	14	(77)
Government Institutes/Clinics	4	(17)	2	(11)
University Laboratories	2	(9)	1	(6)
Private Hospitals	2	(9)	1	(6)
Total	23	(100)	18	(100)

Table 2. Returned Results by Laboratories

	Number of Laboratories			
	Histopathological Staining		Immunohistochemical Staining	
Survey	Late Return	Nil Return	Late Return	Nil Return
One	0	1	0	4
Two	0	3	0	5
Three	0	0	0	4
Four	0	1	0	1

I. Survey Format

This year, we continued to use the formats of the questionnaire, report and marking scheme of the previous year. The marking scheme aimed at standardizing the marking of the assessors and to remove their subjectivity and bias. The maximum score of each test was 10 marks. A score equal to or greater than 5 indicated an acceptable quality of staining, whilst a score less than 5 was considered to be unsatisfactory. Those participants with unsatisfactory results would receive comments of the assessors. In each survey, the protocol of the best performance would be published in the report for the participants' reference so that they can compare their own protocol with the best one.

Table 3 and 4 summarised the various staining methods tested in this year. The inclusion of a questionnaire in each survey asked questions relevant to the details of the staining procedure was used as a means to allow the panel of assessors to spot particular technical problems or pitfalls, which had led to unsatisfactory staining performance of a particular participant. The data derived from the questionnaire was compiled in each survey report to allow individual participating laboratories to evaluate their own method with those used by others. This provided them with an opportunity to improve their own methodology further. In addition, the panel was also valuable for advice or other remedies.

Table 3. Histopathological Staining Programme

Survey	Code Number	Staining Methods
One	HC701	H & E
	HC702	Masson Fontana Silver Method for Melanin
	HC703	Schmorl Method for Melanin
Two	HC707	H & E
	HC708	Gordon & Sweets' Method for Reticulin
	HC709	Highman's Congo Red Method for Amyloid
Three	HC713	H & E
	HC714	Phosphotungstic Acid Haematoxylin Method for Fibrin
	HC715	Martius Scarlet Blue Method for Fibrin Four
	HC719	Papanicolaou (EA50) Method for Bronchial Aspiration Smear
	HC720	Papanicolaou (EA65) Method for Bronchial Aspiration Smear
	HC721	Ziehl-Neelsen Method for Acid Fast Bacilli

Table 4. Immunochistochemical Staining Programme

Survey	Code Number	Antigen Demonstrated
One	HC704	AE1 / AE3 (primary antibody provided)
	HC705	AE1 / AE3 (primary & linking antibodies provided)
	HC706	AE1 / AE3 (in-house antibodies)
Two	HC710	CA125 (primary antibody provided)
	HC711	CA125 (primary & linking antibodies provided)
	HC712	CA125) (in-house antibodies)
Three	HC716	CA19-9 (primary antibody provided)
	HC717	CA19-9 (primary & linking antibodies provided)
	HC718	CA19-9 (in-house antibodies)
Four	HC722	Lambda Light Chain (primary antibody provided)
	HC723	Lambda Light Chain (primary & linking antibodies provided)
	HC724	Lambda Light Chain (in-house antibodies)

Table 5. Criteria and scores for H & E staining

Criteria	Scores
Nuclear staining	0-3
Cytoplasmic counterstain	0-3
Staining contrast	0-2
Staining consistency	0-1
Slide appearance	0-1

II. Methods of Analysis

i. Histological Staining Programme

ii. Immunohistochemical Staining Programme

The above scoring system was also adopted for the assessment of the immunohistochemical staining programme with different criteria (Table 6).

Table 6. Criteria and scores for immunohistochemical staining programme

Criteria	Scores
Intensity of positive staining	0-3
Positive signal to background staining	0-3
Immunostaining consistency	0-1
Nuclear counterstain	0-1
Slide appearance	0-1
Proteolysis	0-1

III. Survey Analysis

i. Histological Staining Programme

a. Survey One

Twenty-two out of twenty-three laboratories participated in this survey with one laboratory only returned H&E, one returned without Masson Fontana Silver Method.

