3.0 RESULTS

3.1 Overview:

The fifty samples evaluated included FNA's from the lung, 24/50 (48%); liver, 23/50 (46%) and lymph node, 3/50 (6%). The immunocytochemistry stains consisted of 44/50 (88%) CK7; 44/50 (88%) CK20; 18/50 (36%) TTF1; 10/50 (20%) synaptophysin; 10/50 (20%) Hepar-1 and 7/50 (14%) AE1/3.

There was no overall agreement in preservation of cytomorphological detail and ICC staining between the two methods of sample preparation. The Pap stain on FNA smears was the best method for the evaluation of nuclear and morphologic characteristics. The ICC staining worked better in the cell block samples due to lack of background and aberrant staining. The same result was obtained when cell block samples that had a dedicated needle aspiration (n = 30) were compared to that of FNA samples.

3.2 Comparison of cellularity between FNA and cell block samples

A poor agreement (K – statistic = -0.01) and a statistically significant difference (p-value = 0.00), was obtained in the assessment of cellularity between the two methods of sample preparation. Conventionally prepared (FNA smears) had higher cellularity assessment grading scores than cell block samples. This was illustrated in the symmetry matrix (figure 3.2.1) by a dominant deviation of FNA samples from the diagonal in comparison to the cell block samples. No FNA samples (0/50) scored zero for cellularity but 8% (4/50) of cell block samples were acellular. Of these samples, 6% (3/50) were obtained by rinsing the needle and the hub of the syringe and 2% (1/50) were obtained by performing a dedicated needle aspiration for the cell block sample. One paired FNA sample (of the 4 acellular cell block samples), scored 1+ for cellularity and the other 3 samples scored 2+. There were a

total of 17 samples of FNA smears and cell blocks in grading cateogory score 2+. Only 29% (5/17) samples scored 2+ for both FNA smears and cell blocks. The remainder (12/17) samples scored 2+ for the assessment of cellularity of cell block samples while their paired FNA samples scored 3+. Low cellularity (score 1+) was obtained for 38% (19/50) of total cell block samples whereas that of the FNA samples was only 10% (5/50). Of further note is that more (90%) total FNA samples (45/50) displayed a higher score (score 2+ and 3+) for cellularity than cell block samples, 54% (27/50). This trend is further displayed in Figure 3.2.3 where the dissimilarity is illustrated in the distribution of the 50 samples across the various grading score categories.

A similar result, a poor agreement (K – statistic = -0.01) and a statistically significant difference (p-value = 0.01), was obtained when the cellularity in the cell block samples that had a dedicated needle pass (n = 30) was compared with that of FNA samples. Conventionally prepared (FNA smears) had higher cellularity assessment grading scores than cell block samples. This was illustrated in the symmetry matrix (figure 3.2.2) by a dominant deviation of FNA samples from the diagonal in comparison to the cell block samples. There were no FNA samples (0/30) that scored zero for cellularity but 3.3% (1/30) of cell block samples with a dedicated needle aspiration was acellular. The respective paired FNA sample scored 2+. Low cellularity (score 1+) was obtained for 33.3% (10/30) of these cell block samples whereas that of FNA samples were only 13.3% (4/30). Thirteen cell block samples scored 2+. In 31% (4/13) of these samples both FNA and cell block samples scored 2+ while the 9/13 cell block samples that scored 2+ for cellularity had their paired FNA samples score 3+. More FNA samples (87%) (26/30) also displayed a higher score (score 2+ and 3+) for cellularity than cell block samples (63%) (19/30) with a dedicated

needle pass. This trend is further displayed in Figure 3.2.4 where the dissimilarity is illustrated in the distribution of the 50 samples across the various grading score categories.

FNA Cellularity	Cell Block (CB) Cellularity SCORES				
	0	1+	2+	3+	**Total FNA's**
SCORES					
0	0	0	0	0	**0**
1+	1	2	0	2	**5**
2+	3	7	5	2	**17**
3+	0	10	12	6	**28**
Total CB	**4**	**19**	**17**	**10**	**50**

FNA fine needle aspiration, CB cell block
0 = absent, 1+ = low (tumour cells represent <10% of present), 2+ = moderate (tumour cells represent 10-50% of cells present), 3+ = high (tumour cells represent >50% of cells present).
Scores shaded blue = diagonal; sum of numbers shaded yellow (34) = FNA samples; sum of numbers shaded purple (4) = cell block samples

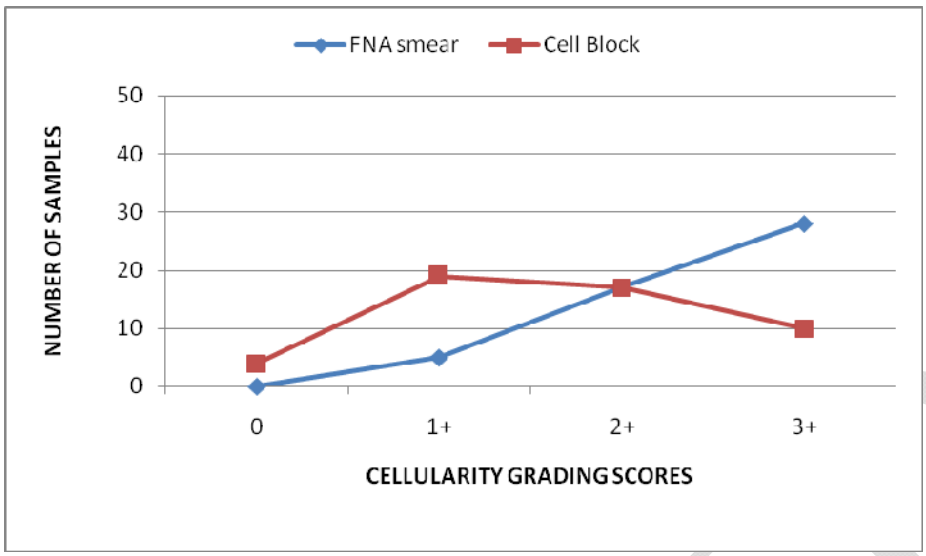
Figure 3.2.1 Symmetry matrix for cellularity of FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse (n = 50).

(K– statistic = -0.01; p-value = 0.00).

FNA Cellularity	Cell Block Cellularity SCORES				
	0	1+	2+	3+	**Total FNA's**
SCORES					
0	0	0	0	0	**0**
1+	0	2	0	2	**4**
2+	1	5	4	1	**11**
3+	0	3	9	3	**15**
Total CB	1	10	13	6	30

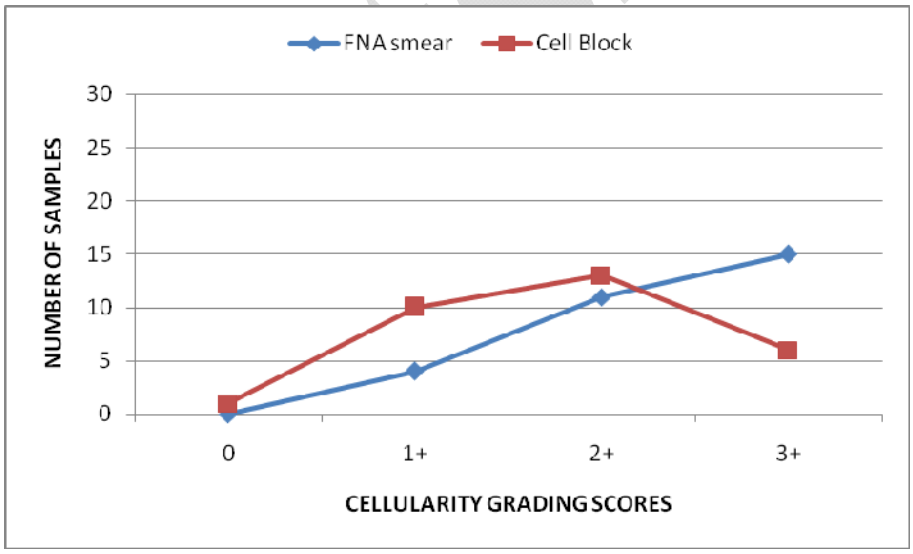
FNA fine needle aspiration, CB cell block
0 = absent, 1+ = low (tumour cells represent <10% of present), 2+ = moderate (tumour cells represent 10-50% of cells present), 3+ = high (tumour cells represent >50% of cells present).
Scores shaded blue = diagonal; sum of numbers shaded yellow (18) = FNA samples; sum of numbers shaded purple (3) = cell block samples.

Figure 3.2.2 Symmetry matrix for cellularity of FNA and cell block samples with dedicated aspiration (n = 30). (K – statistic = -0.01; p-value = 0.01).



FNA fine needle aspiration, CB cell block
0 = absent, 1+ = low (tumour cells represent <10% of present),
2+ = moderate (tumour cells represent 10-50% of cells present),
3+ = high (tumour cells represent >50% of cells present) (K = -0.01; p-value = 0.00)

Figure 3.2.3 Comparison of cellularity grading scores between FNA with dedicated aspiration and cell block samples with either dedicated aspiration or needle rinse (n = 50). (K– statistic = -0.01; p-value = 0.00).



FNA fine needle aspiration, CB cell block
0 = absent, 1+ = low (tumour cells represent <10% of present), 2+ = moderate (tumour cells represent 10-50% of cells present), 3+ = high (tumour cells represent >50% of cells present) (K = -0.01; p = 0.01)

Figure 3.2.4 Comparison of cellularity grading scores between FNA and cell block samples with dedicated aspiration (n = 30). (K–statistic = -0.01; p-value = 0.01).

3.3 COMPARISON OF MORPHOLOGICAL PRESERVATION BETWEEN FNA AND CELL BLOCK SAMPLES

In FNA samples, morphology was well preserved in 100% (50/50) of samples compared to 88% (44/50) in cell block samples (K = 0.00; p = 0.01). A similar result was obtained when the assessment of morphology was compared in the cell block samples that had a dedicated pass (n=30) with that of FNA samples (K = 0.00; p = 0.08). All FNA samples (100%) displayed the presence of morphological preservation (score 1+) compared to 90% (27/30) cell block samples a with dedicated aspiration.

FNA MP	**Cell Block Morphological Preservation (MP) SCORES**		
	0	1+	**Total FNA's**
SCORES			
0	0	0	**0**
1+	6	44	**50**
Total CB	**6**	**44**	**50**

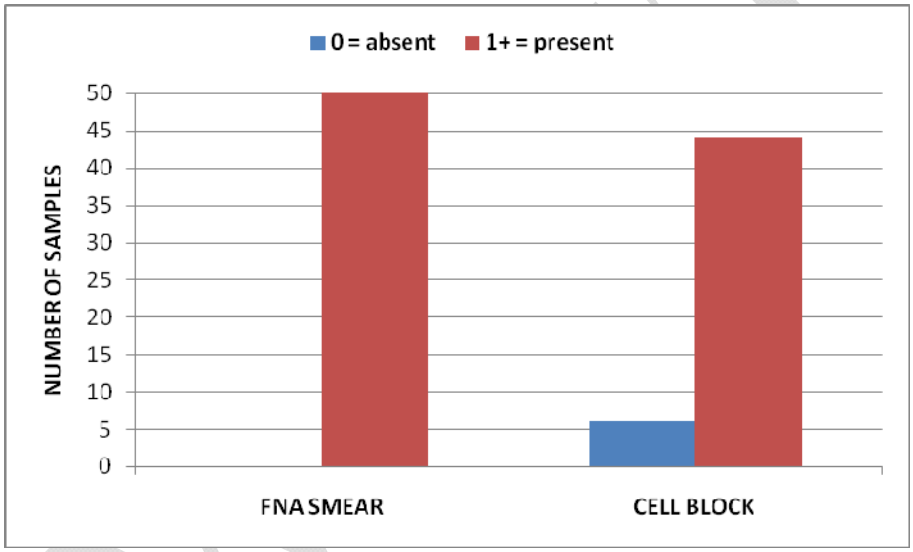
FNA fine needle aspiration, CB cell block; 0 = poorly preserved, 1+ = well preserved

Figure 3.3.1 Symmetry matrix for morphological preservation (of nuclear and cellular detail) of FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse (n = 50). (K-statistic 0.00; p-value 0.01).

FNA MP	Cell Block Morphological Preservation (MP) SCORES		
	0	1+	Total FNA'S
SCORES			
0	0	0	**0**
1+	3	27	**30**
Total CB	**3**	**27**	**30**

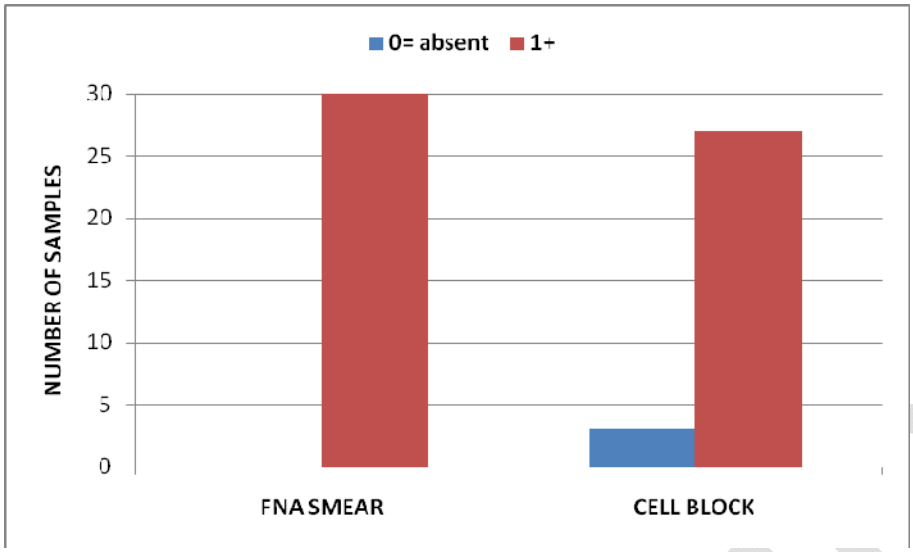
FNA fine needle aspiration, CB cell block; 0 = poorly preserved, 1+ = well preserved

Figure 3.3.2 Symmetry matrix for morphological preservation (of nuclear and cellular detail) of FNA and cell block samples with dedicated aspiration (n = 30). (K-statistic 0.00; p-value 0.08).



FNA fine needle aspiration, CB cell block; 0 = poorly preserved, 1+ = well preserved
(K = 0.00; p = 0.01).

Figure 3.3.3 Comparison of morphological preservation grading scores of FNA with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (n = 50). (K-statistic 0.00; p-value 0.01).



FNA fine needle aspiration, CB cell block; 0 = poorly preserved, 1+ = well preserved (K = 0.00; p = 0.08).

Figure 3.3.4 Comparison of morphological preservation grading scores of FNA and cell block samples with dedicated aspiration (n = 30). (K-statistic 0.00; p-value 0.08).

3.4 COMPARISON OF ARCHITECTURAL PRESERVATION BETWEEN FNA AND CELL BLOCK SAMPLES

All FNA samples (50/50) displayed architectural preservation compared to only 44% (22/50) cell block samples. (K = 0.00; p = 0.00). In the majority (56%) of the cell block samples architectural preservation was absent. A similar result was obtained when the preservation of architecture was compared in the cell block samples that had a dedicated needle pass (n=30) with that of FNA samples. (K = 0.00; p = 0.00). All FNA samples (30/30) displayed architectural preservation compared to 57% (17/30) cell block samples. Architectural preservation was absent in 43% (13/30) cell block samples.

