ISSN No:-2456-2165

Assessment of Staining Quality of Histological Specimens Differentiated by 1% HCL in Alcohol and 5% Acetic Acid in Alcohol During Routine Staining at MNH

RESEARCH REPORT

Aveth F. Mwanyika
Department of Pathology Muhimbili University of Health and Allied Sciences (Muhas)
School of Medicine

Registration Number : 2019-04-13173

Degree Program : Bachelor of Medical Laboratory Sciences in Histotechnology

A Research Report to be submitted for Partial Fulfillment for Award of Bachelor of Medical Laboratory Science in Histotechnology

ISSN No:-2456-2165

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences, a research report entitled "**assessment of staining quality of histological specimens differentiated by 1% HCL in alcohol and 5% Acetic Acid in alcohol during routine staining at MNH**" in (partial) fulfillment of the requirement for the Bachelor degree of Medical Laboratory Science in Histotechnology of Muhimbili University of Health and Allied Sciences.

ISSN No:-2456-2165

DECLARATION AND COPYRIGHT

I, Aveth F. Mwanyika declare that this Research report is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature …… Date……

ISSN No:-2456-2165

ACKNOWLEDGEMENT

I would like to express my sincere gratitude goes to Almighty God and for granting me health during whole time of my studies and Dr. Edda Vuhahula for her supervision, support and guidance towards the preparation and completion of this Research report, may God bless you and thank you very much.

I would like to thank all staffs at Histopathology unity at MNH for their support and cooperation during data collection.

My appreciation to **Dr. Jabu** for her support during data analysis and results interpretation.

My thanksgiving to my colleagues, Bachelor of Medical Laboratory Sciences in Histotechnology (2019-2022) for their hand-to-hand support and kind help during my time at MUHAS and my senior technologist **Ms. Mwasiti A Zakaria** for her undivided support

My sincere gratitude goes to the sponsor of this research project, my parents, for providing me with funds to cover part of the costs of this work.

I would like to appreciate my friends their encouragement, and support during my all time of study and toward completion of this research.

Lastly, I would like to appreciate my family for their tireless support from beginning of my studies up to now.

ISSN No:-2456-2165

DEDICATION

I dedicate this wok to my beloved mother **Mrs. Mary Mwanyika** for her endless support during my all time of studying, for providing for me and never tired of me, God bless her.

TABLE OF CONTENTS

ISSN No:-2456-2165

LIST OF FIGURES

ISSN No:-2456-2165

LIST OF DIAGRAMS

ISSN No:-2456-2165

LIST OF TABLES

ISSN No:-2456-2165

LIST OF ABBREVATIONS

MUHAS	Muhimbili University of Health and Allied sciences.
MNH	Muhimbili National Hospital.
H&E	Haematoxylin and Eosin.
HCL	Hydrochloric Acid
SSPS	Statistical Package for the Social Science.
FFPE	Formalin Fixed Paraffin Embedded SOP Standard Operating Procedures.

ISSN No:-2456-2165

DEFINITION OF TERMS

For the purpose of the research the following terms need to be understood.

- *Histology*
  Is the study of the microscopic structure of tissue.

- *Histopathology*
  This is the diagnosis and study of diseases of the tissues, and involves examining tissues under microscope.

- *Staining Efficacy*
  This is the ability of a staining technique to produce a desired or intended contrast in histological samples generally at microscopic level.

- *Hematoxylin and Eosin Staining (H&E)*
  This is a routine staining technique at MNH histopathology laboratory. H&E is a combination of haematoxylin and eosin. The haematoxylin stains cell nuclei purplish blue, and eosin stains extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combination of these colours.

- *Staining Quality*
  It is a term used to describe the standard of staining process by assessing various parameters such as nucleus, cytoplasm, nucleolus, chromatins, nuclear membrane and others as they are observed under microscope.

- *Staining*
  A technique in which cell and thin sections of biological tissue that are normally transparent are immersed in one or more colored dye(stains) to make them more clearly visible through the microscope.

- *Stain*
  It is a colored material in a solution form with the ability to impart its color to another substance.

- *Differentiation*
  Is the process of removing excess dye from tissue in order to accentuate a structure which retains the dye while all about are losing theirs

ISSN No:-2456-2165

ABSTRACT

Histological staining throughout history has undergone significant changes in the techniques used during routine staining of histological specimens. One of the crucial stages for a good staining quality is the differentiation stage of the primary stain (hematoxylin). Differentiation of stains allows selective removal of excess stain onto undesirable parts of the section to the satisfaction of the technician.

In the routine staining, the common differentiator used is 1% Hydrochloric acid in 70% alcohol however in other laboratories use less strong acids during differentiation. At MNH 1% Hydrochloric acid in 70% alcohol is used which has shortcomings during differentiation process.

- ***Objective***

To compare the staining quality of histological sections between hydrochloric acid-alcohol and acetic acid-alcohol used as differentiators at MNH.