All laboratories produced acceptable H&E, Masson Fontana Silver and Schmorl staining. No particular pitfalls were found.

b. Survey Two

Twenty out of twenty-three laboratories returned the survey material for assessment. One laboratory returned only H&E.

All laboratories produced acceptable H&E, Gordon & Sweets' Silver and Highman's Congo Red staining and no particular pitfalls were found.

c. Survey Three

Twenty-one out of twenty-three laboratories returned the survey material for assessment. One laboratory returned only H&E and one laboratory returned only H&E and Martius-Scarlet-Blue Staining.

All laboratories produced acceptable H&E, PTAH and MSB staining except one failed in the MSB. The pitfall for MSB staining was weak in demonstration of fibrin which was not stained distinctly from the surrounding tissue.

d. Survey Four

Twenty-two out of twenty-three laboratories returned the survey material for assessment. One laboratory returned only Papanicolaou (EA50) Staining.

All laboratories produced acceptable Papanicolaou (EA50) staining. One laboratory failed in Papanicolaou (EA65) staining with heavy nuclear and cytoplasmic stain. Two laboratories produced under differentiated Ziehl-Neelsen staining smears with heavy pinkish background stain.

ii Immunohistochemical Staining Programme

a. Survey One

Fifteen out of eighteen laboratories returned the survey material for assessment.

With regard to the demonstration of AE1/AE3 on paraffin section using supplied primary antibody and in-house linking antibodies, four out of fifteen laboratories showed unsatisfactory performance. Those laboratories failed to stain the entire stratified epithelium and the staining was weak which did not attribute to individual antigen retrieval method or reagents used. There was room for them to improve their techniques.

b. Survey Two

Thirteen out of eighteen laboratories returned the survey material for assessment.

With regard to the demonstration of CA125 on paraffin section using supplied primary antibody and in-house linking antibodies, six out of thirteen laboratories showed unsatisfactory performance. Non-specific staining were found on the luminal surface of the ductal epithelium of the breast on all sections and certain areas in the adenocarcinoma of the colon.

c. Survey Three

Thirteen out of eighteen laboratories returned the survey material for assessment.

Most of the participating laboratories could demonstrate CA19-9 successfully with varying intensities. With regard to the demonstration of CA19-9 on paraffin section using supplied antibody and in-house linking antibodies, only one laboratory showed unsatisfactory performance due to non-specific staining on the luminal surface of the ductal epithelium of the breast. Different antigen retrieval methods produced different effects on the sections: those treated with enzymes had less non-specific staining whereas those treated by pressure cooker have a higher staining intensity with the drawback of a relatively heavier background and are generally more prone to non-specific staining.

d. Survey Four

Seventeen out of eighteen laboratories returned their survey material for assessment.

With regard to the demonstration of Lambda Light Chain on paraffin section using supplied primary antibody and in-house linking antibodies. Seven out of seventeen laboratories showed unsatisfactory performance. These laboratories were unable to demonstrate the Lambda Light Chain in the majority of the neoplastic cells of which were centrocyte-like in morphology.

IV. Conclusion

After all the survey results in 1997 were complied, the laboratory staining performance was divided into three groups for the purpose of convenience in comparison. They were classified according to the criteria that scored less than 5 was unsatisfactory, scored between 5-6 was satisfactory and those scored more than 6 was found to be above standard.

i. Histopathological Staining Programme

Figure 1 showed the H&E staining performance of the 23 laboratories in 1997. The results indicated that a consistent performance was found from Survey One to

Survey Three. There was about 65-86.4% of participants scored more than 6 marks and about 13.6-35% participants scored 5-6 marks in the three survey. These indicated that the H&E staining performance of over 65% of our participants were found to be above standard except in Survey Four.