FNA AP	**Cell Block Architectural Preservation (AP) SCORES**		
	0	1+	**Total FNA'S**
SCORES			
0	0	0	**0**
1+	28	22	**50**
Total CB	**28**	**22**	**50**

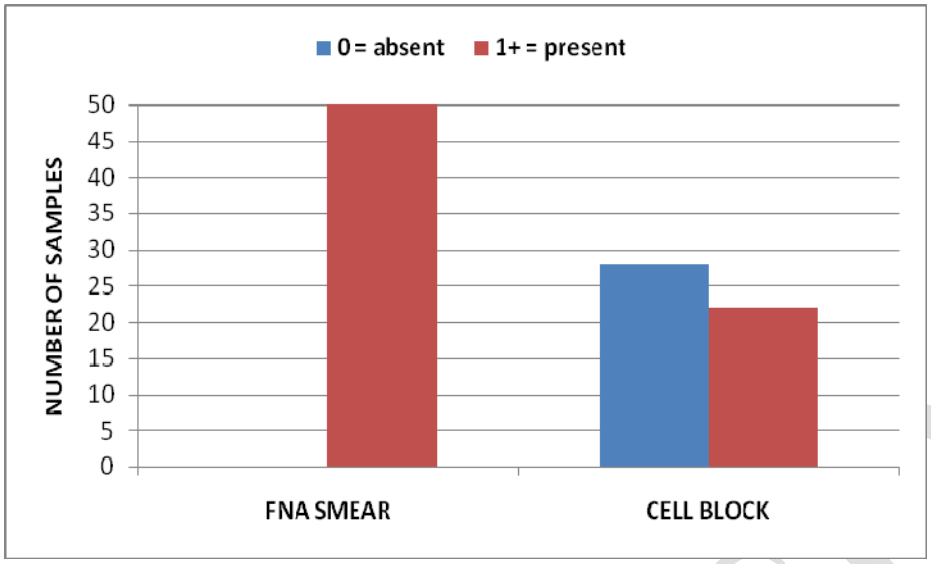
(FNA fine needle aspiration, 0 = absent architectural preservation; 1+ = architectural preservation present)

Figure 3.4.1 Symmetry matrix for architectural preservation (AP) of FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse (n = 50). (K-statistic 0.00; p-value 0.00).

FNA AP	**Cell block Architectural Preservation (AP) SCORES**		
	0	1+	**Total FNA'S**
SCORES			
0	0	0	**0**
1+	13	17	**30**
Total CB	**13**	**17**	**30**

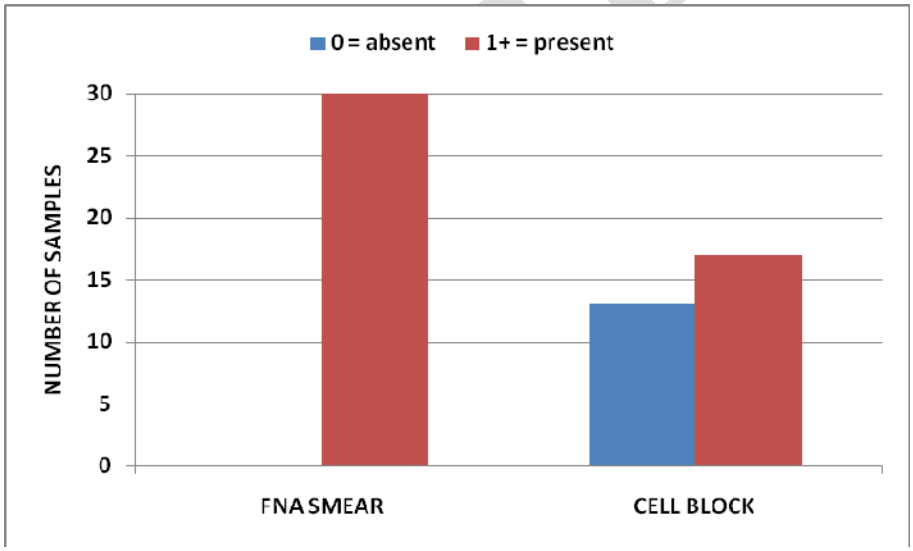
(0 = absent architectural preservation; 1+ = architectural preservation present**)**

Figure 3.4.2 Symmetry matrix for architectural preservation of FNA and cell block samples with dedicated aspiration (n = 30). (K-statistic 0.00; p-value 0.00).



(0 = absent architectural preservation , 1+ = architectural preservation present)

Figure 3.4.3 Comparison of architectural preservation grading scores of FNA with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (n = 50). (K = 0.00; p = 0.00).



(0 = absent architectural preservation , 1+ = architectural preservation present)

Figure 3.4.4 Comparison of architectural preservation grading scores of FNA and cell block samples with dedicated aspiration (n = 30). (K = 0.00; p = 0.00).

3.5 COMPARISON OF IMMUNOSTAINS BETWEEN FNA AND CELL BLOCK SAMPLES

Table 3.5.1 Comparison of number of FNA samples (with dedicated aspiration) and cell block samples (with either dedicated aspiration or needle rinse) in respective ICC grading score categories.

	CK7		CK20		TTF1		SYN		HEP-1		AE1/3	
Scores	FNA	CB	FNA	CB	FNA	CB	FNA	CB	FNA	CB	FNA	CB
0	6	15	35	41	11	10	4	5	5	7	0	1
1	3	8	3	0	1	1	0	1	1	0	0	1
2	3	6	3	1	2	6	2	2	1	0	0	1
3	4	0	0	0	0	0	0	0	0	0	1	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	6	7	2	1	1	0	1	0	1	0	0	2
6	22	8	1	1	3	1	3	2	0	1	6	2
n =	44	44	44	44	18	18	10	10	8	8	7	7
K	0.22		0.20		0.18		0.57		0.09		-0.14	
p-value	0.02		0.14		0.36		0.39		0.41		0.31	

FNA fine needle aspirate, CB cell block, SYN synaptophysin.
0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity, 2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity

3.5.1 Overview

Overall, there was a poor agreement between immunostains on FNA smears and cell block samples. There was a poor agreement (K-statistic 0.22) in the CK7 immuno-stain which was the only test that displayed a statistically significant difference (p-value 0.02) between methods. A statistically significant difference was not obtained in immunocytochemical staining between the two methods of sample preparation for the following tests: CK20, TTF-1, Synaptophysin, Hep1 and AE1/3. This could be due to the small sample size of the respective tests: AE1/3, n =7, Hep-1, n =8, synaptophysin, n =10 and TTF1, n = 18.

The asymmetry obtained was a random event. Although an adequate sample size (n =44) was obtained for CK20, the asymmetry (K- statistic 0.20) lacked statistical significance (p-value 0.14).

Table 3.5.2 Comparison of number of FNA and cell block samples (with dedicated aspiration) in respective ICC grading score categories.

	CK7		CK20		TTF1		SYN		HEP-1		AE1/3	
Scores	FNA	CB	FNA	CB	FNA	CB	FNA	CB	FNA	CB	FNA	CB
0	5	11	20	25	6	6	3	3	5	7	0	1
1	3	5	3	0	1	0	0	1	1	0	0	0
2	2	5	2	1	0	2	1	1	1	0	0	1
3	2	0	0	0	0	0	0	0	0	0	1	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	4	4	2	1	0	0	0	0	1	0	0	2
6	12	3	1	1	1	0	3	2	0	1	4	1
n =	28	28	28	28	8	8	7	7	8	8	5	5
K	0.27		0.20		0.14		0.58		0.17		-0.19	
p-value	0.12		0.20		0.39		0.37		0.39		0.28	

FNA fine needle aspirate, CB cell block, SYN synaptophysin.
0 = negative / absent staining,
1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity

3.5.2 Overview:

Similar analyses was performed on FNA samples (obtained by dedicated aspiration) and cell block samples (with a dedicated aspiration). Since none of the immunostains displayed a statistically significant difference between the two methods of sample preparation; the moderate agreement obtained for the synaptophysin immunostain and the poor agreement obtained for the rest of the immunostains was a random event. This could be due to the small sample size of the respective tests since only a small number of cases in this study warranted those tests.

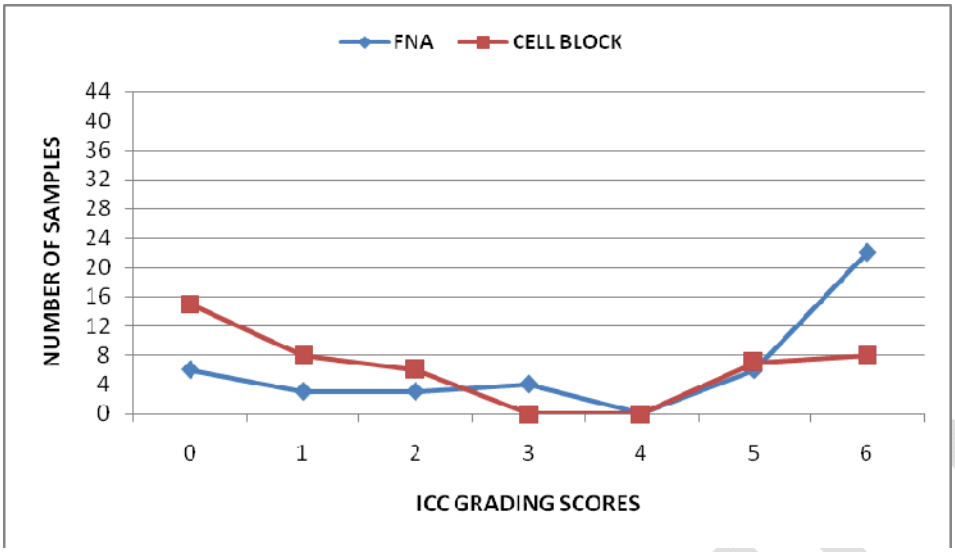
3.5.3 CK7 Immunostain

There was a poor agreement (K-statistic 0.22) in the CK7 immuno-stain which was the only test that displayed a statistically significant difference (p-value 0.02) between methods. Samples prepared conventionally (FNA smears) (28/44) had higher ICC grading scores (score 5 & 6) in CK7 than cell block samples (15/44). This was illustrated in the symmetry matrix (Figure 3.5.3.1) by a dominant deviation of FNA samples from the diagonal in comparison to the cell block samples. Of note, is the higher number (15/44) of cell block samples in the negative (score 0) category. CK7 was performed on 44 samples. Of these, 34% (15/44) were negative (score 0) in the cell block sample and only 13.6% (6/44) in their paired FNA samples.

	CELL BLOCK CK7 SCORES						
FNA CK7	0	1	2	3	5	6	**Total FNA'S**
SCORES							
0	6	0	0	0	0	0	**6**
1	1	2	0	0	0	0	**3**
2	1	0	2	0	0	0	**3**
3	1	2	0	0	1	0	**4**
5	2	2	0	0	0	2	**6**
6	4	2	4	0	6	6	**22**
Total CB	**15**	**8**	**6**	**0**	**7**	**8**	**44**

FNA fine needle aspiration, CB cell block
0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity, 2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity

Figure 3.5.3.1 Symmetry matrix for CK7 immunostain in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K-statistic 0.22; p-value 0.02).



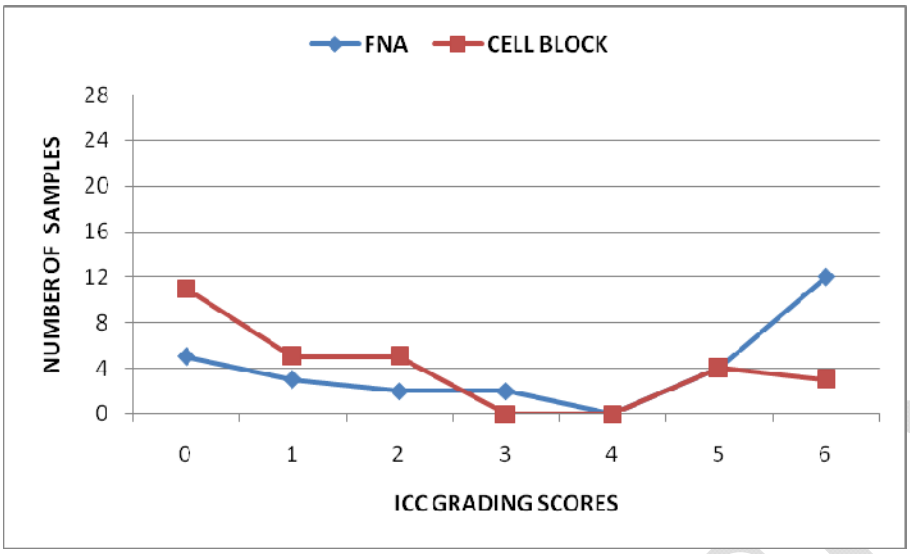
FNA fine needle aspiration, CB cell block
0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity, 2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity

Figure 3.5.3.2 Comparison of CK7 immunostain grading scores of FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K = 0.22; p = 0.02).

	CELL BLOCK CK7						
	SCORES						
FNA CK7	0	1	2	3	5	6	Total FNA'S
SCORES							
0	5	0	0	0	0	0	**5**
1	1	2	0	0	0	0	**3**
2	0	0	2	0	0	0	**2**
3	0	1	0	0	1	0	**2**
5	2	1	0	0	0	1	**4**
6	3	1	3	0	3	2	**12**
Total CB	11	5	5	0	4	3	28

FNA fine needle aspiration, CB cell block
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity, 2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity)

Figure 3.5.3.3 Symmetry matrix for CK7 immunostain in FNA and cell block (CB) samples with a dedicated aspiration. (K = 0.27; p = 0.12).



FNA fine needle aspiration, CB cell block
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity, 2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity)

Figure 3.5.3.4 Comparison of CK7 immunostain grading scores of FNA samples and cell block sample with a dedicated aspiration. (K = 0.27; p = 0.12).

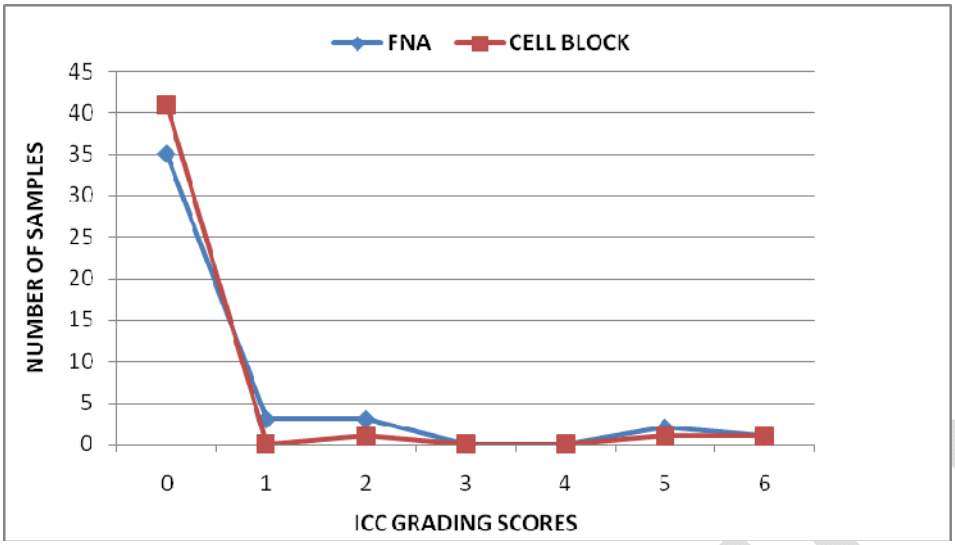
3.5.4 CK 20 Immunostain

A statistically significant difference was not obtained in immunocytochemical staining between the two methods of sample preparation for the CK20 immunostain. Although there was an adequate sample size (n =44), the asymmetry (K- statistic 0.20) lacked statistical significance (p-value 0.14) (Figures 3.5.4 1and 3.5.4.2). FNA samples had higher CK20 immunostain grading scores as depicted in the symmetry matrix (Figure 3.4.5) by a dominant deviation of FNA samples from the diagonal in comparison to the cell block samples but this difference lacked statistical significance. The distribution of the samples across the various ICC grading score categories for CK20 is displayed in figure 3.4.6. The same trend was observed when CK20 immunostaining was compared in the cell block samples that had a dedicated needle pass (n = 28) with that of FNA samples. (Figures 3.5.4.3 and Figure 3.5.4.4; p = 0.20; K = 0.20).

