# COMPARATIVE STUDY OF EFFICACY OF PAPANICOLAOU AND ACETO-ORCEIN STAINS IN DEMONSTRATING BARR BODIES IN BUCCAL MUCOSAL SMEARS

**By**

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**Dissertation submitted to the**

**BLDE (DEEMED TO BE UNIVERSITY), Vijayapura, Karnataka**

**In partial fulfilment of the requirements for the award of the degree of**

**DOCTOR OF MEDICINE**

**IN**

**PATHOLOGY**

**Under the Guidance of**

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## ACKNOWLEDGEMENT

First and foremost, I thank God, the Almighty, for His blessings throughout my life and in accomplishing the present study successfully.

I thank my parents **Mrs. Neeraja V.** and **Dr. Rami Reddy V.** for their constant support and encouragement.

I thank my husband **Dr. Ashok Reddy P.** for his constant support and encouragement.

The study of this project has been accomplished to the maximum refinement through months of endeavour and hard work. It is my humble duty to express my sincere gratitude and acknowledgement to a number of persons who have inspired, supported and guided me during my entire project works.

I remain indebted and express my deep sense of gratitude to my esteemed and honoured teacher and guide **Dr. B.R. YELIKAR** **M.D**, Professor & Head, Department of Pathology, Shri B.M. Patil Medical College, Hospital and Research Centre, B.L.D.E. University, (Deemed to be University)Vijayapur for providing me invaluable guidance towards the correct and precise approach, constructive criticism, seamless encouragement and meticulous supervision to constantly update and improve my study and the project works.

I am extremely fortunate to have such an approachable and supportive department. I am thankful to **Dr. S.U. Arakeri** Prof**, Dr. R.M. Potekar** Prof, **Dr. S.B. Hippargi** Prof **Dr. Mahesh H. Karigoudar** Prof, **Dr. Prakash M. Patil** Assoc Prof, **Dr. Girija S. Patil** Assoc Prof, **Dr. Vijayalaxmi S. Patil** Asst Prof, **Dr. Savitri M. Nerune** Asst prof, **Dr. Mamatha K.** Asst Prof, **Dr. Sneha Jawalkar** Asst Prof and for their supervision, concern and positive feedback during this period.

Special thanks to my senior and staff **Dr. Anil Reddy K.** Asst Prof and my friend **Dr. Rama Devi P.** who have helped and encouraged me during my work.

Special thanks to my senior **Dr. Mahmood Nawaz khan** and my friends **Dr. Neha, Dr. Ankur Kumar and Dr. Deepthi CH.** who have encouraged me during my work. My sincere thanks to all my batch mates, seniors and juniors who have helped me during my work.

My heartfelt thanks to my sister **Mrs. Bhavana V.** and my in - laws for their help, constant encouragement and moral support that led me to successfully complete this work.

My heartfelt gratitude to statisticians, **Mohd. Shannawaz** for his valuable suggestions towards the completion of this project.

I am also thankful to all the non-teaching staff of the Department of Pathology especially **Mrs. Jessy John** and others who were very helpful during my work.

Last but not the least**,** my sincere gratitude to all my study subjects whose cooperation has contributed to this study.

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## LIST OF ABBREVATIONS USED

| | | |
|---|---|---|
| AO | – | Aceto-Orcein |
| D.P.X | – | Distyrene, Plasticizer and Xylene |
| DNA | – | Deoxyribonucleic acid |
| EA 36 | – | Eosin Azure 36 (Proportion of the dye ) |
| FISH | – | Fluorescent In Situ Hybridization |
| H & E | – | Hematoxylin and Eosin |
| LAMP | – | Loop mediated isothermal amplification. |
| OG 6 | – | Orange G dye with added phosphotungstic acid. |
| PAP | – | Papanicolaou stain |
| % | – | Percentage |
| PCR | – | Polymerase chain reaction |
| SRY | – | Sex determining region Y |
| TFS | – | Testicular Feminization syndrome |
| TDF | – | Testis-determining factor |
| XX | – | Genetically Female |
| XY | – | Genetically Male |

# ABSTRACT

**Introduction**

Establishing individuality plays a vital role in medical conditions like ambiguous genitalia and in crime investigations. Among the various methods like hair root sheath, dental pulp, tooth dimensions and advanced techniques like DNA analysis available to establish the identity of an individual, buccal smears for Barr body evaluation forms the first line of investigation. The reason for this being it is a non - invasive procedure and causes minimal discomfort to the subjects. Barr body (sex determination) estimation in buccal mucosal scrapes has been demonstrated by using different stains like Papanicolaou, Orcein, Feulgen, Guard, Cresyl violet, Carbol fuchsin and fluorescent staining methods. In buccal smears the percentage of nuclei containing Barr body ranges from 0 to 4 in males and 20 to 80 in females.