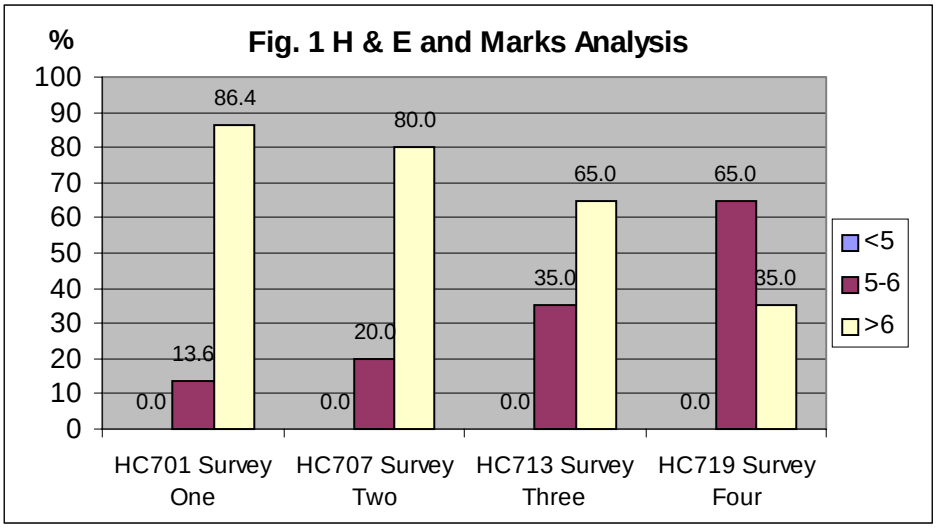
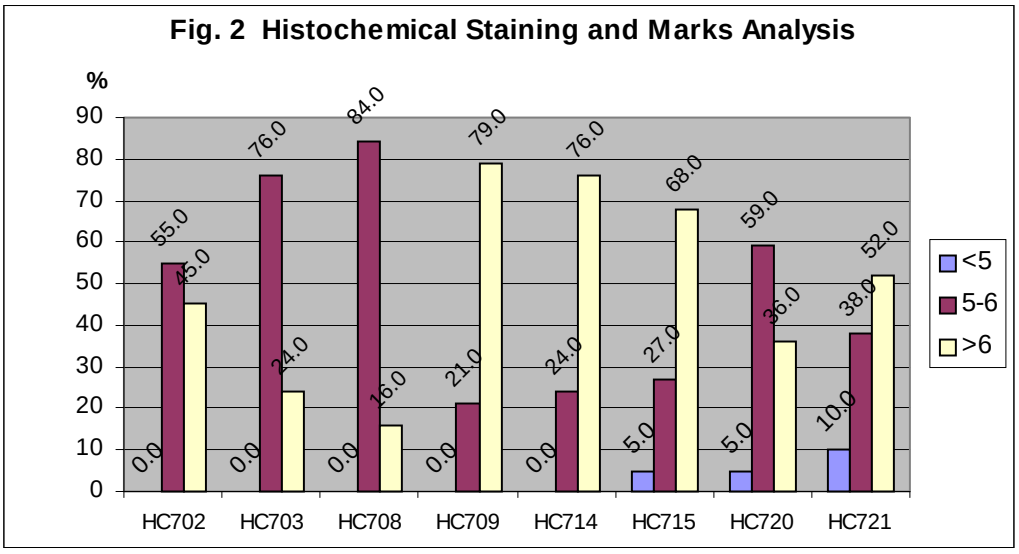
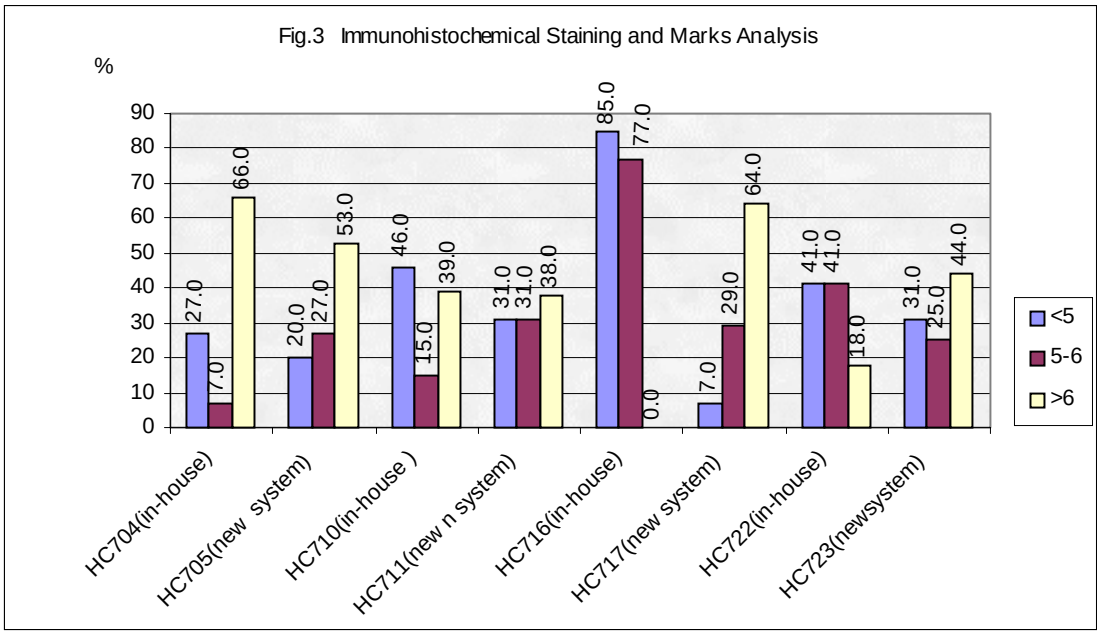