	CELL BLOCK CK20 SCORES					
FNA CK20	0	1	2	5	6	**Total FNA's**
SCORES						
0	35	0	0	0	0	**35**
1	3	0	0	0	0	**3**
2	3	0	0	0	0	**3**
5	0	0	1	0	1	**2**
6	0	0	0	1	0	**1**
Total CB	**41**	**0**	**1**	**1**	**1**	**44**

FNA fine needle aspiration, CB cell block
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity, 2+ = focal moderate intensity 10-50% of tumour cells showing positivity, 3+ = focal strong intensity > 50% of tumour cells showing positivity, 4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity)

Figure 3.5.4.1 Symmetry matrix for CK20 immunostain in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K-statistic 0.20; p-value 0.14).



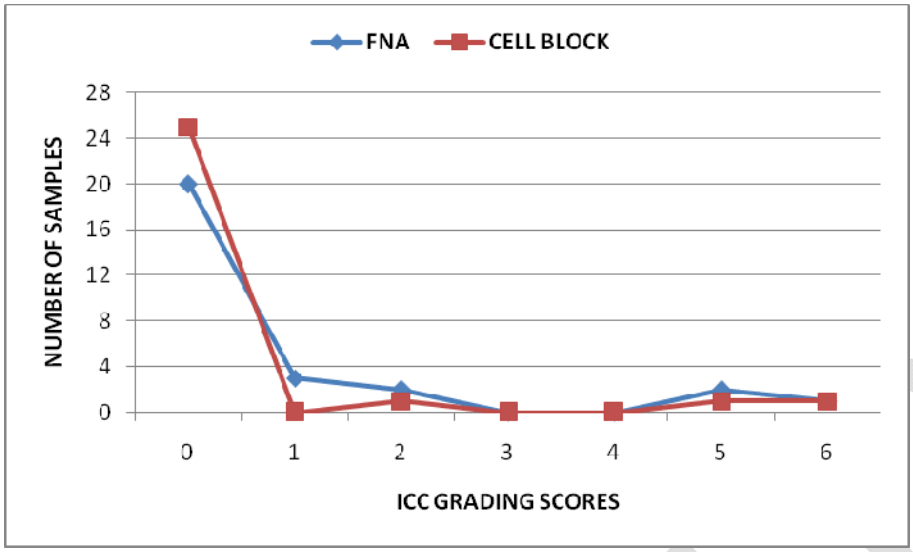
FNA fine needle aspiration, CB cell block
0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity, 2+ = focal moderate intensity 10-50% of tumour cells showing positivity, 3+ = focal strong intensity > 50% of tumour cells showing positivity, 4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity

Figure 3.5.4.2 Comparison of CK20 immunostain grading scores of FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K-statistic 0.20; p-value 0.14).

	CELL BLOCK CK20 SCORES					
FNA CK20	0	1	2	5	6	**Total FNA's**
SCORES						
0	20	0	0	0	0	**20**
1	3	0	0	0	0	**3**
2	2	0	0	0	0	**2**
5	0	0	1	0	1	**2**
6	0	0	0	1	0	**1**
Total CB	25	0	1	1	1	28

FNA fine needle aspiration, CB cell block
0 = negative / absent staining,
1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity

Figure 3.5.4.3 Symmetry matrix for CK20 immunostain in FNA and cell block (CB) samples with a dedicated aspiration (K = 0.20; p = 0.20).



FNA fine needle aspiration, CB cell block
0 = negative / absent staining,
1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity)

Figure 3.5.4.4 Comparison of CK20 immunostain grading scores of FNA and cell block sample with a dedicated aspiration (K = 0.20; p = 0.20).

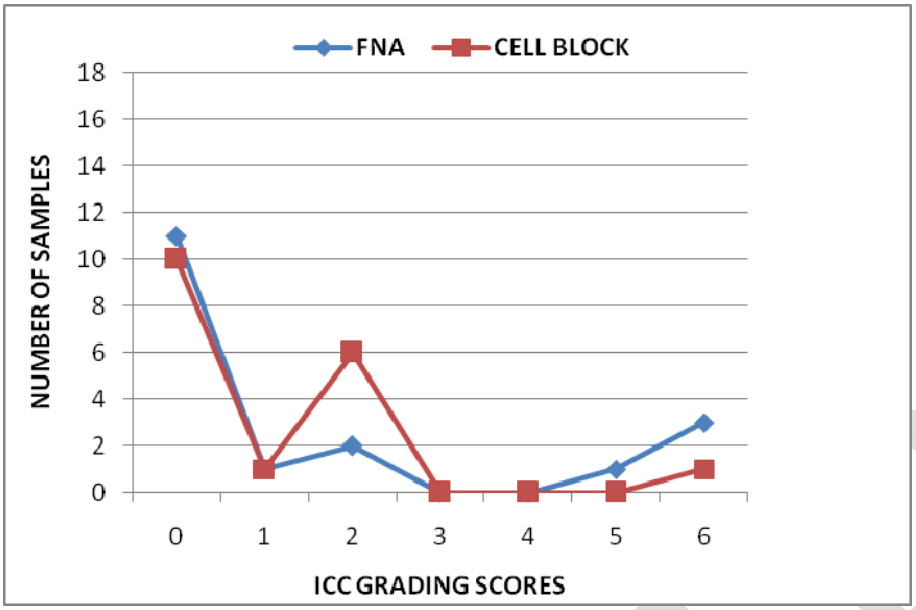
3.5.5 TTF1 IMMUNOSTAIN

A poor agreement (K – statistic = 0.18) between the two methods of sample preparation was obtained in the assessment of TTF1 staining but the discordance was a random event and not statistically significant (p-value = 0.36) (Figures 3.5.5.1 and Figure 3.5.5.2). Although FNA samples had slightly higher TTF1 immunostain grading scores as depicted in the symmetry table (Figure 3.5.5.1) by a dominant deviation of FNA samples from the diagonal (shaded blue) in comparison to the cell block samples,this difference lacked statistical significance. The distribution of the samples across the various ICC grading score categories for TTF1 is displayed in figure 3.5.5.2. The same trend was observed when the TTF1 staining was compared in the cell block samples that had a dedicated pass (n =8) with that of FNA samples. (K = 0.14; p = 0.39 Figures 3.5.5.3 and 3.5.5.4).

	CELL BLOCK TTF1 SCORES					
FNA TTF1	**0**	**1**	**2**	**5**	**6**	**Total FNA's**
SCORES						
0	8	1	2	0	0	**11**
1	1	0	0	0	0	**1**
2	1	0	1	0	0	**2**
5	0	0	0	0	1	**1**
6	0	0	3	0	0	**3**
Total CB	**10**	**1**	**6**	**0**	**1**	**18**

FNA fine needle aspiration, CB cell block
(0 = negative / absent staining,
1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity)

Figure 3.5.5.1 Symmetry matrix for TTF1immunostain in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K – statistic 0.18; p-value 0.36).



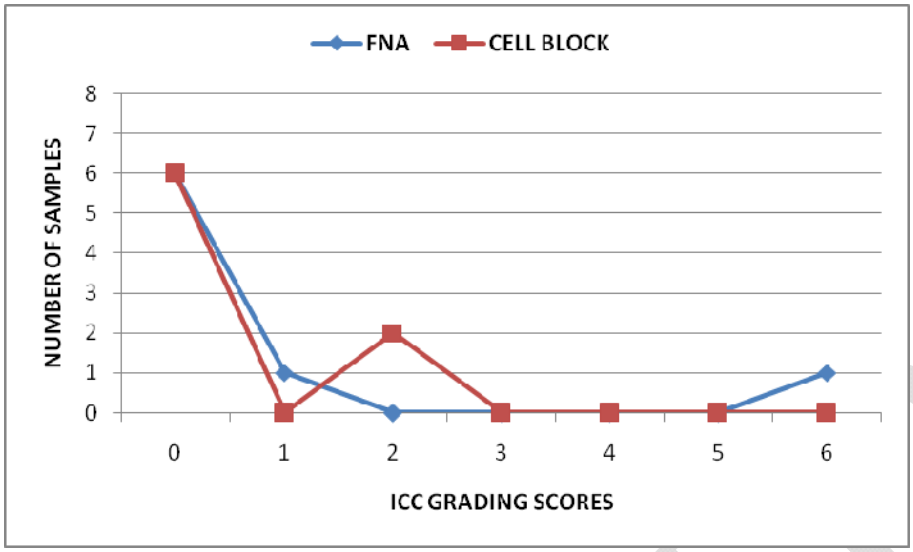
FNA fine needle aspiration, CB cell block
(0 = negative / absent staining,
1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.5.2 Comparison of TTF1immunostain grading scores of FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K-statistic 0.18; p-value 0.36).

	CELL BLOCK TTF1				
	SCORES				
FNA TTF1	0	1	2	6	**Total FNA's**
SCORES					
0	5	0	1	0	**6**
1	1	0	0	0	**1**
2	0	0	0	0	**0**
6	0	0	1	0	**1**
Total CB	6	0	2	0	8

FNA fine needle aspiration, CB cell block
(0 = negative / absent staining,
1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.5.3 Symmetry matrix for TTF1 immunostain in FNA and cell block (CB) samples with a dedicated aspiration. (K-statistic 0.14; p-value 0.39)



FNA fine needle aspiration, CB cell block
(0 = negative / absent staining,
1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.5.4 Comparison of TTF1 immunostain grading scores of FNA and cell block sample with a dedicated aspiration (K-statistic 0.14; p-value 0.39).

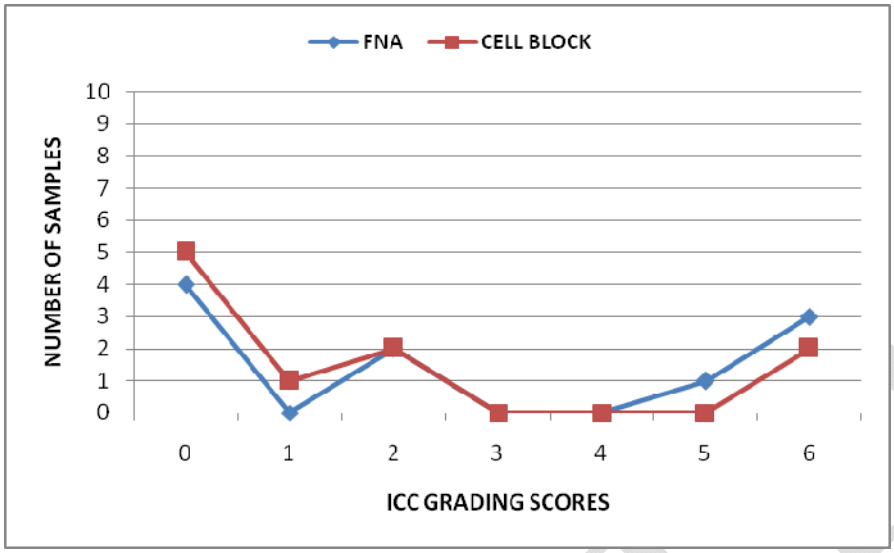
3.5.6 SYNAPTOPHYSIN IMMUNOSTAIN

There was no statistically significant difference (p = 0.39) in the comparison of synaptophysin (syn) immunostains between the two methods of sample preparation which could be due to the small sample size (n =10). The poor symmetry obtained (K = 0.57) was a random event (Figure 3.5.6.1 and 3.5.6.2). Although FNA samples had slightly higher immunostain grading scores for synaptophysin as depicted in the symmetry table (figure 3.5.6.1 by a dominant deviation of FNA samples from the diagonal (shaded blue) in comparison to the cell block samples, this difference lacked statistical significance. The distribution of the samples across the various ICC grading score categories for synaptophysin is displayed in figure 3.5.6.2. The same trend was observed when the synaptophysin staining was compared in the cell block samples that had a dedicated pass (n = 7) with that of FNA samples. (K = 0.58; p = 0.37 Figures 3.5.6.3 and 3.5.6.4).

	CELL BLOCK Syn				
	SCORES				
FNA Syn	0	1	2	5	6	**Total FNA's**
SCORES						
0	4	0	0	0	0	**4**
1	0	0	0	0	0	**0**
2	0	1	1	0	0	**2**
5	1	0	0	0	0	**1**
6	0	0	1	0	2	**3**
Total CB	5	1	2	0	2	10

FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.6.1 Symmetry matrix for synaptophysin (syn) immunostain in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K-statistic 0.57; p-value 0.39).



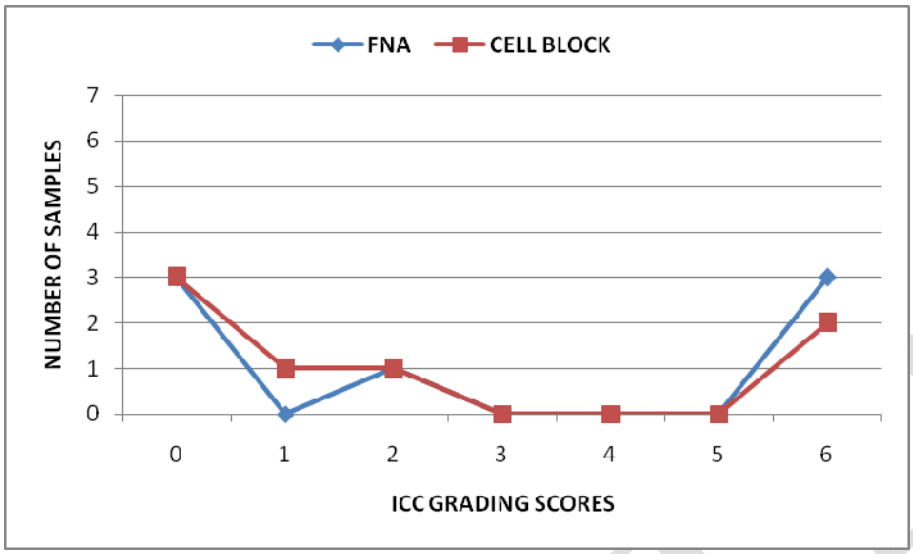
FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.6.2 Comparison of synaptophysin immunostain grading scores of FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K-statistic 0.57; p-value 0.39).

	CELL BLOCK Syn SCORES				
FNA Syn	0	1	2	6	Total FNA's
SCORES					
0	3	0	0	0	3
1	0	0	0	0	0
2	0	1	0	0	1
6	0	0	1	2	3
Total CB	3	1	1	2	7

FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.6.3 Symmetry matrix for synaptophysin (syn) immunostain in FNA and cell block (CB) samples with a dedicated aspiration. (K-statistic 0.58; p-value 0.37).



FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.6.4 Comparison of synaptophysin (syn) immunostain grading scores of FNA and cell block sample with a dedicated aspiration (K-statistic 0.58; p-value 0.37).

