LIST OF CORRECTIONS

EXAMINER 1:

LITERATURE REVIEW

COMMENT BY EXAMINER 1:

Section 1:6 , page 4 (copy attached) — the implication of the first two sentences of section 1.6 imply that surgical pathology (histology) is losing its diagnostic value and being replaced by cytology. This perception is incorrect and surgical pathology remains the gold standard, notwithstanding the fact increasing diagnostic accuracy is being achieved in Cytology. It is suggested that these two sentences be amended to reflect this. This is indeed reflected later on in the middle of that page.

CORRECTION / RESPONSE BY CANDIDATE:

1.6 Past and present status of the problem: literature survey

In the past, surgical pathology was deemed to be of higher diagnostic value than cytology.[1] However, in recent years there has been a change in perception due to cytological tests displaying increased diagnostic specificity for preliminary diagnosis.[1]

LITERATURE REVIEW

COMMENT BY EXAMINER 1:

Spelling of"ancillary" on page 6 to be corrected.

The spelling of "embedded" on page 7 to be corrected.
The spelling of "peroxidase" and "contributes"

CORRECTION / RESPONSE BY CANDIDATE:

All spelling corrected respectively.

LITERATURE REVIEW

COMMENT BY EXAMINER 1:

The spelling of "peroxidase" and "contributes" on page 8 to be corrected and in addition the grammar of the last sentence in section 1.8 tabe amended such that the statement is made clearer.

CORRECTION / RESPONSE BY CANDIDATE:

Pg 8:

This detection system is also ideal for use with normal and neoplastic cells namely hepatocytes, erythrocytes, granulocytes and eosinophils that contain endogenous peroxidase activity which contributes to background and anomalous staining. [37]

LITERATURE REVIEW

COMMENT BY EXAMINER 1:

The literature review is comprehensive and well referenced. No errors in referencing were identified. However section 1.10 an page 1.2 and section 1.18 on page 15 refer to biomarkers and immunohistochemistry in tumours metastatic to lung and lymph nodes and those metastatic to liver and lymph nodes respectively. The relevance of this separation is questioned given that most tumours that metastasize to the liver also metastasize to the lung. Could the candidate explain why these have been separated out?

CORRECTION / RESPONSE BY CANDIDATE:

Section 1.10 and 1.18

These sections have been separated out for ease of understanding and display of data.

TABLE OF CONTENTS

COMMENT BY EXAMINER 1:

This is good and clearly laid out, however there is no list of abbreviations including-the immunohistoehcmistry antibodies. A table of abbreviations should be included in the dissertation and appropriately referenced in the table of contents (page viii).

CORRECTION / RESPONSE BY CANDIDATE:

Nomenclature included on pg xxviii.

MATERIALS & METHODS

COMMENT BY EXAMINER 1:

For the most part the materials and method's have been clearly laid out and are easy to follow. However, the method does not state that the FNA analysis was confined to specific diagnostic categories e.g. adenocarcinoma of the lung. This is confusing to the reader of the dissertation, who when reading the document, is left with the impression that FNA's were performed on a sample of patients in which numerous diagnostic entities could be expected. It only becomes apparent later on in the thesis that specific diagnostic categories were included in the study whilst others were excluded. This

should be corrected in the study design and the materials and methods

CORRECTION / RESPONSE BY CANDIDATE:

2.0 Materials and Methods / 2.1 Study sample and sampling method

Fifty FNAs of primary or metastatic adenocarcinoma of the lung and metastatic adenocarcinoma to liver or lymph nodes were performed from February 2009 to December 2009. Palpable masses e.g. superficial lymph nodes are aspirated by cytology staff trained in FNA technique.

1.5 Preview of the organisation of the rest of dissertation / Study Design

This was a prospective study. Material for the preparation of FNA smears on glass slides (direct smears) and cell block samples were collected simultaneously during fine needle aspiration of 50 cases comprising of primary or metastatic adenocarcinoma of the lung and metastatic adenocarcinoma to liver or lymph nodes.