Figure 2 showed the histochemical staining and marks analysis. We found that the performance of the participating laboratories varied among the four surveys. The reusults indicated that there were varying from 16-79% participants scored more than 6 marks, 21-84% participants scored 5-6 marks and 0-10% participants scored below 5 marks. The trend was that the participants performed well in those routine techniques such as Highmans' Congo Red, Phosphotungstic Acid Haematoxylin, Martius Scarlet-Blue and Ziehl-Neelsen but worse in Masson Fontana and Schmorl techniques.



ii. Immunohistochemical Staining Programme

Same as the year 1996, we provided both the primary antibodies and also the linking antibodies to participants in each survey and assessed their performance. Figure 3 showed the analysis of the performance of participants using the same primary antibodies but with different detection systems (i.e. we compared the results of in-house detection system with provided detection system). There was improvement of individual performance when compared the performance of two different systems irrespective of what kind of primary antibodies were used. In general the participating laboratories achieved better results with the new detection system This indicated that the participants were acquainted with the performance of the new detection system and identify the optimal staining condition or incubation time of the system.



In 1998, twenty-three laboratories participated in the histological staining program and 19 laboratories joined the immunohistochemical staining program. The laboratories belong to various institutes, including Hospital Authority, government institutes/clinics, university laboratories as well as private hospital.

I. Survey Format

Table 1 and 2 summarised the various staining methods and antibodies assessed in this year QAP. The survey format was more or less the same as last year; briefly, 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 1. Histological Staining Program

Survey	Code Number	Staining Methods
	HC801	H&E
One	HC802	Alcian Blue-Periodic Schiff
	HC803	Southgate's Mucicarmine
	HC807	H&E
Two	HC808	Kinyouins Cold Ziehl-Neelsen
	HC809	Standard Ziehl-Neelsen
Three	HC813	H&E
	HC814	Taenzer-Unna Orcein
	HC815	Humberstone's Victoria Blue
Four	HC819	Cytology

Table 2. Immunohistochemical Staining Program

Survey	Code Number	Staining Methods
	HC804	34βE12
One	HC805	34βE12 with Provided Linking antibody
	HC806	34bE12 (in house)
	HC810	CD30
Two	HC811	CD30 with Provided Linking antibody
	HC812	CD30 (in house)
	HC816	CD3
Three	HC817	CD3 with Provided Linking antibody
	HC818	CD3 (in house)
	HC820	Hepatitis B Core Antigen
Four	HC821	Hepatitis B Core Antigen with Provided Linking antibody
	HC822	Hepatitis B Core Antigen (in house)

II. Method of Analysis

i. Histological Staining Programme

For H&E staining, all the slides were assessed according to the following criteria:

(1) Depth of nuclear staining
(2) Depth of cytoplasmic staining
(3) Nuclear differentiation
(4) Contrast of cytoplasmic staining
(5) Complete dehydration, clearing, mounting and labelling

Scores were given to each criterion as follows:

0 - Fail / Not Done
1 - Unsatisfactory
2 - Pass
3 - Good
4 - Excellent

Getting scores <10 in the H&E staining is considered unsatisfactory. Similar marking scheme but with a different set of criteria was used for special staining methods.