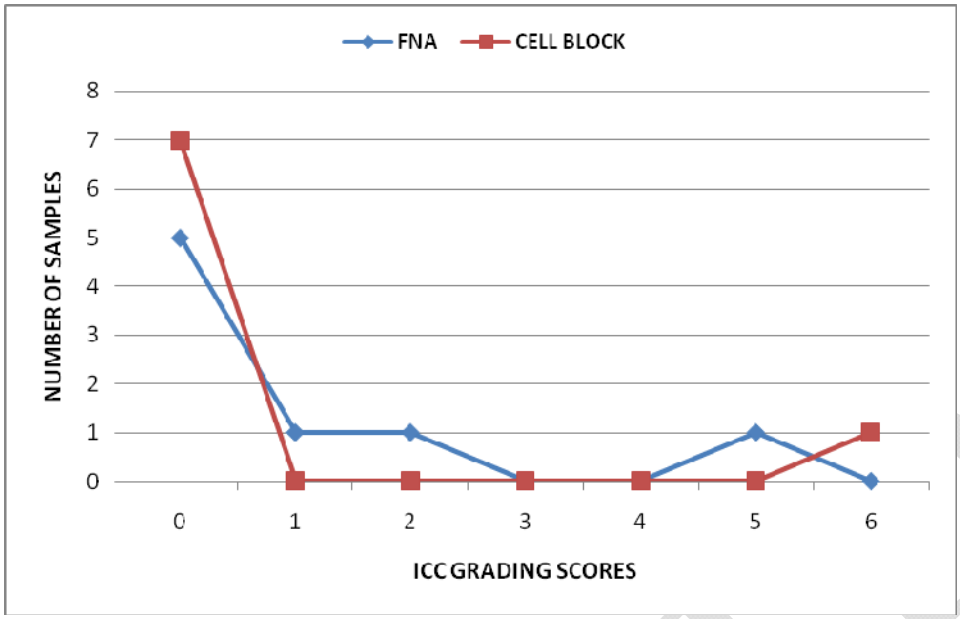
3.5.7 HEPAR-1 IMMUNOSTAIN

The poor symmetry (K = 0.09) obtained in the comparison of Hepar-1 (Hep-1) immunostains between the two methods of sample preparation was a random event which lacked statistical significance (p = 0.41). This could have been due to the small sample size (n =10). As depicted in the symmetry matrix (Figure 3.5.7.1), both FNA and cell block samples displayed the same deviation score from the diagonal. The distribution of the samples across the various ICC grading score categories for Hep-1 is displayed in Figure 3.5.7.2. The same trend was observed when the Hep-1staining was compared in the cell block samples that had a dedicated pass (n = 8) with that of FNA samples. (K-statistic 0.17; p-value 0.39 Figures 3.5.7.3 and Figure 3.5.7.4).

	CELL BLOCK Hep1				
	SCORES				
FNA Hep1	0	1	2	5	6	**Total FNA's**
SCORES						
0	6	0	0	0	1	**7**
1	1	0	0	0	0	**1**
2	1	0	0	0	0	**1**
5	0	0	0	0	1	**1**
6	0	0	0	0	0	**0**
Total	8	0	0	0	2	10

FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.7.1 Symmetry matrix for hepar-1 (hep-1) immunostain in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K-statistic 0.09; p-value 0.41).



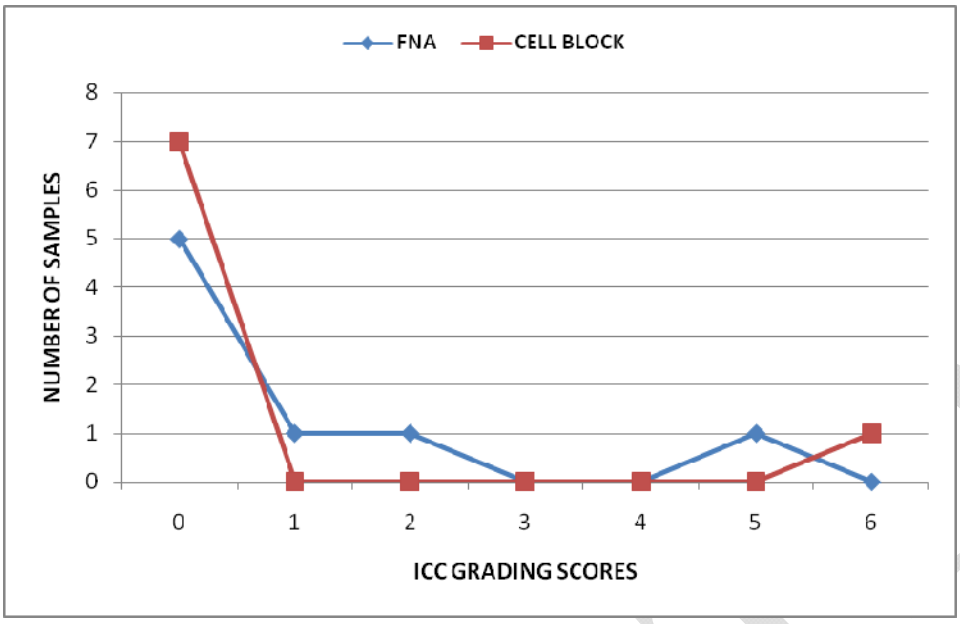
FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.7.2 Comparison of hepar-1 (hep-1) immunostain grading scores of FNA samples with dedicated aspiration and cell block samples with either dedicated aspiration or needle rinse (K-statistic 0.09; p-value 0.41).

	CELL BLOCK Hep-1 SCORES					
FNA Hep-1	0	1	2	5	6	**Total FNA's**
SCORES						
0	5	0	0	0	0	**5**
1	1	0	0	0	0	**1**
2	1	0	0	0	0	**1**
5	0	0	0	0	1	**1**
6	0	0	0	0	0	**0**
Total CB	7	0	0	0	1	8

FNA fine needle aspiration, CB cell block, SYN synaptophysin
0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity

Figure 3.5.7.3 Symmetry matrix for hepar-1 (hep-1) immunostain in FNA and cell block (CB) samples with a dedicated aspiration. (K-statistic 0.17; p-value 0.39)



FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.7.4 Comparison of hepar-1 (hep-1) immunostain grading scores of FNA and cell block sample with a dedicated aspiration (K-statistic 0.17; p-value 0.39).

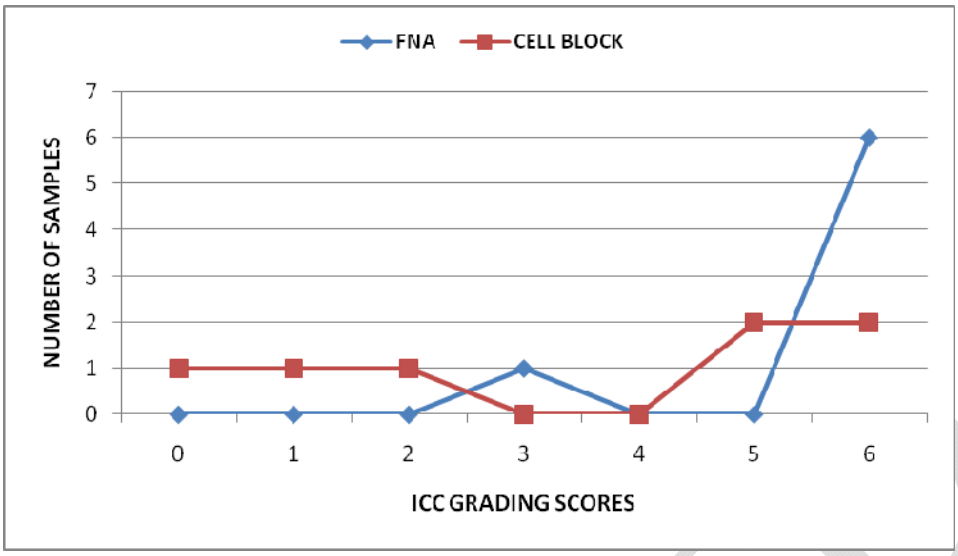
3.5.8 AE1/3 IMMUNOSTAIN

The comparison of AE1/3 immunostains between the two methods of sample preparation did not display any statistically significant difference (p = 0.31), which could have been due to the small sample size (n = 7). The poor symmetry obtained (K = - 0.14) was a random event. Although FNA samples had higher immunostain grading scores for AE1/3 as depicted in the symmetry table (Figure 3.5.8.1) by a dominant deviation of FNA samples from the diagonal in comparison to the cell block samples, this difference lacked statistical significance. The distribution of the samples across the various ICC grading score categories for AE1/3 is displayed in Figure 3.5.8.2. The same trend was observed when the AE1/3 staining was compared in the cell block samples that had a dedicated pass (n = 5) with that of FNA samples. (K-statistic -0.19; p-value 0.28, Figures 3.5.8.3 and 3.5.8.4).

	CELL BLOCK AE 1/3						
	SCORES						
FNA AE1/3	0	1	2	3	5	6	**Total FNA's**
SCORES							
0	0	0	0	0	0	0	**0**
1	0	0	0	0	0	0	**0**
2	0	0	0	0	0	0	**0**
3	0	0	0	0	0	1	**1**
5	0	0	0	0	0	0	**0**
6	1	1	1	0	2	1	**6**
Total CB	**1**	**1**	**1**	**0**	**2**	**2**	**7**

FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.8.1 Symmetry matrix for AE1/3 immunostain in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K-statistic -0.14; p-value 0.31).



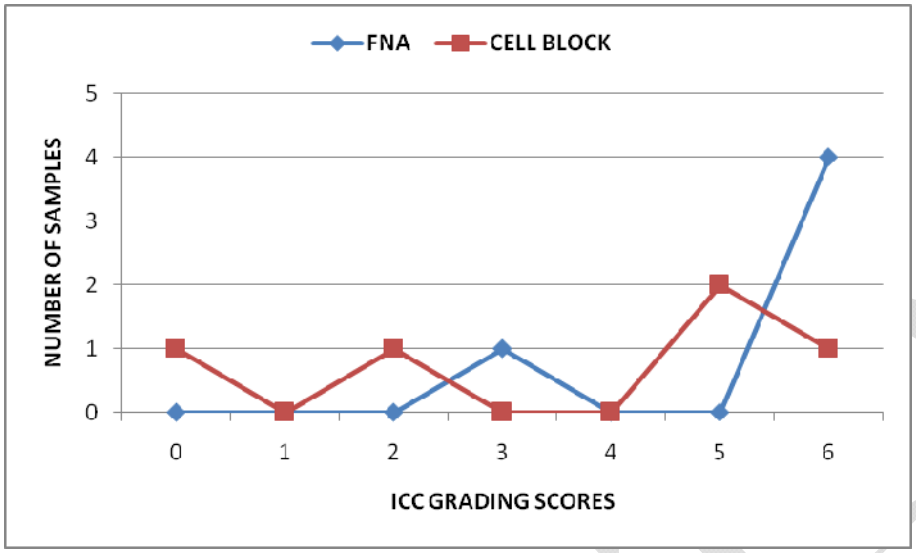
FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.8.2 Comparison of AE1/3 immunostain grading scores of FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K-statistic -0.14; p-value 0.31).

	CELL BLOCK AE1/3 SCORES					
FNA AE1/3	**0**	**2**	**3**	**5**	**6**	**Total FNA's**
SCORES						
0	0	0	0	0	0	**0**
2	0	0	0	0	0	**0**
3	0	0	0	0	1	**1**
5	0	0	0	0	0	**0**
6	1	1	0	2	0	**4**
Total CB	**1**	**1**	**0**	**2**	**1**	**5**

FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.8.3 Symmetry matrix for AE1/3 immunostain in FNA and cell block (CB) samples with a dedicated aspiration. (K-statistic -0.19; p-value 0.28).



FNA fine needle aspiration, CB cell block, SYN synaptophysin
(0 = negative / absent staining, 1+ = focal weak intensity < 10% of tumour cells showing positivity,
2+ = focal moderate intensity 10-50% of tumour cells showing positivity,
3+ = focal strong intensity > 50% of tumour cells showing positivity,
4+ = diffuse weak intensity < 10% of tumour cells showing positivity,
5+ = diffuse moderate intensity 10-50% of tumour cells showing positivity,
6+ = diffuse strong intensity > 50% of tumour cells showing positivity).

Figure 3.5.8.4 Comparison of AE1/3 immunostain grading scores of FNA and cell block sample with a dedicated aspiration (K-statistic -0.19; p-vlaue 0.28).

3.5.9 DISCREPANT RESULT

Discrepant results were obtained for one sample, a liver FNA. Immunocytochemistry performed on this smear demonstrated tumour cell positivity for CK7 and synaptophysin whilst CK20 was negative. The same panel of tests performed on the corresponding cell block sample were negative. All three tests were repeated but the results remained the same.

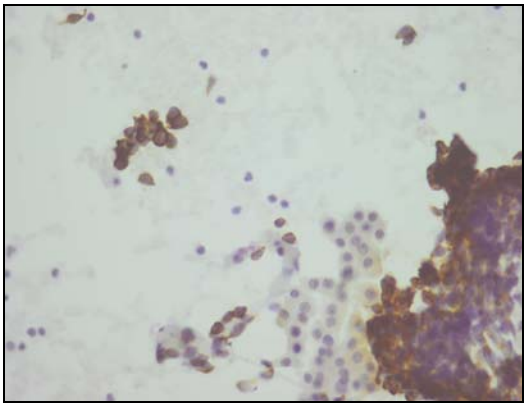


Figure 3.5.9.1 sk38 FNA sample obtained by dedicated needle aspiration.
(Cellularity grading score = 3+).
CK7 immunostain grading score = 3+, lacks background staining.
CK7 immunostain x 40.

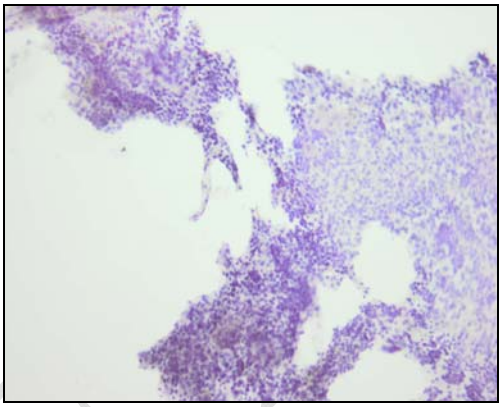


Figure 3.5.9.2 sk38 Paired cell block sample obtained by dedicated needle aspiration.
(Cellularity grading score = 2+).
CK7 immunostain grading score = 0, lacks background staining.
CK7 immunostain x 20.

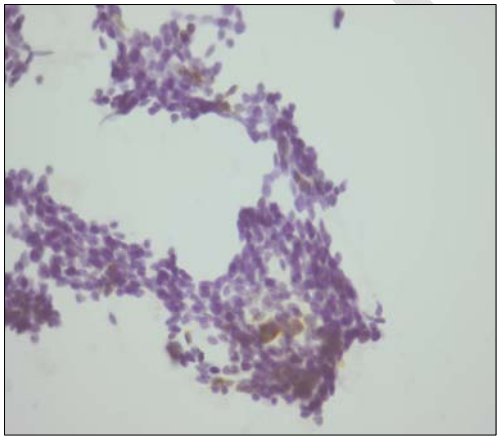


Figure 3.5.9.3 sk38 FNA sample obtained by dedicated needle aspiration.
(Cellularity grading score = 3+).
SYN immunostain grading score = 5+, with mild background staining, grading score = 1.
SYN immunostain x 40.

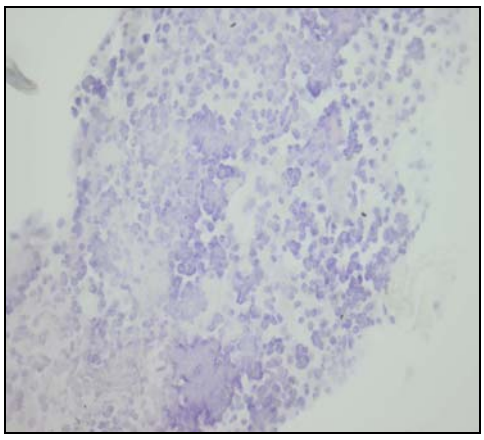


Figure 3.5.9.4 sk38 Paired cell block sample obtained by dedicated needle aspiration.
(Cellularity grading score = 2+).
SYN immunostain grading score = 0, lacks background staining.
SYN immunostain x 40.