- ***Methodology***

The study was a prospective cross-sectional conducted at MNH Anatomical Pathology. Histological specimens were obtained during grossing sessions where some remnants of where by fixed sold massbased tissue were packed in respective cassettes and processed. Sections were routinely stained (hematoxylin and eosin staining) and differentiated using 1% HCL in alcohol and 5% Acetic acid in alcohol. Stained sections were observed and assessed the staining quality using a bright field microscope. Raw data was captured on structured collection sheets and SPSS 28.1.1.1 will be used for data cleaning and analysis. P-values of 0.05 were the cut-off point for statistical significance.

- ***Results***

The study showed that of 5% Acetic acid in alcohol as a differentiator provides a quality histological stain where the nucleus staining quality and cytoplasm staining quality was assessed statistically using SPSS 28.1.1.1 as compared to 1% HCL in alcohol showed that it produces sections that are over-differentiated. Thus, providing poor staining quality

- ***Conclusion***

5% Acetic acid in alcohol as a differentiator produces a more controlled differentiating and a quality routine histological stain compared to 1% HCL in alcohol

- ***Recommedations***

5% Acetic acid in alcohol provides quality and crisp nucleus stain and does not exert stress to the cellular components of the tissue.

ISSN No:-2456-2165

CHAPTER ONE
INTRODUCTION

- *Background*

Histology is the microscopic study of animal and plant cell and tissues through staining and sectioning and examining them under a microscope (electron or light microscope). There are various methods used to study tissue characteristics and microscopic structures of the cells. Histology is used extensively in medicine especially in the study of diseased tissues to aid treatment. [1]

Histological staining is a series of technique processes undertaken in the preparation of sample tissues by staining using histological stains to aid in the microscope study. [3] The process of histological staining takes five key stages which involve; fixation, processing, embedding, sectioning and staining. [4]

Histological staining throughout history has undergone significant changes in the techniques used during routine staining of histological specimens. The routinely used histological stain is Hematoxylin and Eosin. Staining is used to highlight important features of the tissue as well as to enhance the tissue contrast. Hematoxylin is a basic dye that is commonly used in this process and stains the nuclei giving it a bluish color while eosin (another stain dye used in histology) stains the cell's nucleus giving it a pinkish stain. However, there are other several staining techniques used for particular cells and components. [1] Hematoxylin as itself cannot be called a stain but until it goes through a process known oxidation or ripening. Hematoxylin can be grouped into two in terms of ways or means of oxidation as follows: natural oxidation, which use air for oxidation. It usually months for it be oxidized. Ehrlich's and Delafield's hematoxylin solutions are examples of naturally ripened hematoxylins. Another type of hematoxylin is the one which is chemically oxidized, Chemical oxidizing agents convert the hematoxylin to hematein rapidly, so these solutions are ready for use immediately after preparation. Sodium iodate used in Mayer's hematoxylin and mercuric oxide used in Harris's hematoxylin are examples of these agents. These solutions have a shorter useful life than the naturally oxidized hematoxylins because the oxidation process continues in air and light, converting the hematein to a colorless compound. [5] Other types of hematoxylins according to the mordant used are alum hematoxylin, iron hematoxylin, tungsten hematoxylin, molybdenum hematoxylin, lead hematoxylin and hematoxylin without mordant. [6] The most commonly used hematoxylin is the alum hematoxylin (Harris hematoxylin).

One of the crucial stages for a good staining quality is the differentiation stage of the primary stain. Differentiation of stains allows selective removal of excess stain onto undesirable parts of the section to the satisfaction of the technician.

The variation of the stain intensity is often driven by the pathologist's learning experience and personal preference. [1]

The primary stain (hematoxylin) is used illustrate nuclear details in the cells. Depth of the coloration is not related to the amount of DNA in the nuclei but also the length of the time the sample spends in the hematoxylin. Histological staining using hematoxylin can be used either progressively or regressively. Progressive stain is when the tissue as left in hematoxylin until the end point of staining however, it is difficult to get the right intensity for the stain. Regressive staining, the tissue sections are deliberately overstained and then the excess stain is removed through the process of differentiation. Routine histological staining using Harris Hematoxylin uses regressively staining. The differentiation of the stains allows for the ability to selectively remove the stain from tissue to the taste of the viewer, in the case of hematoxylin hydrochloric acid is commonly used as differentiator.

- *Problem Statement*

Differentiation is the crucial step in the routine staining of histological sections. There are various means to achieve differentiation of the primary stain to get good quality stain, throughout history of advancing of histological staining techniques various methods were imploded to achieve differentiation through use of acids-alcohol commonly 1% HCL in 70% ethanol.

At MNH Central Pathology Laboratory the routinely used form of differentiation is the traditional hydrochloric acid-alcohol. However, the use of 1% Hydrochloric acid-alcohol as differentiator as per protocols established in the SOP of MNH Central Pathology Laboratory does not into account the maturity of the hematoxylin stain. This brings about variations in the staining of hematoxylin stain in histological specimens due to the fact that hematoxylin ages with time and differentiation used does not take into account about the maturity of the hematoxylin.

- *Rationale*

The study used the two methods of differentiation of hematoxylin stain thus ending up with a differentiator which have consistent result despite the maturity of the hematoxylin stain in various histological specimens. The study focused on the reproducibility and stainability of the two differentiators in various histological specimens provided that other staining variables are kept constant such as the composition of the staining reagents.