To ensure objectivity in assessing histological staining performance of individual laboratory, the highest and the lowest scores from the assessors were not counted in the calculation. The average of the remaining scores, rounding up to the nearest 0.5, constituted the final score which serves as the performance index for individual laboratory (Table 3).

ii. Immunohistochemical Staining Program

Similar to the special stain's scoring system, but with a different set of criteria, was adopted for the assessment of the immunohistochemical staining program (Table 4).

Table 3. Scoring system

	Scores given by Panel				Final
Participant	Member A	Member B	Member C	Member D	Score
X	12	13	15	10	12.5
Y	15	16	14	16	15.5

Table 4. Scoring System

Criteria	Scores
Intensity of positive staining	0-8
Background staining	0-6
Presence of uneven, patchy positive staining	0-2
Unnecessary chromogen deposits, dirt or stain	0-2
Nuclear counterstaining, dehydration, clearing, mounting and labelling	0-2

Emphasis was placed on crisp and intense positive staining with minimal or no background (good staining contrast), no uneven, no patchy staining nor other unnecessary deposits and with adequate nuclear counterstaining. Scoring <10 was considered as unsatisfactory.

III. Survey Analysis

i. Histological Staining Programme

a. Survey One

Twenty-one (91%) out of 23 laboratories returned the slides for assessment. All the laboratories produced acceptable H&E, Alcian Blue, Periodic Acid-Schiff and Southgate's Mucicarmine staining results. No particular pitfall were found in this survey.

b. Survey Two

Twenty (87%) laboratories returned the H&E, Kinyouins Cold Ziehl-Neelsen and Ziehl-Neelsen stained slides for assessment. They all produced acceptable staining results.

In the demonstration of acid fast bacilli, the Kinyouins Cold Ziehl-Neelsen Method was found to be as good as the Standard Ziehl-Neelsen Method. The Kinyouins Cold Ziehl-Neelsen Method may be a good choice for those laboratories which want to eliminate the fire hazard of the Ziehl-Neelsen method.

c. Survey Three

Nineteen laboratories (83%) return the H&E section for assessment. They all produced acceptable H&E staining.

In the demonstration of elastic fibres, 18 (78%) laboratories return the Taenzer-Unna Orcein stained section and 15 (65%) laboratories return the Humberstone's Victoria Blue stained section for assessment.

All the laboratories performed well in both techniques except one failed to stain the elastic fibres with the Humberstone's Victoria Blue method.

d. Survey Four

Nineteen (83%) laboratories returned the cytology material for assessment. Seventeen (90%) out of nineteen laboratories correctly diagnosed the specimen as the Herpes Simplex virus infection. One laboratory gave the diagnosis of suspicious squamous cell carcinoma with herpes and candida infection and one laboratory did not provide any diagnosis.

e. Consistency of H&E staining

All the H&E survey results in 1998 were compiled. Scores were transformed into percentage against full marks for comparison. Laboratory staining

Table 5. Stratification of Performance

Stratification	Criteria
Consistent	Scores deviated less than 10% among surveys
Deteriorating	Scores deviated greater than 10% in a declining trend
Fluctuating	Scores deviated greater than 10% among surveys
Improving	Scores deviated greater than 10% in a progressing trend
Unsatisfactory	Two or more survey scores less than 40%

performance was classified according to the criteria set in Table 5.

Table 6 showed the overall performance of the 23 participating laboratories in 1998. The results indicated that 8 (35%) had consistent performance, 4 (17%) was deteriorating, 3 (13%) showed fluctuation, 5 (22%) showed improvement, and 3 (13%) was unsatisfactory, since they did not return any survey materials for assessment.

Table 6. Performance Index of Histological Staining Program (H&E)

Performance	Participants (N=19)	Percentage
Consistent	8	35
Deteriorating	4	17
Fluctuating	3	13
Improving	5	22
Unsatisfactory	3	13

ii. Immunohistochemical Staining Programme

a. Survey One

Sixteen (94%) out of 17 laboratories returned the survey materials for assessment and three of them did not have their in-house equivalent. Most of the participating laboratories could demonstrate the 34bE12 antigen successfully.