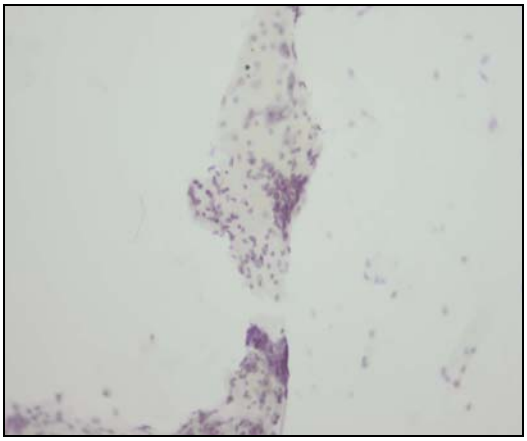


Figure 3.5.9.5 sk38 FNA sample obtained by dedicated needle aspiration.
(Cellularity grading score = 3+).
CK20 immunostain grading score = 0, with mild background staining, grading score = 1.
CK20 immunostain x 40 .

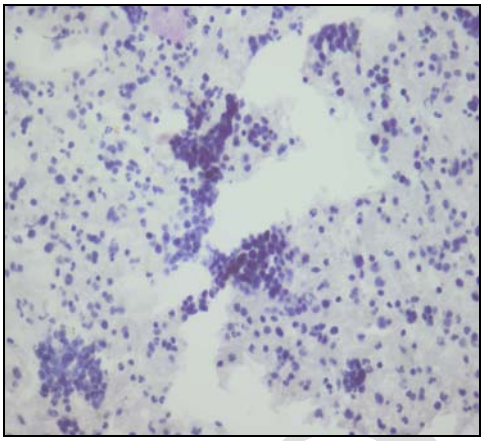


Figure 3.5.9.6 sk38 Paired cell block sample obtained by dedicated needle aspiration.
(Cellularity grading score = 2+).
CK20 immunostain grading score = 0 , lacks background staining.
CK20 immunostain x 40.

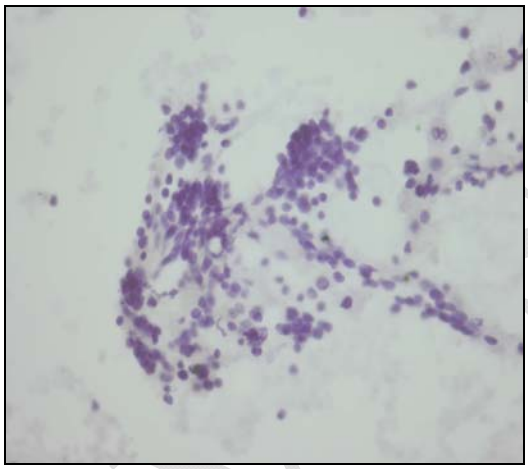


Figure 3.5.9.7 sk38 FNA sample obtained by dedicated needle aspiration.
(Cellularity grading score = 3+).
Negative control for immunostain, without non-specific staining.
Negative control immunostain x 40.

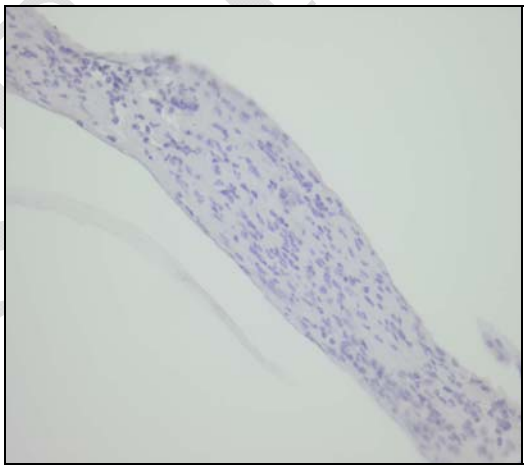


Figure 3.5.9.8 sk38 Paired cell block sample obtained by dedicated needle aspiration.
(Cellularity grading score = 2+).
Negative control for immunostain, without non-specific staining.
Negative control immunostain x 20.

3.6 COMPARISON OF BACKGROUND STAINING (BG) IN IMMUNOSTAINS BETWEEN FNA AND CELL BLOCK SAMPLES

In contrast to the previous assessments performed, lower grading scores (score 0 and 1) for background staining in ICC are desirable in order to facilitate unequivocal interpretation of staining. The bias towards FNA samples which had higher background staining grading scores was illustrated in the symmetry matrices (Figures 3.6.1- Figure 3.6.23). In all immunostains, more cell block samples displayed no background staining (score 0) than FNA samples (Table 3.6.1 and 3.6.2). This trend was represented graphically in figures 3.6.2 – 3.6.24 where the distribution of the respective samples across the various background staining score categories in ICC tests were displayed.

The assessment of background staining in CK 7, CK20, TTF1 and synaptophysin immunostains, displayed a poor agreement (K– statistic = 0.03, 0.01, 0.00 and 0.15 respectively), between the two methods of sample preparation. This discordance was statistically significant for CK 7, CK20 and TTF1 immunostains (p-value = 0.00, 0.00, 0.03 respectively). A marginally significant difference (p-value = 0.08) was obtained in the assessment of background staining in synaptophysin. More samples prepared conventionally (FNA smears) had higher background assessment grading scores (score 2 and 3) in CK 7, CK20 , TTF1 and synaptophysin immunostains than cell block samples. The asymmetry in background assessment of Hepar-1 and AE 1/3 immunostains was not statistically significant: Hepar-1 ($p = 0.16$; $K = 0.00$); AE1/3 ($p = 0.20$; $K = 0.02$).

The same trend was observed in the assessment of background staining of CK 7, CK20 and TTF1 immunostains when cell block samples (n = 28, 28 and 8 respectively) that had a dedicated needle pass was compared with that of FNA samples. The discordance was

statistically significant for CK 7, CK20 and TTF1 immunostains: CK7 ($p = 0.01$; $K = 0.02$); CK20 ($p = 0.00$; $K = 0.01$); TTF1 ($p = 0.10$; $K = 0.00$). For the same comparison in the synaptophysin stain ($n = 7$) no statistically significant difference (p-value = 0.14) was obtained and the poor agreement (discordance) between the methods (K- statistic = 0.00) was a random event. The asymmetry in background assessment of Hepar-1 and AE 1/3 immunostains was not statistically significant: Hepar-1 ($p = 0.16$; $K = 0.00$); AE 1/3 ($p = 0.17$; $K = 0.00$).

Table 3.6.1 Comaprison of number of FNA samples (with dedicated aspiration) and cell block samples (with either dedicated aspiration or needle rinse) in respective background (BG) score categories in immunocytochemistry (ICC) tests.

	CK7 - BG		CK20 - BG		TTF1 - BG		SYN- BG		HEP-1 - BG		AE1/3 - BG	
Scores	FNA	CB	FNA	CB	FNA	CB	FNA	CB	FNA	CB	FNA	CB
0	17	37	19	40	9	18	3	7	8	10	0	5
1	12	5	15	4	5	0	3	0	0	0	1	0
2	9	2	8	0	2	0	1	0	2	0	3	0
3	6	0	2	0	2	0	0	0	0	0	1	0
n =	44	44	44	44	18	18	7	7	10	10	5	5
K	0.03		0.01		0.00		0.15		0.00		0.02	
p-value	0.00		0.00		0.03		0.08		0.16		0.20	

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Table 3.6.2 Comparison of number of FNA and cell block samples (with dedicated aspiration) in respective background (BG) score categories in ICC tests.

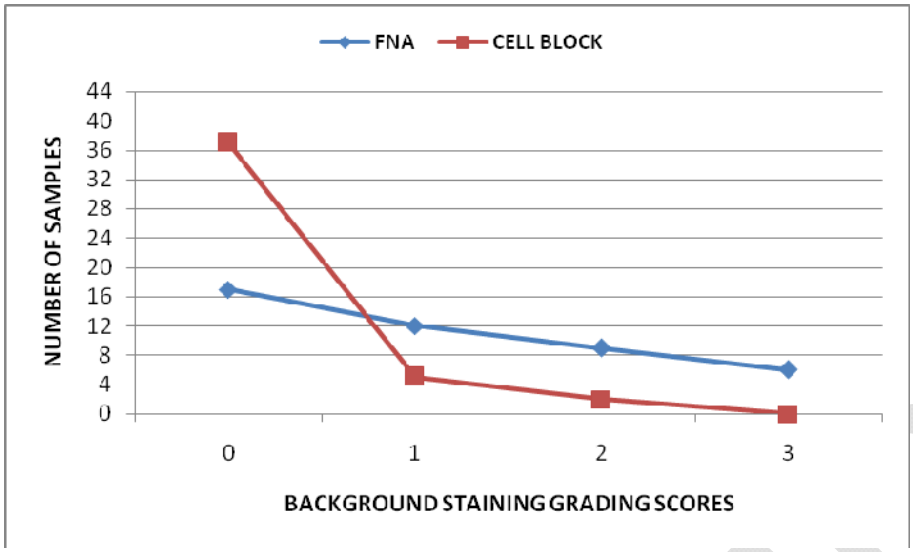
	CK7 - BG		CK20 - BG		TTF1 - BG		SYN- BG		HEP-1 - BG		AE1/3 - BG	
Scores	FNA	CB	FNA	CB	FNA	CB	FNA	CB	FNA	CB	FNA	CB
0	9	23	11	26	2	8	3	7	6	8	0	5
1	7	4	9	2	2	0	3	0	0	0	1	0
2	7	1	7	0	2	0	1	0	2	0	3	0
3	5	0	1	0	2	0	0	0	0	0	1	0
n =	28	28	28	28	8	8	7	7	8	8	5	5
K	0.02		0.01		0.00		0.00		0.00		0.00	
p-value	0.01		0.00		0.10		0.14		0.16		0.17	

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

	Cell Block: CK7 BG				
FNA: CK7 BG	SCORES 0	1	2	3	Total FNA's
SCORES					
0	16	1	0	0	17
1	11	1	0	0	12
2	8	1	0	0	9
3	2	2	2	0	6
Total CB	37	5	2	0	44

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.1 Symmetry matrix for CK7 background (BG) staining in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K = 0.03; p = 0.00).



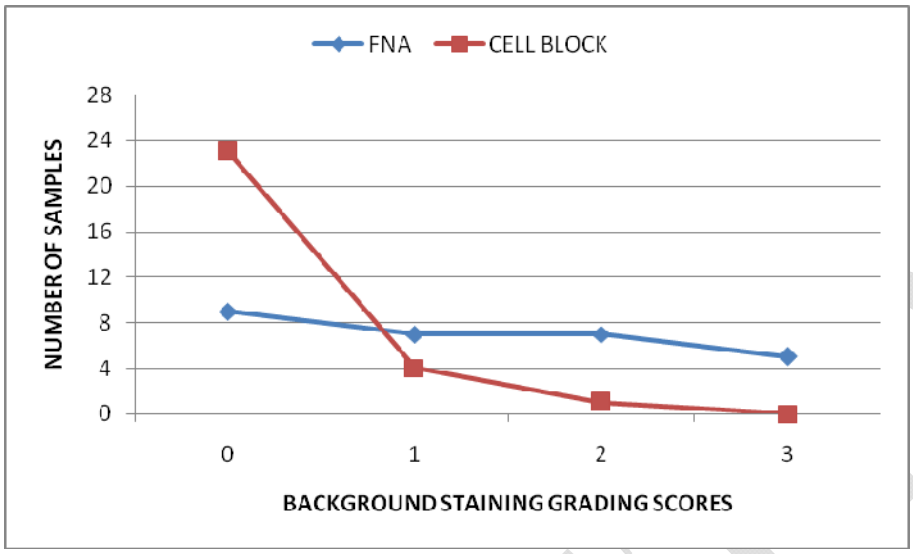
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.2 Comparison of CK7 background (BG) staining grading scores in FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K = 0.03; p = 0.00).

	Cell Block: CK7 BG SCORES			
FNA: **CK7 BG**	0	1	2	3	**Total FNA's**
SCORES					
0	8	1	0	0	**9**
1	6	1	0	0	**7**
2	7	0	0	0	**7**
3	2	2	1	0	**5**
Total CB	**23**	**4**	**1**	**0**	**28**

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.3 Symmetry matrix for CK7 background (BG) staining in FNA and cell block (CB) samples with dedicated aspiration. (K = 0.02; p = 0.01).



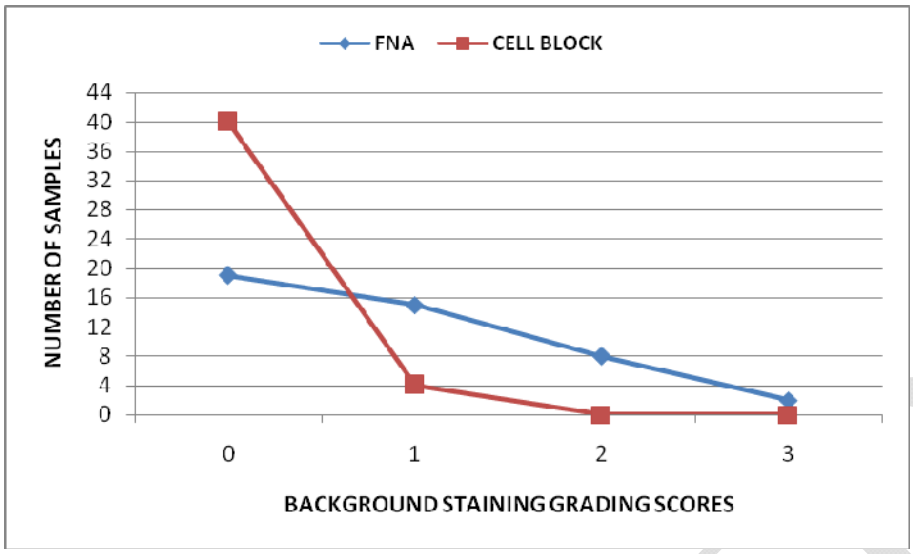
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.4 Comparison of CK7 background (BG) staining grading scores in FNA and cell block sample with dedicated aspiration (K = 0.02; p = 0.01).

FNA: CK20 BG	Cell Block: CK20 BG SCORES 0	1	2	3	Total FNA's
SCORES					
0	18	1	0	0	19
1	14	1	0	0	15
2	6	2	0	0	8
3	2	0	0	0	2
Total CB	40	4	0	0	44

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.5 Symmetry matrix for CK20 background (BG) staining in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K = 0.01; p = 0.00).



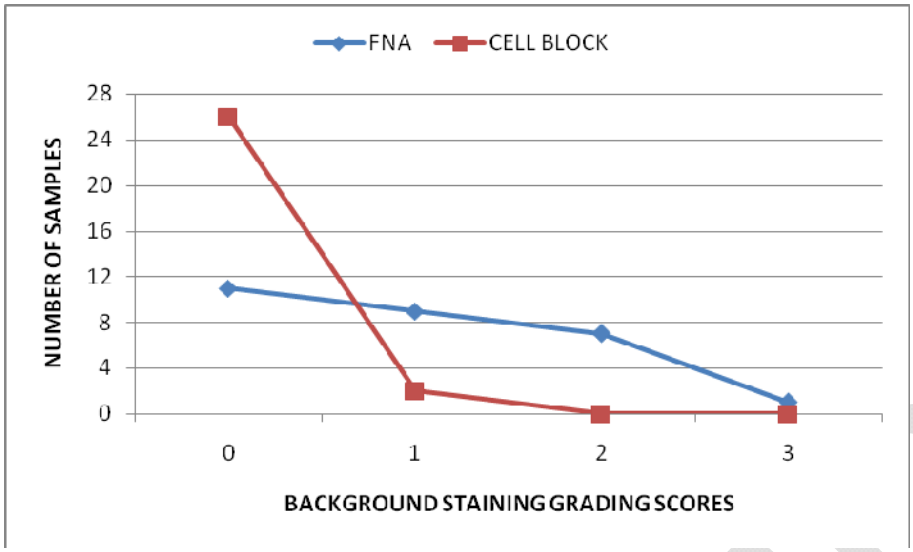
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.6 Comparison of CK20 background (BG) staining grading scores in FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K = 0.01; p = 0.00).