MATERIALS & METHODS

COMMENT BY EXAMINER 1:

Page 17 — please correct the grammar as indicated (were to was).

CORRECTION / RESPONSE BY CANDIDATE:

Grammar corrected.

MATERIALS & METHODS

COMMENT BY EXAMINER 1:

Page 18 — a comment should be made on the possible effect of the destaining of slides and the result that this may have on antigen expression. It should be raised as a possible confounding factor under the limitations of the study and also in the discussion, particularly as a result of the aberrant staining that was identified and reflected in the results.

CORRECTION / RESPONSE BY CANDIDATE:

(The possible effects of destaining slides are discussed p90 – 2nd paragraph).

Discrepant results were obtained for one sample, a FNA from the liver. Immunocytochemistry was performed on the FNA smear and tumour cells were positive for CK7 and synaptophysin while CK20 was negative. This cytokeratin profile is observed in 56% of neurendocrine carcinomas of the lung. [38] All of the ICC tests performed on the cell block sample were negative. Although all tests were repeated the results remained unchanged. A possible explanation could be the sub-optimal preservation of antigencity of tumour cells in this sample which was somehow more susceptible than the others.[37] This could have been due to destaining the cytology slide which results in high levels of background staining and loss of conventionally stained cytological material. [16]

MATERIALS & METHODS

COMMENT BY EXAMINER 1:

Page 20. *Table 2.1 the scoring system for the immunohistochemistry stainimz is confusing. Although this has been referenced., the decision to break the staining patterns down into negative, focal and diffuse is inherently correct, however, to subcategorise these into ˜weak moderate and strong intensity results in a scoring system that implies that a 4+ staining intensity is more significant than a 3+ staining intensity. From a diagnostic point of view it is believed that strong focal staining would trump diffuse weak staining. In addition it is difficult to reconcile the concept of diffuse staining when less than 10% of tumour cells show positivity.*

CORRECTION / RESPONSE BY CANDIDATE:

This scoring system has been referenced and all stains were assessed using this system. The decision to grade staining into the given categories was based on methods used in the literature by Bhatia P et al. (reference # 51) Goldstein et al. (reference #52) and Liberman et al. (reference # 53). However, the examiner's comment has been noted.

RESULTS

COMMENT BY EXAMINER 1:

On page 26 please correct the grammar as indicated (was to were).

Page 27 — the figure 3.1.2 appears to be incorrect. Is it not figure 3.2.2?

Page 37 — the figure referred to appears to be incorrect. Should this not be 3.5.3.1?

Page 46 — figures 14.13 and 3.4.14 are both incorrect.

Page 57 — Please correct the grammar (from is to are).

CORRECTION / RESPONSE BY CANDIDATE:

All correction have been made according

.

RESULTS

COMMENT BY EXAMINER 1:

Page 30 — the text refers to 90% of cell block samples but does not state whether these are made from dedicated passes or otherwise.

CORRECTION / RESPONSE BY CANDIDATE:

All FNA samples (100%) displayed the presence of morphological preservation (score 1+) compared to 90% (27/30) cell block samples with a dedicated aspiration.

RESULTS

COMMENT BY EXAMINER 1:

Page 77 — *section 3.7.3 refers to aberrant staining. As mentioned above possible explanations for aberrant staining, whether it be nuclear or cytoplasmic should be mentioned. For instance this could be a function of the previous stain. Aberrant staining is mentioned on more than one occasion.*

CORRECTION / RESPONSE BY CANDIDATE:

This is raised in the Discussion section – pg 92-93 (2nd paragraph).

Miller et al [3] reported that a potential pitfall present on immunostained cytological smears leading to erroneous staining is the trapping of reagents in the three-dimensional cell clusters. In FNA smears the cytoplasm covers the nucleus, therefore a cytoplasmic stain will in some instances show nuclear staining in addition to cytoplasmic staining.