- *Research Question*

The following are the research questions that spearheaded and guided the entire research process and uncovered other unforeseen challenges concerning the research at hand.

ISSN No:-2456-2165

- Does differentiation using acetic acid-alcohol give similar result like the traditional hydrochloric acid-alcohol?
- Does acetic acid-alcohol differentiator increase or decrease the nuclear stain intensity?
- Does the use of acetic acid-alcohol as a differentiator consistent to different types of tissues?

➢ *Objectives*

- *Broad Objective.*

To compare the staining quality of histological sections between hydrochloric acid-alcohol and acetic acid-alcohol used as differentiators at MNH.

- *Specific Objectives.*

✓ To assess the staining quality of 1% hydrochloric acid-alcohol as a differentiator in different histological specimens.
✓ To assess the staining quality of 5% acetic acid-alcohol as a differentiator in different histological specimens.
✓ To compare the staining quality of hydrochloric acid-alcohol and acetic acid-alcohol used as differentiators in histological specimens.

➢ *Literature Review*

Differentiation is the process of removing the excess dye from the tissue in order to accentuate a structure that retains the dye while all the unintended structure lose theirs. It is similar to decolorizing, but infers a high degree of selectivity. The difference between differentiation and decolorizing is the decolorizing is non-selective and removes almost all the dye from the section non-selectively. Apart from this, the two processes are essentially the same. [8]

The differentiation of stains allows for the ability to selectively remove stain from tissue to the taste of the viewer or technician. In routine hematoxylin and eosin stain, the Harris hematoxylin is used regressively thus hydrochloric acid (for rapid differentiation) and acetic acid (for slower, more controlled differentiation) are most commonly used. Other laboratories tend to use more milder acids in the process of differentiation to provide gentler dye removal. [9]

The alum hematoxylin is chemically ripened with mercuric oxide but Mercury is highly toxic, environmentally unfriendly and has detrimental and corrosive long-term effects on automated staining machines, so sodium or potassium iodate is now generally used for the oxidation. The alum hematoxylin despite to be used commonly as regressive but it can also be used progressively, particularly in situations where a nuclear counterstain is needed to emphasize a cytoplasmic component which has been demonstrated by a special stain and where the acid-alcohol differentiation might destroy or de-colorize the histological section. [5]

The use of acetic acid-alcohol rinse as a differentiator is a more controllable method for removing excess stain from tissue components. The traditional hydrochloric acid-alcohol acts quickly and indiscriminately and since this is more difficult to control it can result in a light nuclear stain. A 5–10% solution of acetic acid in 70–95% alcohol detaches dye molecules from the cytoplasm and nucleoplasm while keeping nucleic acid complexes intact. [7]

ISSN No:-2456-2165

CHAPTER TWO
METHODS AND MATERIAL

➢ *Study Design.*

The study was a cross-sectional study at MNH Central Pathology Laboratory, histopathology unit from May to June 2022.

➢ *Study Population.*

The sample size was obtained from the remnants of histological samples received in MNH which are solid mass based such as cervix, liver, thyroid, lymph nodes and salivary glands.

➢ *Inclusion Criteria.*

Remnants of various solid mass based histological specimens which are well fixed were included into the research such as cervix, liver, thyroid, uterus and colon.

➢ *Exclusion Criteria.*

All bones, unfixed tissues and remnants of various non-solid mass-based histological specimens were not be included into the research such as cervix, liver, thyroid, uterus and colon. Tissue blocks archived were also not be included in the study.

➢ *Sampling Procedures.*

During grossing of histological specimens, the remnants of solid mass-based tissues after pathologist have sectioned the area of pathological interest, some of the remnants were blocked in respective cassettes and processed in automatic tissue processing machines overnight. The remnants of non-solid mass-based tissues such as cystic masses were not be packed for processing. Once the processing is done each tissue specimen were embedded and sectioned into two slides, one was subdued to hydrochloric acid-alcohol differentiation and the other slide was subdued to acetic acid-alcohol differentiation.

- *Sampling was a Follows*

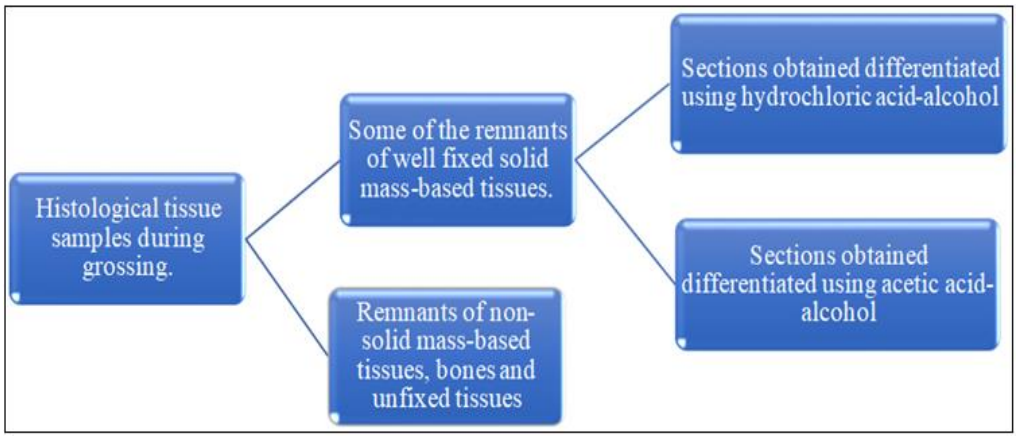

Fig 1 Sampling Procedures for the Research

➢ *Sample Size.*

Since it's a prospective cross-sectional study the sample size for the study was obtained from five different histological tissue sample namely the cervix, uterus, liver, thyroid and colon. Where by each type had five (5) cassettes making a total of the sample size to be twenty-five (25). Each cassette had two slide sections, making a total of 50 slides were stained and observed under a blight field microscope.