**Aim**

To compare the Papanicolaou and Aceto-Orcein stains for their efficacy in demonstration of Barr bodies in both genders.

**Materials and Methods**

A total of 207 medical students were included in the study. Two buccal smears were collected, and subsequently, one was stained with Aceto-Orcein by squash technique and the other with Papanicolaou stain. Both slides were evaluated for percentage of Barr bodies using 1000x magnification and cytomorphological features in 400x.

**Results**

The percentage of Barr bodies in Aceto-Orcein stained slides ranged from 5-18 among females and 0-8 in males, while with Papanicolaou stain the ranges recorded were 4 – 12 in females, 0 – 2 in males. The accuracy of Aceto-Orcein for

detecting sex accurately was 97% and while for Papanicolaou it was 91%. Evaluation of the buccal smears was better in AO stained smears because of the clean background and better cytoplasmic and nuclear contrast in comparison to PAP stain.

**Conclusion**

Aceto-orcein staining method is rapid, economical, accurate, reproducible and comparable to Papanicolaou staining, for the detection of Barr body in buccal mucosal smears.

**Keywords**

Aceto-Orcein, Papanicolaou, Buccal smears, Barr body.

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# 1. INTRODUCTION

Demonstration of identity is a very crucial aspect in any crime investigation. Sex determination plays an important role in this process of knowing the identity of a person especially when insufficient samples are available for examination. In a crime scene or natural disasters determining sexual identity can be done from various tissue samples or saliva stains found on body of the victim or on the weapons.[1]

Demonstration of genetical sex has an important role in sex determination. Sex identification can be done by various methods like morphometric analysis (of tooth, skull and other soft tissues of oral and paraoral region) karyotyping, demonstration of fluorescent body (Y chromatin), polymerase chain reaction and Barr bodies. Among all the techniques, demonstration of Barr bodies is preferred, as it is done by simple stain methods.[2]

Most easily harvestable tissue sample for the Barr body evaluation is obtained from buccal mucosa, which can be performed by simple exfoliative cytology without inflicting trauma to the subject.[3]

Barr bodies develop as a result of inactivation of the X chromosome in normal female somatic cell by lyonization. They are Feulgen positive, heteropyknotic and basophilic intranuclear structures demonstrable during interphase. All nuclear structures are known to fluoresce. Similarly, Barr bodies also fluoresce.[3,4]

Sex determination of a person can be done by demonstrating the percentage of Barr-body positive cells, as two non- overlapping ranges for the percentage of Barr-body positive cells have been obtained for men and women.[3]

Papanicolaou stain has been time tested and is in vogue even today for the estimation of Barr bodies in buccal smears. Even though it offers excellent contrast and is a simple procedure, it is time consuming, which has led to the increasing interest in utilizing stains like Leishman stain, Hematoxylin and Eosin ( H & E ), Thionine, Cresyl violet, Giemsa and Aceto-Orcein.

Amongst these staining methods being studied, Aceto-Orcein is rapid, chromatin specific and cost effective which is best suited for immediate screening of Barr bodies, in comparison to Papanicolaou stain. However, literature with regards to this comparison is scarce. So, to add to the available data, this study was taken up to assess the efficacy of sex determination by a comparison between Papanicolaou and Aceto-Orcein stains for the detection of Barr bodies in buccal mucosal scrapings.

## 2. OBJECTIVE OF THE STUDY

To compare the Papanicolaou and Aceto-Orcein stains for their efficacy in demonstration of Barr bodies in buccal mucosal smears.

# 3. REVIEW OF LITERATURE

Sex determination forms an essential part of investigation in certain situations to establish the sex of an individual in case of ambiguous genitalia, natural disasters and crime scenes. There are many sex determination systems in use like chromosomal, temperature based, environmental based. XX/XY, XX/XO, ZW, UV and haplodiploidy are various chromosomal based sex determination systems. In humans and mammals, it is XX/XY sex determination system which determines the genetical sex of the off spring.[5,6]

Humans have 44 autosomes and two allosomes, which code for all the genes required for somatic and sexual development respectively. X and Y chromosomes are the sex determining allosomes. X chromosome is transmitted to the offspring through maternal inheritance and paternally determined X or Y chromosome determines the sex of the individual. XX karyotype makes the offspring genetical female and XY karyotype makes the offspring genetical male.[5]

Herman Henking in 1890 was the first to discover and use the term X chromosome and Clarence Erwin McClung first discovered its role in sex determination.[7] Later in the year 1905, Nettie Stevens discovered the other sex chromosome and named it as Y chromosome, following the alphabetical order.