	Cell Block: CK20 BG SCORES				
FNA: **CK20 BG**	0	1	2	3	**Total FNA's**
SCORES					
0	11	0	0	0	**11**
1	9	0	0	0	**9**
2	5	2	0	0	**7**
3	1	0	0	0	**1**
Total CB	26	2	0	0	28

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.7 Symmetry matrix for CK20 background (BG) staining in FNA and cell block (CB) samples with dedicated aspiration. (K = 0.01; p = 0.00).



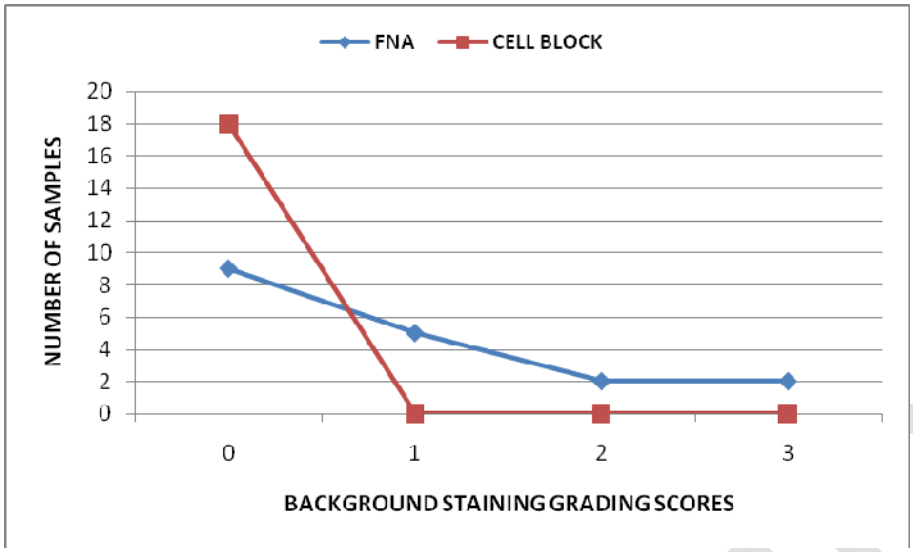
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.8 Comparison of CK20 background (BG) staining grading scores in FNA and cell block sample with dedicated aspiration (K = 0.01; p = 0.00).

FNA: TTF1 BG SCORES	Cell Block: TTF1 BG SCORES				
	0	1	2	3	**Total FNA's**
0	9	0	0	0	**9**
1	5	0	0	0	**5**
2	2	0	0	0	**2**
3	2	0	0	0	**2**
Total CB	**18**	**0**	**0**	**0**	**18**

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.9 Symmetry matrix for TTF1 background (BG) staining in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K = 0.00; p = 0.03).



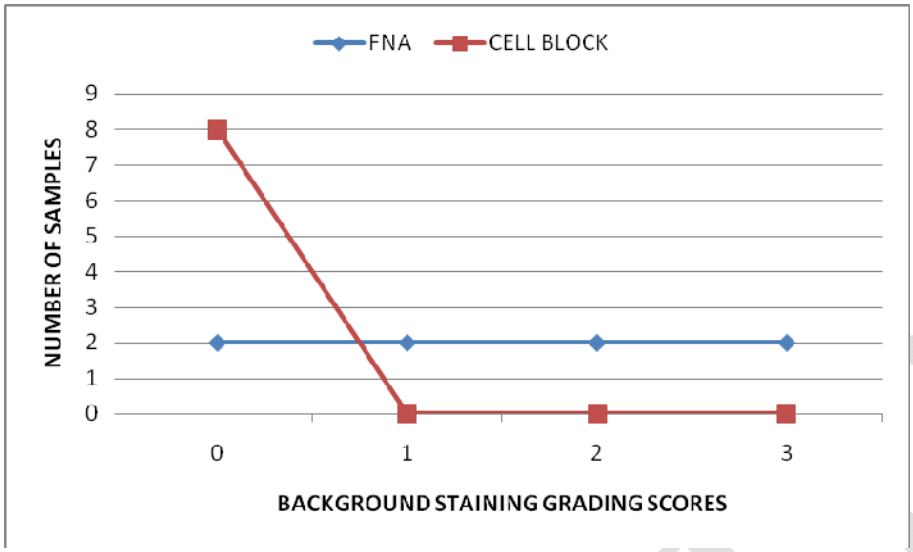
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.10 Comparison of TTF1 background (BG) staining grading scores in FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K = 0.00; p = 0.03).

	Cell Block: TTF1 BG			
	SCORES			
FNA: TTF1 BG	0	1	2	3	**Total FNA's**
SCORES					
0	2	0	0	0	**2**
1	2	0	0	0	**2**
2	2	0	0	0	**2**
3	2	0	0	0	**2**
Total CB	**8**	**0**	**0**	**0**	**8**

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.11 Symmetry matrix for TTF1 background (BG) staining in FNA and cell block (CB) samples with dedicated aspiration. (K = 0.00; p = 0.10).



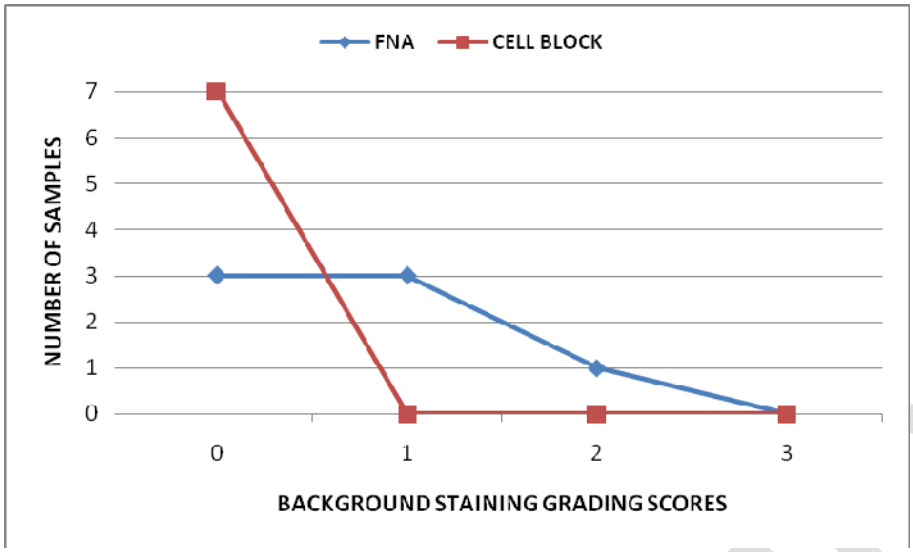
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.12 Comparison of TTF1 background (BG) staining grading scores in FNA and cell block sample with dedicated aspiration (K = 0.00; p = 0.10).

	Cell Block: SYN BG SCORES			
FNA: SYN BG	0	1	2	Total FNA's
SCORES 0	4	0	0	4
1	4	1	0	5
2	1	0	0	1
Total CB	9	1	0	10

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.13 Symmetry matrix for synaptophysin (syn) background (BG) staining in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K = 0.15; p = 0.08).



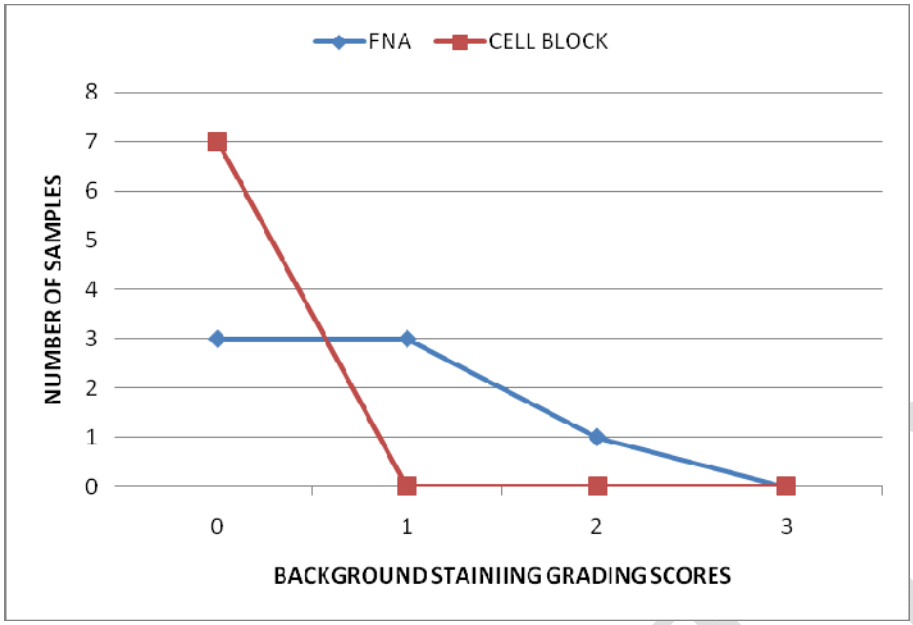
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.14 Comparison of synaptophysin (syn) background (BG) staining grading scores in FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K = 0.15; p = 0.08).

	Cell Block: SYN BG SCORES		
FNA: SYN BG	0	1	2	Total FNA's
SCORES 0	3	0	0	3
1	3	0	0	3
2	1	0	0	1
Total CB	7	0	0	7

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.15 Symmetry matrix for synaptophysin (syn) background (BG) staining in FNA and cell block (CB) samples with dedicated aspiration. (K = 0.00; p = 0.14).



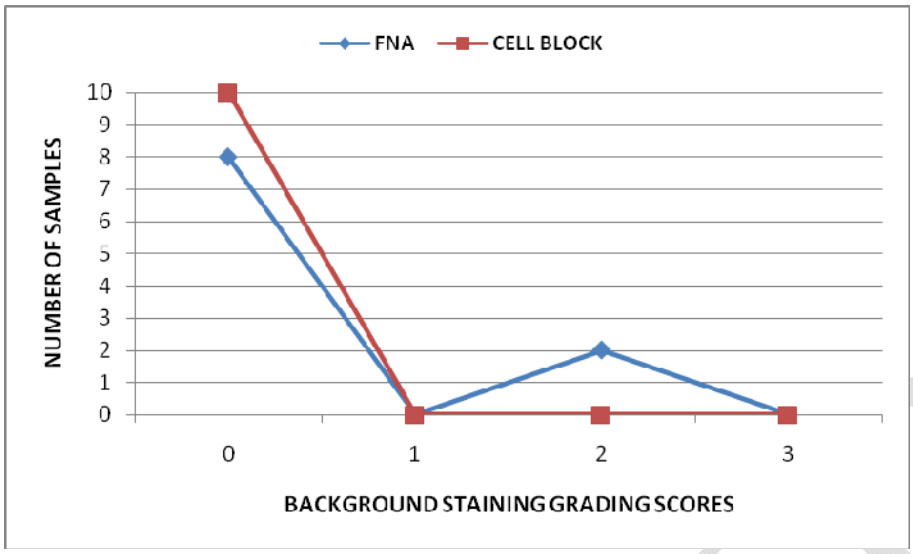
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.16 Comparison of synaptophysin (syn) background (BG) staining grading scores in FNA and cell block sample with dedicated aspiration (K = 0.00; p = 0.14).

	Cell Block: HEP-1 BG		
	SCORES		
FNA:			
HEP-1 BG	0	2	**Total**
SCORES			
0	8	0	**8**
2	2	0	**2**
Total	**10**	**0**	**10**

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.17 Symmetry matrix for hepar-1 (hep-1) background (BG) staining in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K = 0.00; p = 0.16).



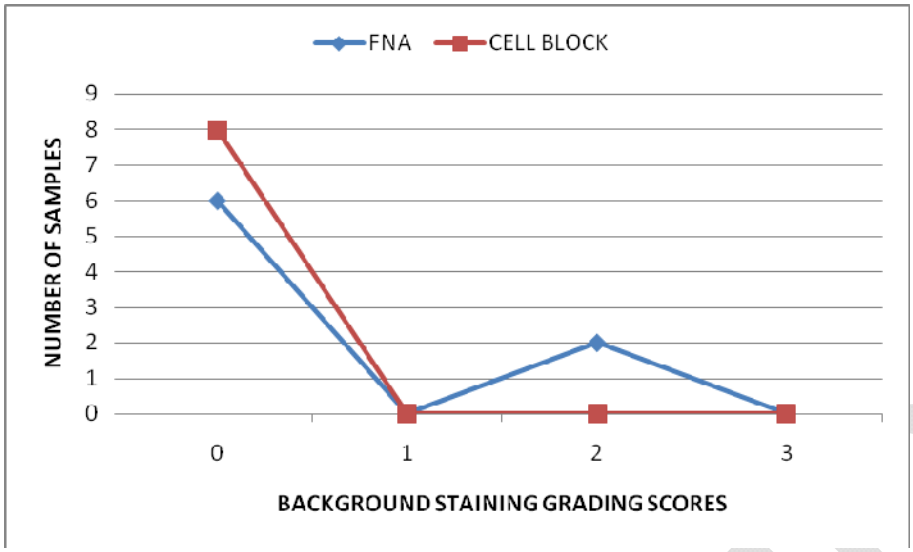
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.18 Comparison of hepar-1 (hep-1) background (BG) staining grading scores in FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K = 0.00; p = 0.16).

	Cell Block: HEP-1 BG SCORES		
FNA: HEP-1 BG	0	2	Total
SCORES			
0	6	0	6
2	2	0	2
Total	8	0	8

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.19 Symmetry matrix for hepar-1 (hep-1) background (BG) staining in FNA and cell block (CB) samples with dedicated aspiration. (K = 0.00; p = 0.16).



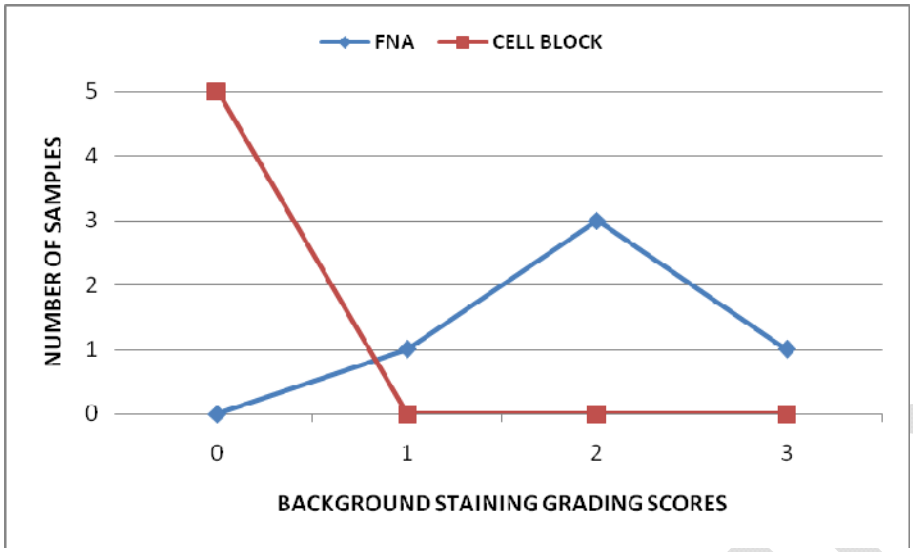
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.20 Comparison of hepar-1(hep-1) background (BG) staining grading scores in FNA and cell block sample with dedicated aspiration (K = 0.00; p = 0.16).