➢ *Variables.*

Variables in this study are going to be staining quality whereby they were evaluated as crisp nucleus, dull nucleus, presence of haematoxylin in cytoplasm and presence of haematoxylin in cytoplasm according to the dyeing affinity to the tissue sections. Whereby the details of the nuclear, background and cytoplasmic stain will be observed.

➢ *Laboratory Methodology.*

In the study, the population was obtained from the histopathology laboratory at the MNH Central Pathological Laboratory. The reagent that will be used in the research will be prepared as follows:

- *Hydrochloric Acid-Alcihol.*

The traditional differentiator, that is, 1% hydrochloric acid-alcohol is prepared by dissolving 1% hydrochloric acid in 70% ethanol. Thus, 5mls of hydrochloric acid will be dissolved in 495mls of 70% ethanol solution.

- *Acetic Acid-Alcohol.*

This will be prepared by dissolving 25mls of concentrated solution of acetic acid in 475mls of 70% ethanol solution to form 5% acetic acid-alcohol solution.

Considering that, the following laboratory methods were employed throughout the study:

- *Microtomy*

Due to the fact that the study population are tissue cassettes that have been formalin fixed paraffin embedded (FFPE), thus the study starts with microtomy of the tissue cassettes. The tissue samples were cooled in the freezer then trimmed followed by sectioning by following the same procedures done at the MNH histopathology laboratory in accordance to the MNH Central Pathology, histopathology laboratory unit SOPs' guidance.

- *Staining and Mounting.*

Sections made after sectioning were stained using the routine histological H&E staining technique as stated by the MNH histopathology laboratory SOP, however some were differentiated using hydrochloric acid-alcohol and others using acetic acid-alcohol. After staining, the slides were mounted and cover slipped using DPX ready to be examined and assessed by a bright field microscope.

➢ *Data Collection Methods*

The sectioned and stained slides will be observed under a bright field microscope. Magnifications that will be used will be X10 and X40. Data were collected in special designed data sheets. The hydrochloric acid-alcohol differentiated slides' data were collected separately from acetic acidalcohol differentiated slides' data.

➢ *Investigation Tools, Validity and Reliability Issues.*

- *Investigation Tools.*

The study used well maintained machines which are found at the MNH histopathology laboratory. Sakura Accu-Cut SRM 200 Rotary Microtome was used for trimming and sectioning the tissue blocks. For mounting and cover-slipping there is an automated mounting machine Tissue-Tek Film Coverslipper at MNH histopathology laboratory for slide mounting and cover slipping. Bright field microscope was used for slides examination. All the procedures for staining using 1% HCL in alcohol and 5% Acetic acid in alcohol as differentiators are provided in the appendix section.

- *Examination and Reporting.*

After the laboratory procedures have been accomplished, the stained slides were examined by the investigator. Also, they were reviewed by a senior laboratory technician as well as anatomical pathologist. Then, then data collected was recorded on a special designed data collection sheets after assessing the slides on the bright field microscope.

- *Quality Assurance.*

All the procedures included in this study was performed at the MNH histopathology laboratory. MNH histopathology laboratory is highly and fully equipped to accomplish this study.

The laboratory contains a number of modern machines which are regularly checked for maintaining their good performance. All the procedures adhered to the MNH histopathology laboratory's SOP.

➢ *Data Analysis.*

Raw data was collected and written down on data collection sheets and then data will be imported in SPSS 28.1.1.1 for data cleaning and analysis. Data was summarized in the form of proportions and frequency tables mainly 2X2 table. Graphics and charts will be drawn using SPSS 28.1.1.1.

P-value was computed and a P-value ≤ 0.05 (two-tailed) will be considered statistically significant. The results were compared to the findings from the literature reasons for differences or similarities were discussed. Recommendations from senior laboratory technicians and anatomical pathologist on useful good practices were highlighted.

➢ *Ethical Consideration.*

This study was presented to Muhimbili University of Health and Allied Sciences, MUHAS, for approval by the University Ethical Clearance Committee. After the ethical clearance have been obtained, it was presented to the management of MNH to get the permission to conduct the study.
The confidentiality of the subjects' information was strictly kept. The study is expected to have no adverse effects on the subject.

ISSN No:-2456-2165

CHAPTER THREE
RESULTS

The study included twenty-five (25) tissue blocks with each 2 sections were obtained making an aggregate of 50 slides included in the study. Twenty-five slides were subjected in routine haematoxylin staining procedure with 1% HCL in Alcohol and the other were subjected to 5% Acetic Acid in Alcohol with each having to be exposed to the differentiator solution in a one dip modality. The outcome was analysed as follows:

➢ *Comparison of Staining Quality of Nuclear Stain.*

In this assessment the quality of the nuclear stain was observed under a bright field microscope and the following is the statistical data obtained.