Interpretation of these smears should be confined to areas where the cells are present in monolayers in order to render definitive results. [3] This phenomenon was not observed in the cell block samples since they are two- dimensional. On sectioning the cell blocks the cytoplasm does not cover the nucleus hence the lack of nuclear staining. This facilitates ease of interpretation of immunostains on cell block samples.[3]

However, due to the delay in fixation of cell block samples in this study, the results for the preservation of cytomorphology in cell block samples were not comparable to similar studies undertaken.[25-32] Consequently in our setting, both smears and cell blocks are needed, the former to assess morphology, and the latter for optimal immunocytochemistry results.

DISCUSSION

COMMENT BY EXAMINER 1:

On page 87 there is discussion regarding suboptimal preservation linked to the type of fixative and time of fixation. It should also be mentioned that the fixation delay could have contributed to the discrepant and aberrant immunohistochemistry staining.

CORRECTION / RESPONSE BY CANDIDATE:

Page 87 - The sub-optimal preservation and discrepant aberrant immunocytochemical staining observed could have been due to the pre-fixation time lag.

COMMENT BY EXAMINER 1:

Pg 18 (from Material and methods section)

Page 18 — a comment should be made on the possible effect that the destaining of slides and the result that this may have on antigen expression. It should be raised as a possible confounding factor under the limitations of the study and also in the discussion, particularly as a result of the aberrant staining that was identified and reflected in the results.

CORRECTION / RESPONSE BY CANDIDATE:

Pg 90- *Discussion*

Discrepant results were obtained for one sample, a FNA from the liver. Immunocytochemistry was performed on the FNA smear and tumour cells were positive for CK7 and synaptophysin while CK20 was negative. This cytokeratin profile is observed in 56% of neurendocrine carcinomas of the lung. [38] All of the ICC tests performed on the cell block sample were negative. Although all tests were repeated the results remained unchanged. A possible explanation could be the sub-optimal preservation of antigencity of tumour cells in this sample which was somehow more susceptible than the others.[37] This could have been due to destaining the cytology slide which results in high levels of background staining and loss of conventionally stained cytological material. [16]

COMMENT BY EXAMINER 1:

Pg77 (from results section)

Page 77 — section 3.7.3 refers to aberrant staining. As mentioned above possible explanations for aberrant staining, whether it be nuclear or cytoplasmic should be mentioned. For instance this could be a function of the previous stain. Aberrant staining is mentioned. on more than one occasion.

CORRECTION / RESPONSE BY CANDIDATE:

This had already been discussed previously , however examiners explanation is included (blue text).

Pg 92 – *Discussion-2nd paragraph*

Miller et al [3] reported that a potential pitfall present on immunostained cytological smears leading to erroneous staining is the trapping of reagents in the three-dimensional cell clusters. In FNA smears the cytoplasm covers the nucleus, therefore a cytoplasmic stain will in some instances show nuclear staining in addition to cytoplasmic staining. Interpretation of these smears should be confined to areas where the cells are present in monolayers in order to render definitive results. [3] This phenomenon was not observed in the cell block samples since they are two- dimensional. On sectioning the cell blocks the cytoplasm does not cover the nucleus hence the lack of nuclear staining. This facilitates ease of interpretation of immunostains on cell block samples.[3]

REFERENCES:

COMMENT BY EXAMINER 1:

A sample of the references has been checked. Please correct the title of reference 52 to "The impact of limited specimen size.

CORRECTION / RESPONSE BY CANDIDATE:

Corrected accordingly.

EXAMINER 2:

COMMENT BY EXAMINER 2:

Pg 94: Discussion - 4.7

This is well written and the literature review is adequate. However one of the deficits which does need to be corrected is that there is no overview of the various techniques of cellblock preparation which are currently available. There is only a small section of this under discussion 4.7. A rationale as to why Shandon was selected for the study should be given.