	Cell Block: AE1/3 BG			
FNA: AE1/3 BG	SCORES			
	0	1	2	3	**Total FNA's**
SCORES					
0	0	0	0	0	**0**
1	1	0	0	0	**1**
2	3	1	1	0	**5**
3	1	0	0	0	**1**
Total CB	**5**	**1**	**1**	**0**	**7**

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.21 Symmetry matrix for AE 1/3 background (BG) staining in FNA samples with dedicated aspiration and cell block (CB) samples either dedicated aspiration or needle rinse. (K = 0.02; p = 0.20).



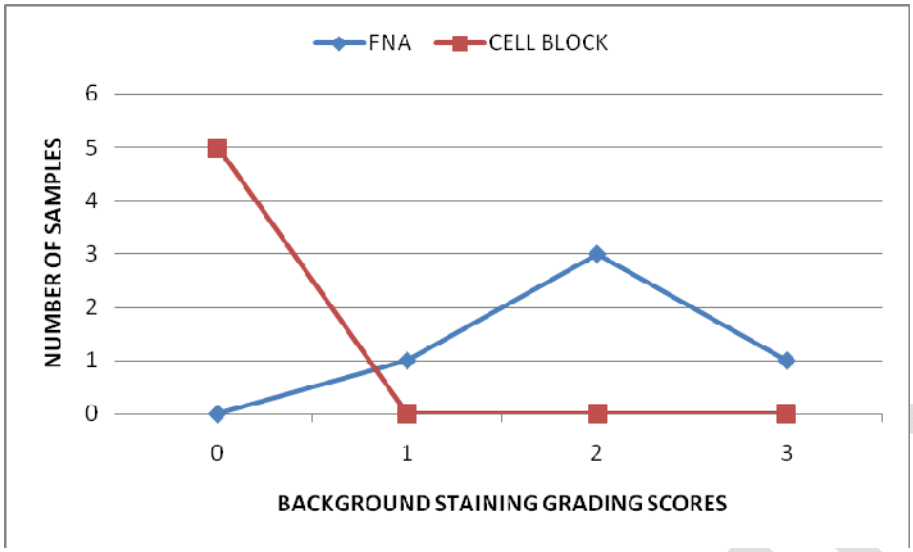
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.22 Comparison of AE 1/3 background (BG) staining grading scores in FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K = 0.02; p = 0.20).

	Cell Block: AE1/3 BG SCORES				
FNA: **AE1/3 BG**	0	1	2	3	**Total**
SCORES					
0	0	0	0	0	**0**
1	1	0	0	0	**1**
2	3	0	0	0	**3**
3	1	0	0	0	**1**
Total	**5**	**0**	**0**	**0**	**5**

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.23 Symmetry matrix for AE 1/3 background (BG) staining in FNA and cell block (CB) samples with dedicated aspiration. (K = 0.00; p = 0.17).



0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.6.24 Comparison of AE 1/3 background (BG) staining grading scores in FNA and cell block sample with dedicated aspiration (K = 0.00; p = 0.17).

3.7.0 COMPARISON OF BACKGROUND / ABERRANT STAINING IN NEGATIVE CONTROLS OF IMMUNOSTAINS BETWEEN FNA AND CELL BLOCK SAMPLES

Non specific aberrant staining was observed in the FNA negative control of the CK7, CK20, TTF1, synaptophysin and Hepar-1 immuno stains. This phenomenon was not displayed in the respective cell block negative controls and in the paired AE 1/3 immunostains.

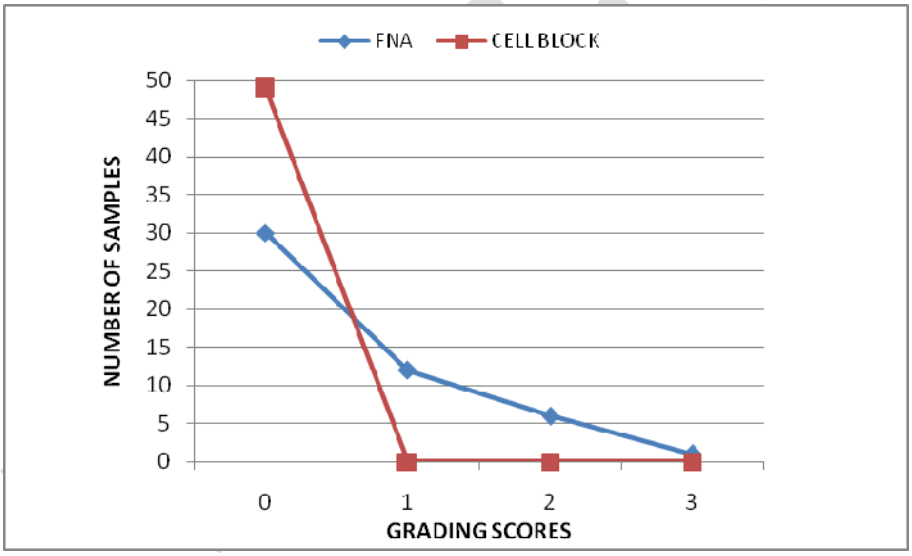
A statistically significant difference (p-value 0.00) was obtained for the evaluation of the background staining of negative controls in ICC of samples prepared by both techniques. The poor agreement (K = 0.00) observed for this evaluation illustrated a bias towards samples prepared conventionally (FNA smears) which had higher background assessment grading scores in negative controls than cell block samples. This was illustrated in the symmetry matrix (Figure 3.7.0.1) by a dominant deviation of FNA samples from the diagonal in comparison to the cell block samples. All the cell block samples, 100% (49/49), displayed no background staining compared to 61% (30/49) of FNA samples. (The cellularity of one sample was too sparse therefore it was not possible to perform immunocytochemistry on the FNA smear and the cell block sample).

A similar trend was obtained when the background assessment of negative controls in ICC, in the cell block samples that had a dedicated needle pass (n = 30) was compared with that of FNA samples. A statistically significant difference (p-value = 0.01) was obtained and the poor agreement (discordance) between the methods (K- statistic = 0.00) was not a random event. Samples prepared conventionally (FNA smears) had higher background assessment grading scores in negative controls than cell block samples. This was illustrated in the symmetry matrix (Figure 3.7.0.3) by a dominant deviation of FNA samples from the diagonal in comparison to the cell block samples.

	Cell Block: BG - Negative Controls				
FNA:BG		SCORES			
Negative Controls					
	0	1	2	3	**Total FNA's**
SCORES					
0	30	0	0	0	**30**
1	12	0	0	0	**12**
2	6	0	0	0	**6**
3	1	0	0	0	**1**
Total CB	**49**	**0**	**0**	**0**	**49**

0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.7.0.1 Symmetry matrix for background (BG) / aberrant stanining in negative controls (for immunostains) of FNA with dedicated aspiration and cell block (CB) samples with either dedicated aspiration or needle rinse (K = 0.00; p = 0.00).



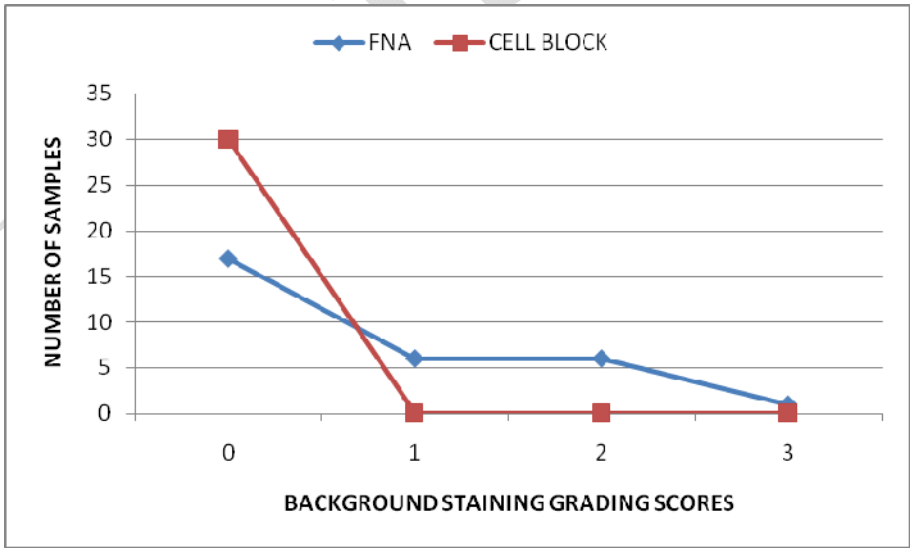
0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.7.0.2 Comparison of background (BG) / aberrant staining in negative controls (for immunostains) of FNA samples with dedicated aspiration and cell block sample either dedicated aspiration or needle rinse (K = 0.00; p = 0.00).

	Cell Block: BG -Negative Controls				
FNA: BG	SCORES				
Negative Controls					
	0	1	2	3	**Total**
SCORES					
0	17	0	0	0	**17**
1	6	0	0	0	**6**
2	6	0	0	0	**6**
3	1	0	0	0	**1**
Total	30	0	0	0	30

FNA fine needle aspiration, CB cell block, 0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.7.0.3 Symmetry matrix for background (BG) / aberrant staining in negative controls (for immunostains) of FNA and cell block (CB) samples with dedicated aspiration. (K = 0.00; p = 0.01).



FNA fine needle aspiration, CB cell block, 0 = no background; 1 = mild background (< 10% of smear / section); 2 = moderate background (10-50% of smear / section); 3 = severe background (> 50% of smear / section)

Figure 3.7.0.4 Comparison of background (BG) / aberrant staining in negative controls

(for immunostains) of FNA and cell block sample with dedicated aspiration

(K = 0.00; p = 0.01).

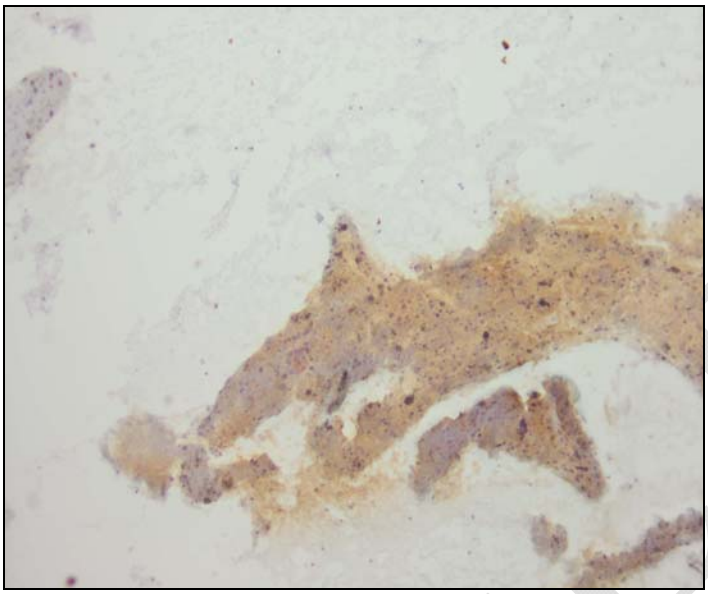


Figure 3.7.0.5 (Sk36) FNA Right Lung mass. This figure demonstrates non- specific background staining of the negative control (of the de-stained Papanicaloau stained slide of the FNA), of the immnocytochemistry assay. This non-specificity makes interpretation of the panel of immuno stains difficult. Negative control for immunocytochemistry stain x 10.

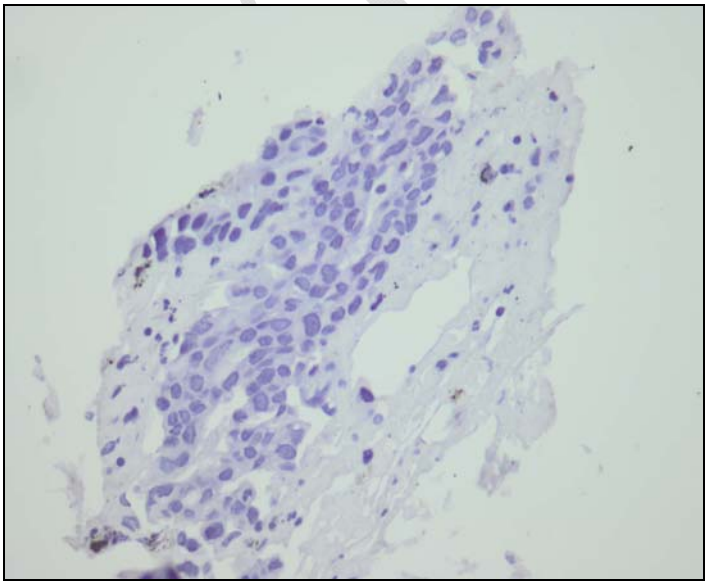


Figure 3.7.0.6 **(Sk36)** FNA Right Lung mass – Paired Cell block sample (of above FNA sample).
Lack of non-specific background staining of negative control, which facilitates ease of interpretation of immunocytochemistry stains. Negative control for immunocytochemistry stain x 40.

3.7.1 Aberrant Staining : FNA CK 7 Immunostain

Cells labelled by the CK 7 antibody displays a cytoplasmic staining pattern.[39] However, aberrant nuclear staining was displayed in 9% of samples (4/44) prepared conventionally (FNA smears) whilst this phenomenon was not observed in samples prepared using the cell block technique.

3.7.2 Aberrant Staining : FNA CK 20 Immunostain

A cytoplasmic staining pattern is expected for cells labelled with CK 20 antibody.[39]

In 10/44 (23%) of samples prepared conventionally, aberrant nuclear staining was displayed. This was not observed in cell block samples.

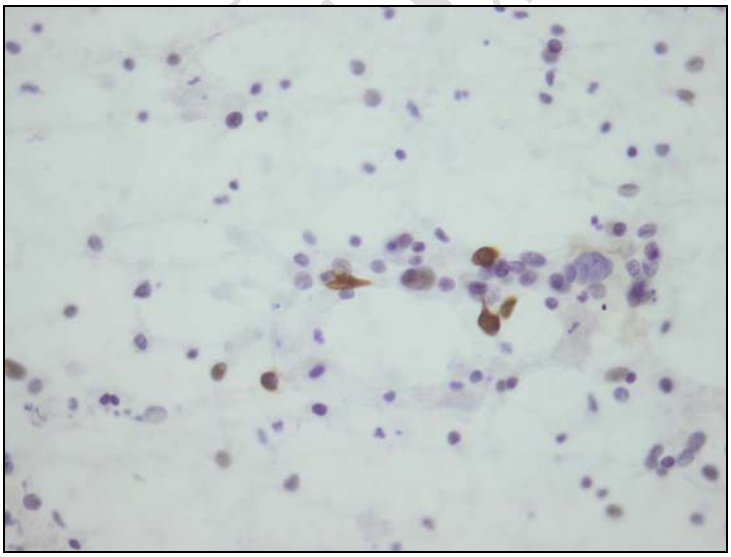


Figure 3.7.2.1 sk07 FNA liver aspirate – diagnosed as metastatic adenocarcinoma. CK20 immunostain shows aberrant nuclear staining. FNA CK20 immunostain X 40.