- *Crosstab*

Table 1 Comparison of Nuclear Stain Quality between 5% Acetic Acid in Alcohol and 1% HCL in Alcohol

		Nucleus Staining		
		Crisp Nucleus detail	Dull Nucleus detail	Total
Differentiator solution	5% Acetic acid in Alcohol for 5 min	23	2	25
	1% HCL Acid in Alcohol	14	11	25
Total		37	13	50

- *Chi-Square Test*

Chi-square test was conducted to fully test and support the hypothesis under this study using SPSS 2.28.1.1 where by the p-value was determined as follows:

Table 2 Chi-Square Test for the Nuclear Stain Quality between 5% Acetic Acid in Alcohol and 1% HCL in Alcohol

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.420[a]	1	.004		
Continuity Correction[b]	6.653	1	.010		
Likelihood Ratio	9.071	1	.003		
Fisher's Exact Test				.008	.004
Linear-by-Linear Association	8.252	1	.004		
N of Valid Cases	50				

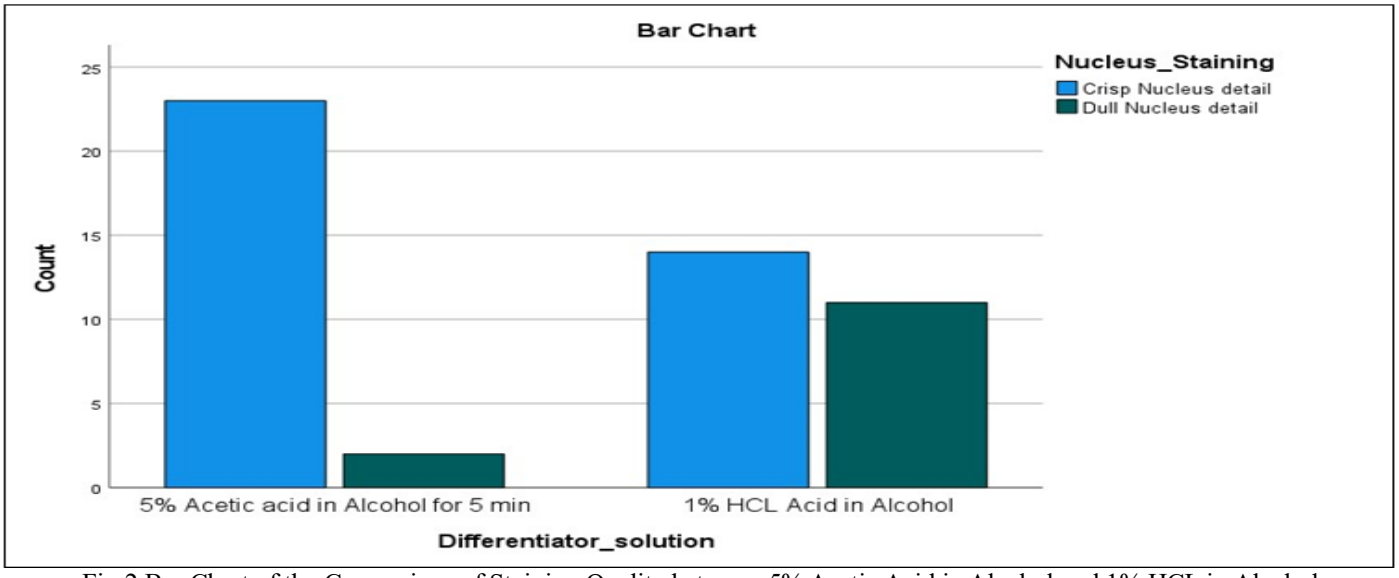

Fig 2 Bar Chart of the Comparison of Staining Quality between 5% Acetic Acid in Alcohol and 1% HCL in Alcohol

➢ *Comparison of Staining Quality of Cytoplasm Stain.*

In the staining of the slides the capabilities of the differentiators were tested through the absence of the haematoxylin stain in the cytoplasm of the cell in each slide. The overall number of slides having haematoxylin and not having haematoxylin satin in the cytoplasm were recorded and presented statistically as follows:

- *Crosstab*

Table 3 Comparison of the Cytoplasm Stain Quality between 5% Acetic Acid in Alcohol and 1% HCL in Alcohol

		Cytoplasm Absence of hematoxylin stain	**Staining Presence of hematoxylin stain**	**Total**
Differentiator solution	5% Acetic acid in Alcohol for 5 min	21	4	25
	1% HCL Acid in Alcohol	18	7	25
Total		39	11	50

- *Chi-Square Test*

Chi-square test was conducted to fully test and support the hypothesis under this study using SPSS 2.28.1.1 where by the p-value was determined as follows:

Table 4 Chi-Square Test for the Nuclear Stain Quality between 5% Acetic Acid in Alcohol and 1% HCL in Alcohol