CORRECTION / RESPONSE BY CANDIDATE:

This has already been discussed however a further explanation is included (in blue text and table).

4.7 Other techniques (pg 94)

Many other methods (agar, plasma, thromboplastin[4, 13, 14, 15, 16] to prepare cell blocks have been utilised successfully by other teams which is also aimed at recovering sparse material

obtained during the FNA procedure. The use of the Shandon Cytoblock Cell Block Preparation System proved beneficial in previous studies undertaken in this department and a review of the literature did not reveal any negative views on its use. [25, 27, 28, 29, 30, 31] Consequently it was utilized in this study since it was possible to capture small groups of cells which would have otherwise been impossible to achieve with routine tissue processing.

Name of technique	**Method of action (very brief 1-2 lines)**	**Advantages**	**Disadvantages**	**Comments**
Plasma Thromoplastin / thrombin-based [13] [4, 14]	Small cellular fragments are captured by the clotting action of the plasma.	Good cellularity obtained.	Success depends on high cellularity of the aspirate.	-
Rapid cell block technique [15]	Automated system. Deposits needle rinse at one plane.	Higher yield of tissue fragments obtained.	Automated system. Expensive. Success depends on high cellularity of the aspirate.	Cost effectiveness must be assessed before routine implementation.
Agar [16]	Molten agar is used to capture small cellular fragments after hardening at 4ºC.	Good cellularity obtained.	Success depends on high cellularity of the aspirate.	-
Shandon Cytoblock system [25, 27, 28, 29, 30, 31]	A cell button is formed by gelling action of Reagent 1 and 2 available in the kit. This results in the capture of small cellular fragments.	Cost effective for our setting. Previously utilized successfully in our department.	Success depends on high cellularity of the aspirate.	Available in kit form thereby providing convenience, consistency and reliability of reagents and consumables used.

EXAMINER 2:

COMMENT BY EXAMINER 2:

The list of figures is redundant as these are all listed in the table of contents.

CORRECTION / RESPONSE BY CANDIDATE:

The list of figures ensures convenience in locating tables.

EXAMINER 2:

COMMENT BY EXAMINER 2:

Page 13 tables 1.2 and 1.3 should be combined into one table.

CORRECTION / RESPONSE BY CANDIDATE:

Tables are separated out for ease of understanding and display of data.

EXAMINER 2:

COMMENT BY EXAMINER 2:

Pg2: 1.3 line 10 - it is preferable to refer to antibodies rather than immuno stains

CORRECTION / RESPONSE BY CANDIDATE:

Corrected accordingly.

COMMENT BY EXAMINER 2:

Results :

The student noted that aberrant nuclear staining was displayed in the FNA smears whilst this phenomenon was not observed in the cellblock techniques. This may be explained as follows: FNA smears are three-dimensional and cytoplasm covers the nucleus, therefore a cytoplasmic stain will show in some instances nuclear staining in addition to cytoplasmic staining whilst a cellblock preparation is two-dimensional and on sectioning the cytoplasm will not be covering the nucleus therefore not showing nuclear staining.

CORRECTION / RESPONSE BY CANDIDATE:

See Discussion pg 92-93 (and above under Examiner 1)

COMMENT BY EXAMINER 2:

3.5.9 Discrepant Results (pg 55)

It was noted here that the smear was positive for CK 7 and negative for CK 20 whilst the corresponding cellblock was negative for both. The figure demonstrating the positive CK 7 immunostaining shows a group of positive cells on the left which have the appearance of bile duct which would be positive for CK 7.

CORRECTION / RESPONSE BY CANDIDATE:

Pg 55: (3.5.9 Discrepant Results)

The corresponding cell block was negative for CK7 except for a group of positive cells on the left that indicates bile duct epithelium (built-in internal control) that would be expected to stain positively with CK7. The examiner's comment is acknowledged.