For the antigen retrieval, various techniques were employed. Microwave treatment being the most popular (47%), followed by pressure cooking (35%) and enzymatic treatment (18%).

The medians of the provided and the in-house antibodies using EnVision detection system were both 6.5, whereas the median of the in-house antibody detected with Labelled Strept-Avidin Biotin/Avidin Biotin was 6.0.

b. Survey Two

Fifteen (88%) out of 17 laboratories returned the survey materials for assessment and two of them did not have their in-house equivalent. About half of the participating laboratories failed in the demonstration of the CD30 antigen with the provided antibodies, whereas only three of the laboratories failed with their in-house antibodies and linking system.

For the antigen retrieval, various techniques were employed. Microwave treatment being the most popular (53.5%), followed by pressure cooking (33.3%) and enzymatic treatment (13.3%).

The medians of the provided and the in-house antibodies using EnVision detection system were 4.5 and 5.0 respectively, whereas the median of the in-house antibody detected with Labelled Strept-Avidin Biotin/ Avidin Biotin was 5.5.

c. Survey Three

Fifteen (88%) out of 17 laboratories returned the survey materials for assessment and only one of them did not have their in-house equivalent. Most of the participating laboratories could demonstrate the CD3 antigen successfully.

For the antigen retrieval, various techniques were employed. 47% employed microwave treatment, 20% employed pressure cooking and 20% applied both hydrating heat method and enzymatic treatment simultaneously, the remaining 13% did not provide sufficient information.

The medians of the provided and the in-house antibodies using EnVision detection system were 6.0 and 5.0 respectively, the median of the in-house antibody detected with Labelled Strept-Avidin Biotin/Avidin Biotin was 6.5.

d. Survey Four

Fourteen (82%) laboratories returned the survey materials for assessment and six of them did not have their in-house equivalent. Most of the participating laboratories could demonstrate the HBcAg antigen successfully.

For the antigen retrieval, as usual microwave treatment being the most popular followed by pressure cooking and enzymatic treatment. Some participants however did not perform any antigen retrieval procedure.

The medians of all the three techniques were 6.0.

e. Consistency of Immunohistochemical Staining Performance Analysis

Similar definitions of laboratory performance as in the histological staining programme were adopted (Table 5). Table 7 shows the results of the performance analysis. The results indicated that 14(82%) with fluctuating performance, 1 (6%) was deteriorating and 2 (12%) showed unsatisfactory performance due to failure to return survey materials for assessment in more than 2 surveys.

Table 7. Performance Analysis of Immunohistochemical Staining Program

Performance	Participants (N=17)	Percentage
Consistent	0	0
Deteriorating	1	6
Fluctuating	14	82
Improving	0	0
Unsatisfactory	2	12

IV. Summary

i. Histological Staining Programme

For the performance consistency of H&E staining of individual laboratory, 35% was consistent, 17% was deteriorating, 13% was fluctuating, 22% was improving and 13% was unsatisfactory.

The Histochemical Staining Marks Analysis (Figure 1) shows that all the participants' performance was satisfactory in this year's surveys.

ii. Immunohistochemical Staining Program

For the performance consistency of immunohistochemical staining of individual laboratory, 82% was fluctuating, 6% was

deteriorating and 12% was unsatisfactory.

The Immunohistochemical Staining Marks Analysis (Figure 2) shows that over 50% of the participants' performance was satisfactory in this year's surveys except the CD30 demonstration using the EnVision detection system (★) of survey 2.

The comparison of the performance index (median) between the Envision detection system (EnV) and the in-house detection system (IN)(Figure 3) reveals that the median of EnV(☆)was in general lower than the median of IN (●). Presumably, the participants are more familiar/experience in their in-house detection system, which allows them to achieve better staining result.