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.049[a]	1	.306		
Continuity Correction[b]	.466	1	.495		
Likelihood Ratio	1.060	1	.303		
Fisher's Exact Test				.496	.248
Linear-by-Linear Association	1.028	1	.311		
N of Valid Cases	50				

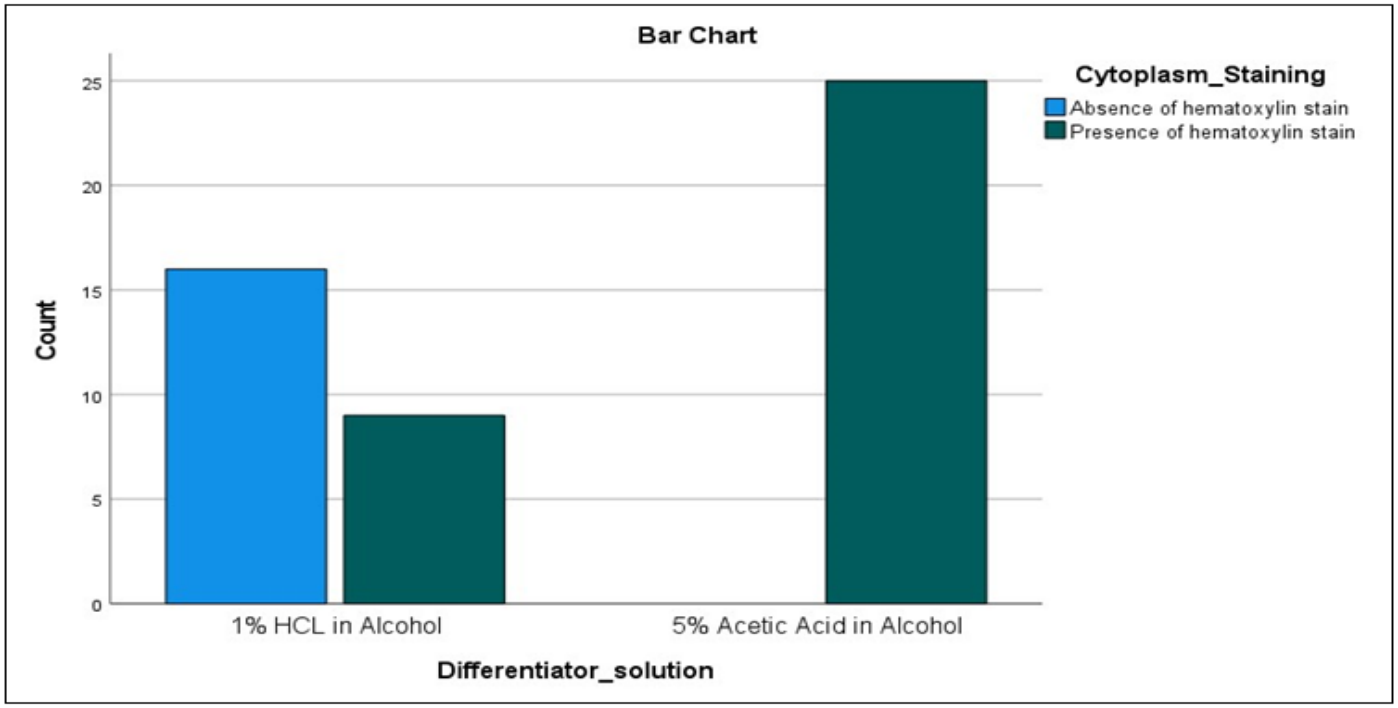

Fig 3 Bar Chart of the Comparison of Cytoplasm Staining Quality between 5% Acetic Acid in Alcohol and 1% HCL in Alcohol

ISSN No:-2456-2165

- *The following are Pictorial Presentations of the Staining Quality using 5% Acetic Acid in Alcohol and 1 % HCL in Alcohol.*