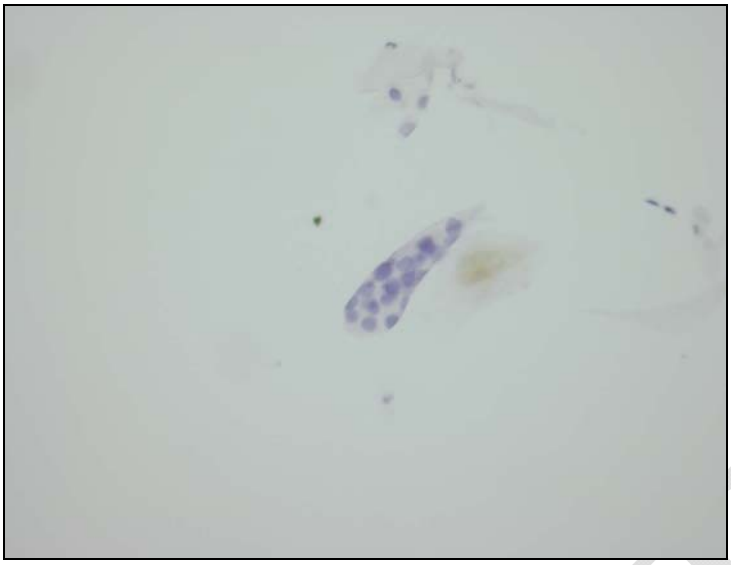


Figure 3.7.2.2 Sk07 Paired Cell Block of liver aspirate.
CK20 negative without aberrant nuclear staining.
Cell Block CK20 X 40.

3.7.3 Aberrant Staining: FNA TTF1 Immunostain

A TTF1 stain was performed on 36% of samples (18 /50). Aberrant nuclear staining was displayed in the negative control of 28% of conventionally stained samples (5/18).

No abberant staining was noticed in the negative control of the cell block samples.

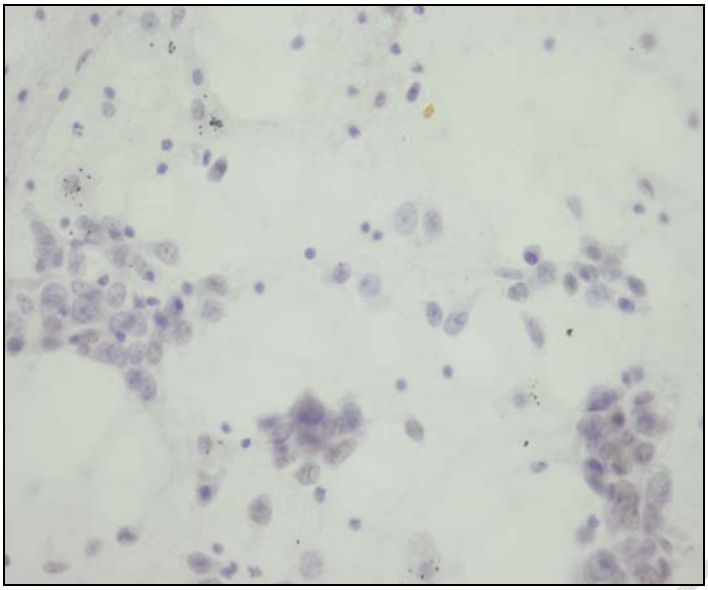


Figure 3.7.3.1 FNA lung aspirate diagnosed as bronchiolar-alveolar carcinoma.
Negative control for immunostain showing faint aberrant nuclear staining. Immunostain Negative Control X 40.

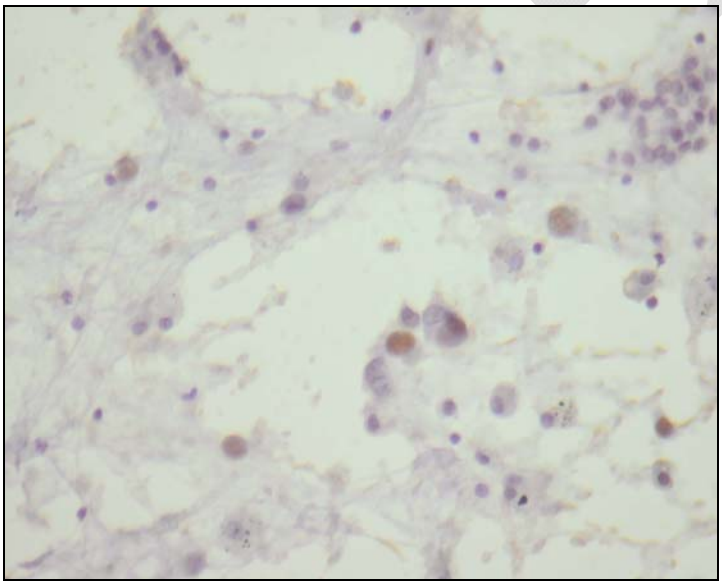


Figure 3.7.3.2 FNA TTF1 immunostain which cannot be interpreted definitively due to the presence of aberrant nuclear staining of the negative control (above).
TTF1 immunostain x 40.

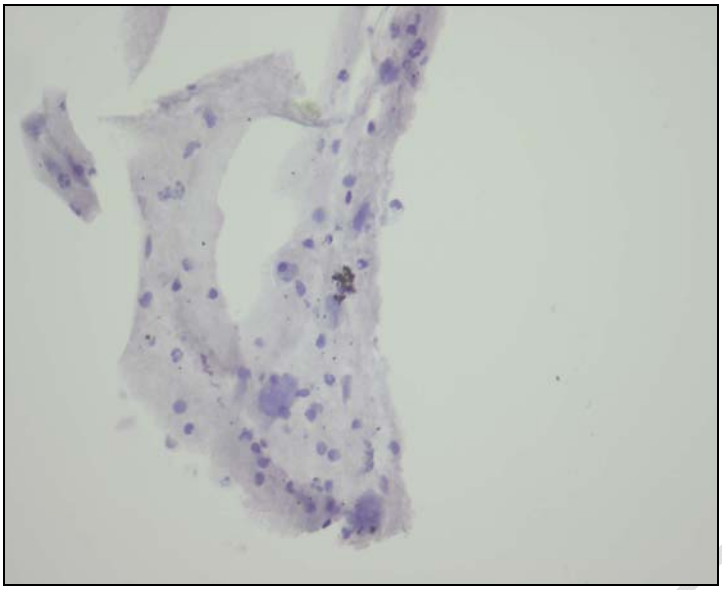


Figure 3.7.3.3 Paired Cell Block Negative Control for immunostain without aberrant staining.
Immunostain Negative Control X 40.

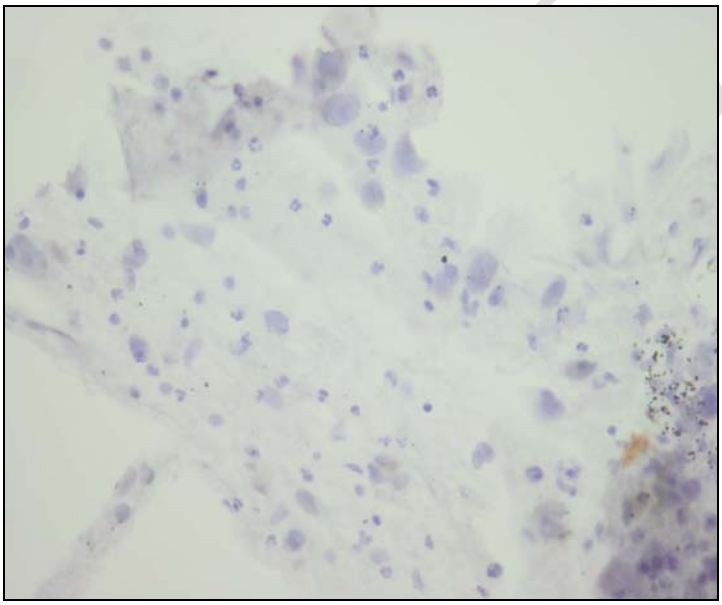


Figure 3.7.3.4 Paired Cell Block of lung aspirate.
TTF1 immunostain was negative and negative control does not display aberrant nuclear staining.
TTF1 immunostain X 40.

3.7.4 Aberrant Staining: FNA Synaptophysin Immunostain

Cells labelled by the synaptophysin antibody display a cytoplasmic staining pattern.[46]

Aberrant nuclear staining pattern was observed in 10% (1/10) of conventionally prepared

(FNA) samples labeled with this antibody whilst this phenomenon was not observed in cell block samples.

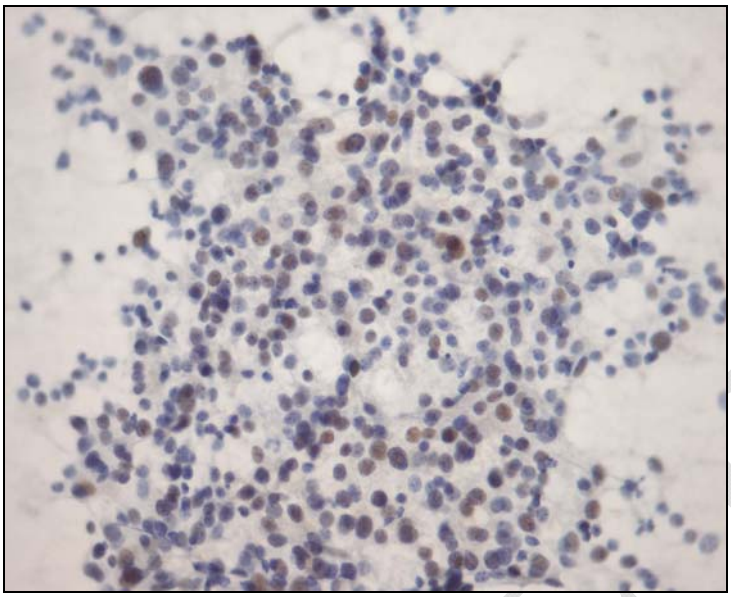


Figure 3.7.4.1 Sk139 FNA liver aspirate- diagnosis favoured hepatocellular carcinoma and neuro-endocrine component could not be excluded. Synaptophysin immunostain with aberrant nuclear staining. Synaptophysin immunostain X 40.

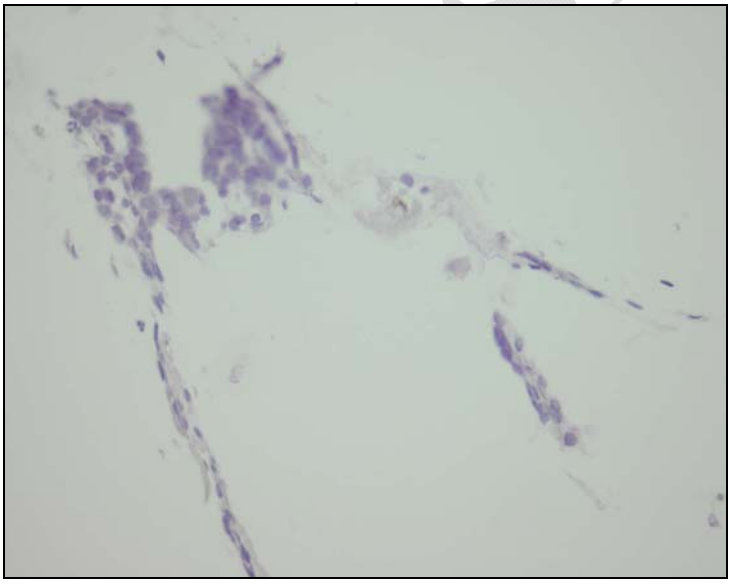


Figure 3.7.4.2 sk 139 Paired Cell block sample of liver aspirate. Synaptophysin immunostain without aberrant staining. Synaptophysin immunostain X 40.

3.7.5 Aberrant Staining : FNA Hepar-1 Immunostain

Aberrant nuclear staining pattern was also observed for 30% (3/10) conventionally prepared (FNA) samples labelled with Hepar-1 antibody whilst this phenomenon was not displayed in cell block samples.

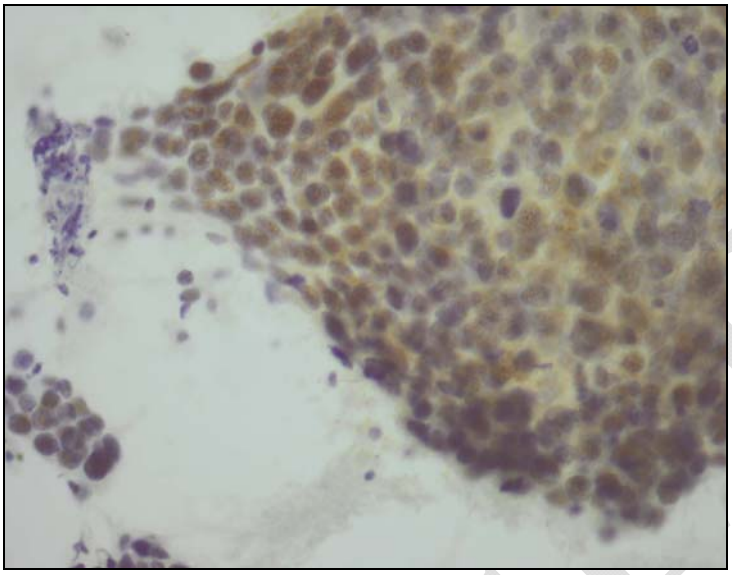


Figure 3.7.5.1 SK 15 FNA liver aspirate, diagnosed with hepatocellular carcinoma.
Hepar-1 immunostain with aberrant nuclear staining
FNA Hepar-1 immunostain X 40.

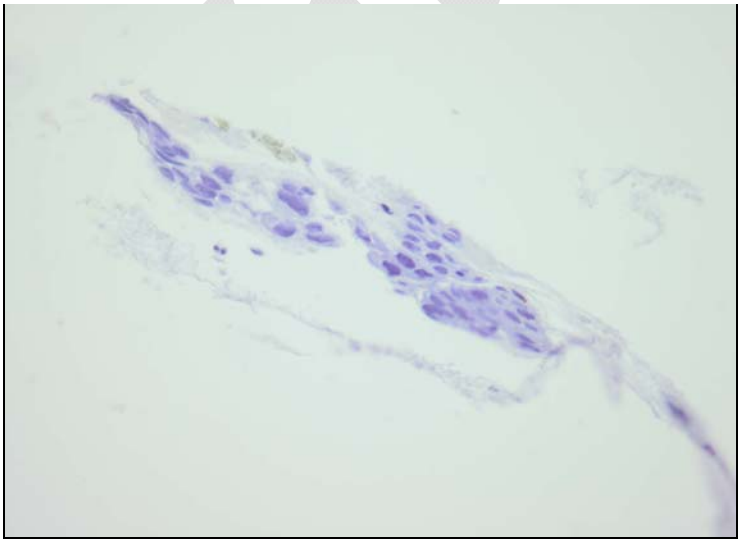


Figure 3.7.5.2 SK 15 Cell Block Hepar-1 immunostain without aberrant nuclear staining. Cell Block Hepar-1 immunostain X 40.

3.7.6 No Aberrant staining in FNA and cell block AE 1/3 Immunostain:

No aberrant staining was observed in samples prepared by both methods.

3.8 Percentage adequacy of samples collected by pathologists, radiology registrars, medical officers and nurses

Table 3.8.1 Tabulation of percentage adequacy of samples collected by pathologists, radiology registrars, medical officers and nurses

Cellularity: grade score	Pathologists	Radiology registrars	Medical Officers	Nurses
0	11% (n=4/50)	11% (n=1/9)	11% (n=1/9)	0% (n=0/2)
2	30% (n=8/27)	35% (n=6/17)	22% (n=2/9)	50% (n=1/2)
3	19% (n=5/27)	17% (n=2/12)	22% (n=2/9)	50% (n=1/2)