Figure 1. Histochemical Staining Marks Analysis

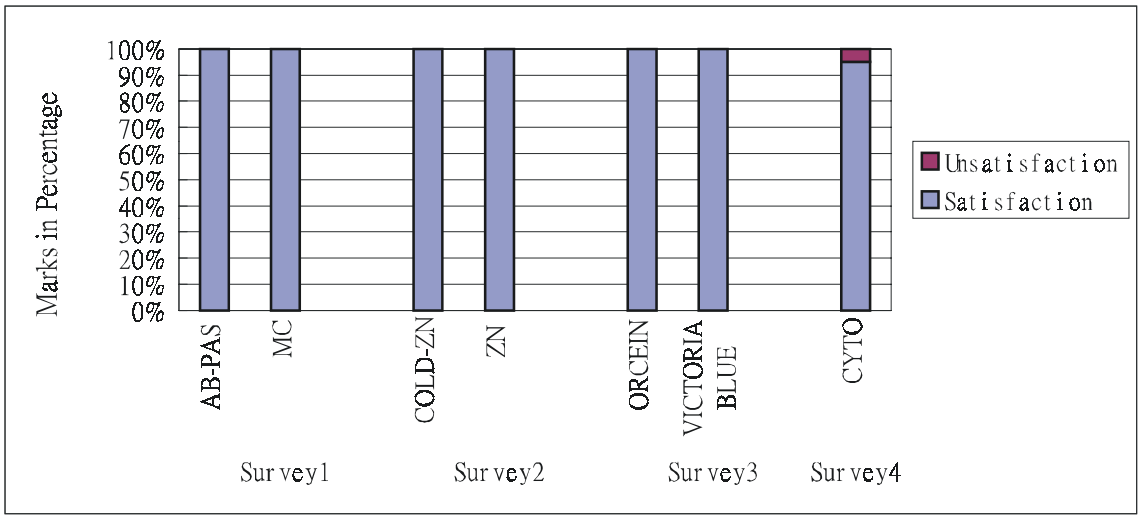


Figure 2. Immunohistochemical Staining Marks Analysis

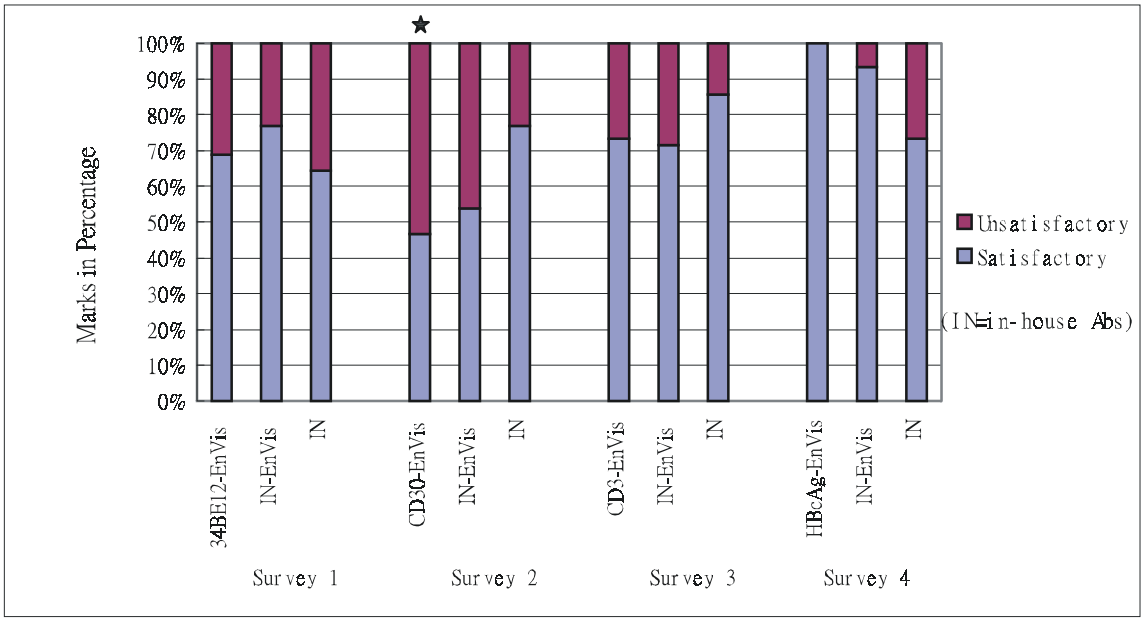
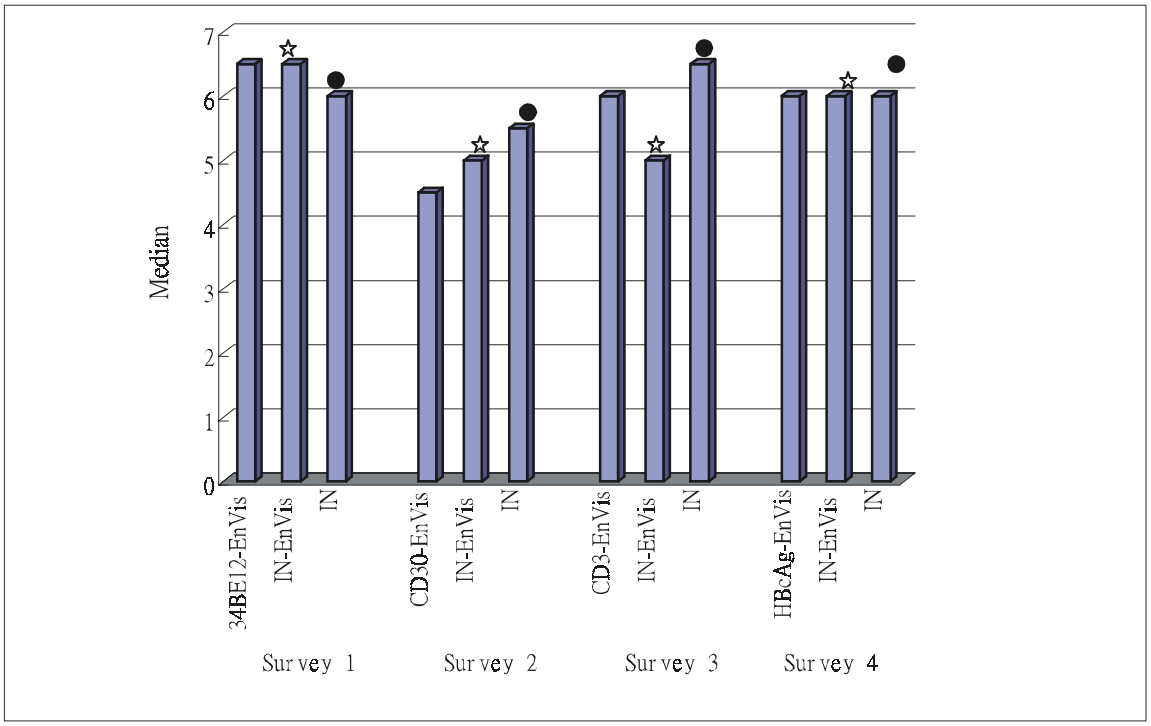


Figure 3. Comparison of the Performance Index (median)



References

1. Chiu KY, Li YW, Yau WL, Lo TH, Khoo CP, Ng HK, Chan KC, Leung CY, Chow TC and Leung KF. Histopathology and Cytology. In Hong Kong Medical Technology Association Quality Assurance Programme: Annual Report 1992; 41-53.

2. Dranoff G, Bigner DD. A word of caution in the use of neuron-specific enolase expression in tumor diagnosis. Arch Pathol Lab Med 1984; 108:535-540.

3. Esteban JM., Ahn C, Battifora H. Mehta P. Biologic significance of quantitative estrogen receptor immunohistochemical assay by image analysis in breast cancer. Am J Clin Pathol 1994; 102(2): 158-162.

4. Lo TH, Chiu KY, Li YW, Yau WL, Khoo CP, Ng HK, Chan KC, Leung CY, Chow TC and Leung KF. In Hong Kong Medical Technology Association Quality Assurance Programme: Annual Report 1993; 45-57.

5. Perentes E, Rubinstein LJ. Recent applications of immunoperoxidase histochemistry in human neuro-oncology. An update. Arch Pathol Lab Med 111 (9), 796-812 (1987) Arch Pathol Lab Med 1987; 111: 796-812.

6. Pollard K, Lunny D, Holgate CS, Jackson P, Bird CC. Fixation, processing, and immunochemical reagent effects on preservation of T-lymphocyte surface membrane antigens in paraffin-embedded tissue. J Histo Cyotchem 1987; 35 (11): 1329-1338.