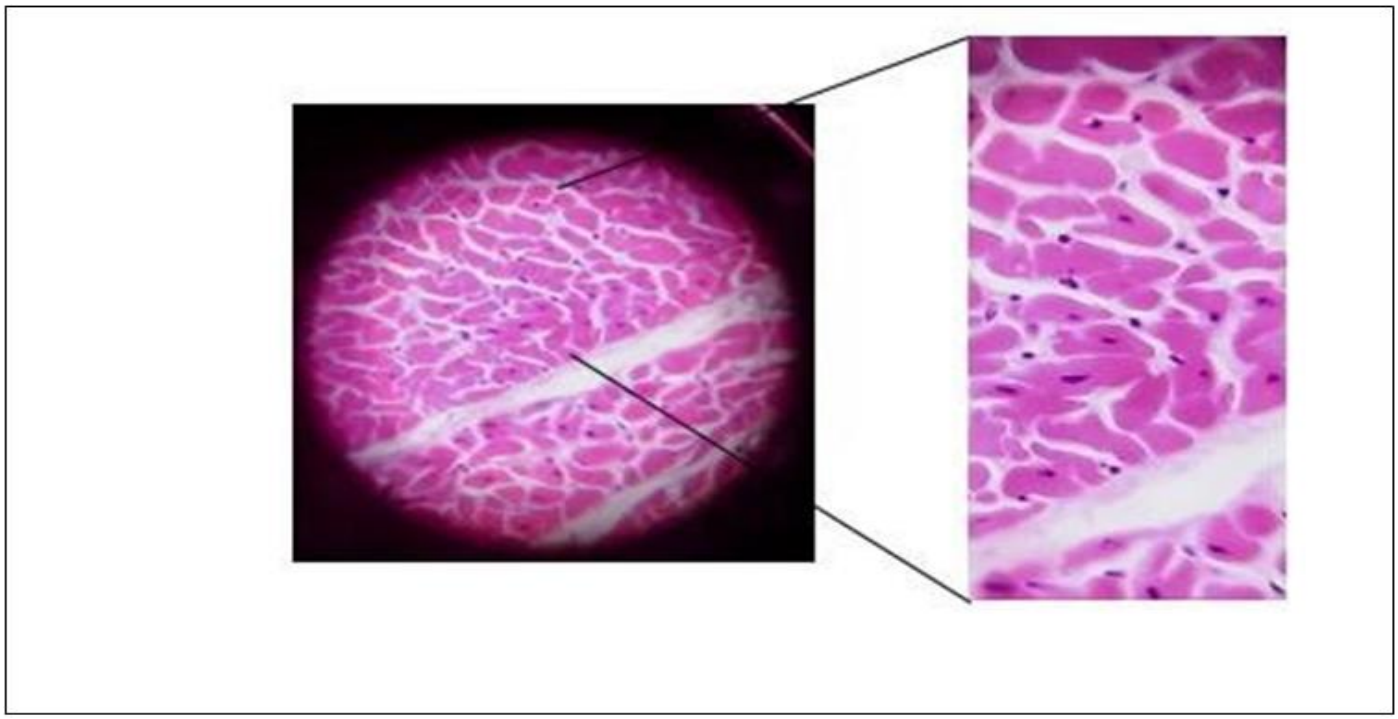

Diagram1: Section Showing Good Nucleus Staining when 5% Acetic Acid in Alcohol was used During the Study

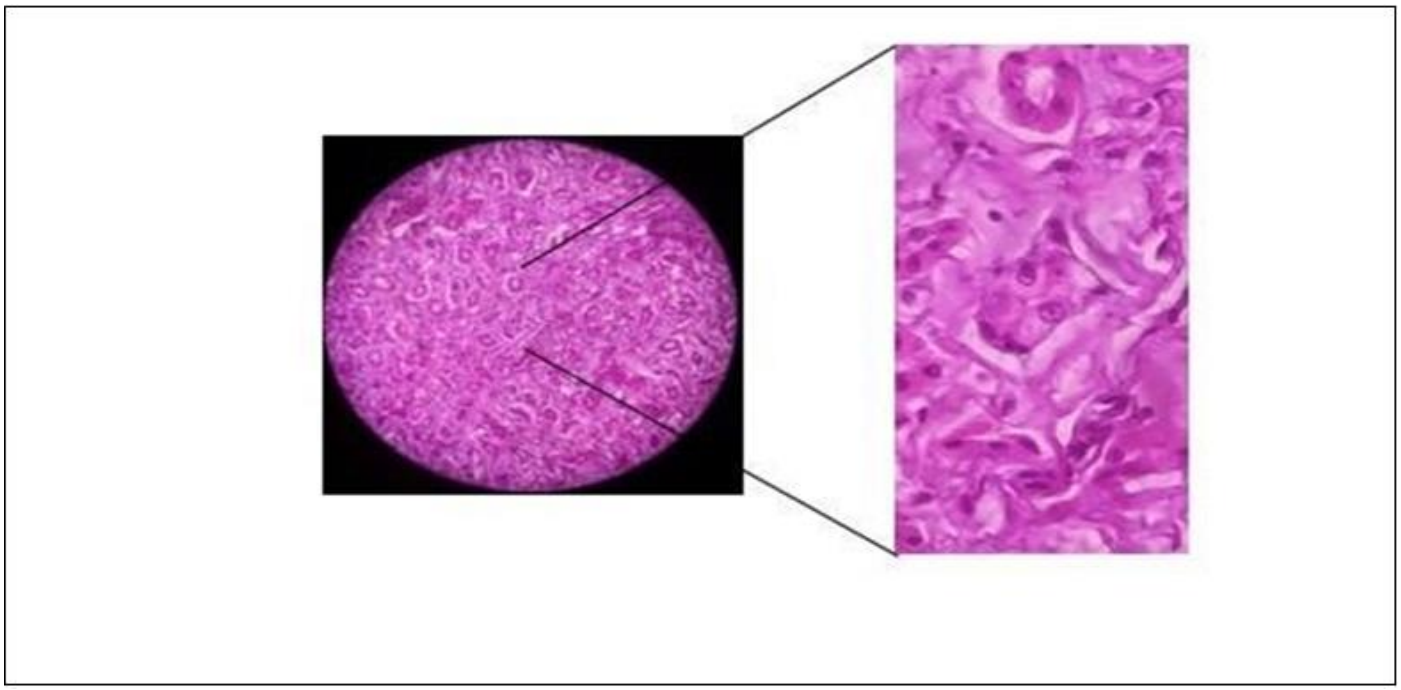

Diagram 2: Showing Bad Nucleus Stainingwhen 1% HCL in Alcohol was used in the Study Causing over Differentiation of the Nucleus Stain

ISSN No:-2456-2165

CHAPTER FOUR
DISCUSSION

Hematoxylin and eosin stain is a routine histological stain which has been used for a long time due to its simplicity in the staining procedure. Hematoxylin stains the cell nuclei blue-black, showing clear intranuclear detail, while eosin stains cell cytoplasm and most connective tissue fibers in varying shades and intensities of pink, orange and red. In the traditional method the quality of the stain depended in the differentiating solution. Hematoxylin was differentiated using acid-alcohol while eosin was differentiated using subsequent ascending grades of alcohol which were constant throughout the history of the stain. In recent years there has been the use of various types of acidalcohol differentiators.

In the study, the quality of histological stain was assessed when 1% HCL in alcohol and 5% Acetic Acid in alcohol as differentiators. The staining qualities of the two (2) differentiators were evaluated in three parameters; Nuclear staining, Cytoplasm staining and Background staining.

- *Nuclear Staining.*

From Table 1 which shows the comparison of the nucleus staining quality between 5% Acetic acid in alcohol and 1% HCL in alcohol shows that there were 21 sections which is equivalent to 85% have crisp nuclear staining while the other 15% have dull nucleuses. These results shows that the quality of the nucleus staining while using 5% Acetic acid produce better quality staining compared to 1% HCL in alcohol. Feldman & Dapson, 1985 et al The traditional hydrochloric acidalcohol acts quickly and indiscriminately and since this is more difficult to control it can result in a light nuclear stain. A 5% Acetic acid in alcohol detaches dye molecules from the cytoplasm and nucleoplasm while keeping nucleic acid complexes intact.

- *Cytoplasm Staining.*

In this study the quality of the cytoplasmic stain as a parameter shows that 5% Acetic acid in alcohol produces the best results since it's a slow and controlled differentiating properties which give the best results. However, the use of 1% HCL in alcohol can produce the same results but the cytoplasm of some tissue sections contained hematoxylin stain due to dye-tissue interaction in the study.

ISSN No:-2456-2165

CHAPTER FIVE
STUDY LIMITATIONS

In the study the concentration of acetic acid used is 5%. Other ascending concentration may have other effects as a differentiator.

ISSN No:-2456-2165

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

- *Conclusion.*

In conclusion, the use of 5% Acetic acid in alcohol as a differentiator produces good results due to its slowed and more controlled differentiating to obtain a crisper nuclear staining by hematoxylin. The use of 1% HCL in alcohol is a fast reliable differentiator it has a risk of overdifferentiating of the hematoxylin stain and result in a lighter stain.

- *Recommendations.*

I recommend the use of 5% Acetic acid in alcohol for best results in research purposes since it takes a lot of time. Acetic acid being an organic acid it doesn't not exert stress to the cellular components of the tissue sections.

REFERENCES

[1]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4804027/?report=reader
[2]. Black J. Microbiology: Principles and exploration. 8th ed. John Wiley Sons; 2012. p. 68.
[3]. [Google Scholar]
[4]. Anderson J. An introduction to Routine and special staining. 2011.
[5]. Titford M. Progress in the development of microscopical techniques for diagnostic pathology. J
[6]. Histotechnology. 2009; 32:9–19. http://dx.doi.org/10.1179/his.2009.32.1.9 . [Google Scholar]
[7]. Bancroft's Theory and Practice of Histological Techniques, Eighth Edition. 2019
[8]. Histotechnology. A Self-Instructional Text, Fourth Edition.
[9]. Feldman, A., & Dapson, R. (1985). Newsletter, Winter. ANATECH
[10]. StainsFile - Stain theory - Differentiation https://stainsfile.info/theory/diff.htm
[11]. https://www.leicabiosystems.com/knowledge-pathway/he-staining-overview-a-guide-to-bestpractices/

ISSN No:-2456-2165

APPENDICIES

➢ *APPENDIX 1: Conventional H&E Stain Procedure*

- Dewax in xylene (2 changes) …….. 10 minutes
- Bring sections down to water (absolute ethyl alcohol, 95%ethyl alcohol, tap water)
- Hematoxylin staining…….10minutes
- Rinse in tap water and differentiation in 1 % HCL – alcohol (single dip)
- Blue in running tap water……10 minutes
- Counter stain in 1% Eosin……3 minutes
- Rinse in tap water and dehydrate sections (95% ethyl alcohol to absolute ethyl alcohol)
- Clear in xylene (2 changes) and mounting.

➢ *APPENDIX 2: H&E Stain Procedure using 5% Acetic Acid in alcohol as a differentiator.*

- Dewax in xylene (2 changes) …….. 10 minutes
- Bring sections down to water (absolute ethyl alcohol, 95%ethyl alcohol, tap water)
- Hematoxylin staining…….10minutes
- Rinse in tap water and differentiation in 5 % acetic acid – alcohol (5 minutes)
- Blue in running tap water……10 minutes
- Counter stain in 1% Eosin……3 minutes
- Rinse in tap water and dehydrate sections (95% ethyl alcohol to absolute ethyl alcohol)
- Clear in xylene (2 changes) and mounting.