In the early stages of embryonic development there is no sexual differentiation between either sex and both have equal internal structures. The presence of SRY gene on Y chromosome plays the main role in release of various hormones which help in the development of testes in males Fig (1) and regression of paramesonephric ducts making them phenotypically male. In genetical females mesonephric ducts regress making them phenotypically female.[5] However, there are some exceptions resulting in intersexuality resulting in Turner syndrome and Klinefelter syndrome.

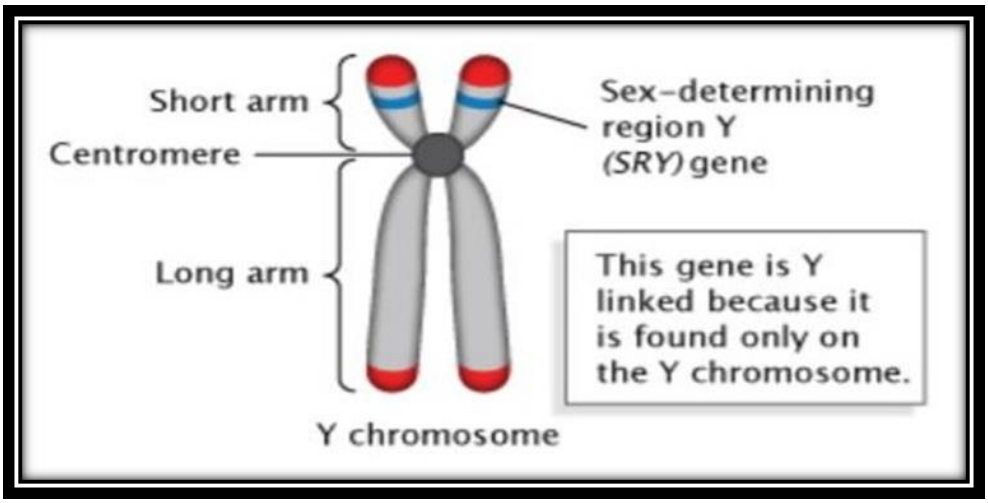



**Figure 1- Presence of SRY gene in Y chromosome[5]**

Turner syndrome occurs when one of the two X chromosomes normally found in females is missing or incomplete. Exact cause of Turner syndrome is not known and this occurs as a result of a random error during the formation of either the egg or sperm. Few individuals with Turner syndrome do have two X chromosomes, out of which one is incomplete. In other scenario called as Mosaicism, there is presence of cells with two X chromosomes as well as cells with only one X chromosome.[8]

In case of Klinefelter syndrome, it usually occurs as a random event in the form of a mutation during the formation of reproductive cells. So instead of the normal 46XY pattern they have an 47XXY pattern.[9]

The degree of symptoms vary according to the number of cells with extra X chromosomes. Klinefelter syndrome is when males have an extra X sex chromosome in most of their cells.

**BARR BODY – HISTORICAL REVIEW:**

Murray Barr & Bertram in 1949, described a small body present in the nuclei of neurons of female cats but absent in neurons of male cats. This body was first named as 'nucleolar satellite', but the name was changed to 'sex chromatin' when it was found that in many types of cells the body was not associated with the nucleolus. Later the name was changed from sex chromatin to Barr body after Dr. Murray Llewellyn Barr.[4,10]

Sex chromatin can be demonstrated in practically all tissues of most of the female mammals except marsupials like Kangaroos, Opossums etc. On the other hand, sex chromatin cannot be demonstrated, or only with great difficulty, in rodents, such as rats and mice. Sex chromatin is present in a large variety of human tissues including blood, semen, hair root, dental pulp, bone cells[11], cells of the retina[12] and saliva containing buccal mucosal cells.[13]

Two sources which have proved of great practical value were cells scraped off the oral mucosa, and the polymorph nuclear leucocytes, in which a sex difference (drumstick) was found by Davidson & Smith in 1954 Fig (2). Sex chromatin bodies other than drumsticks are now often called 'Barr bodies'. [14,15]

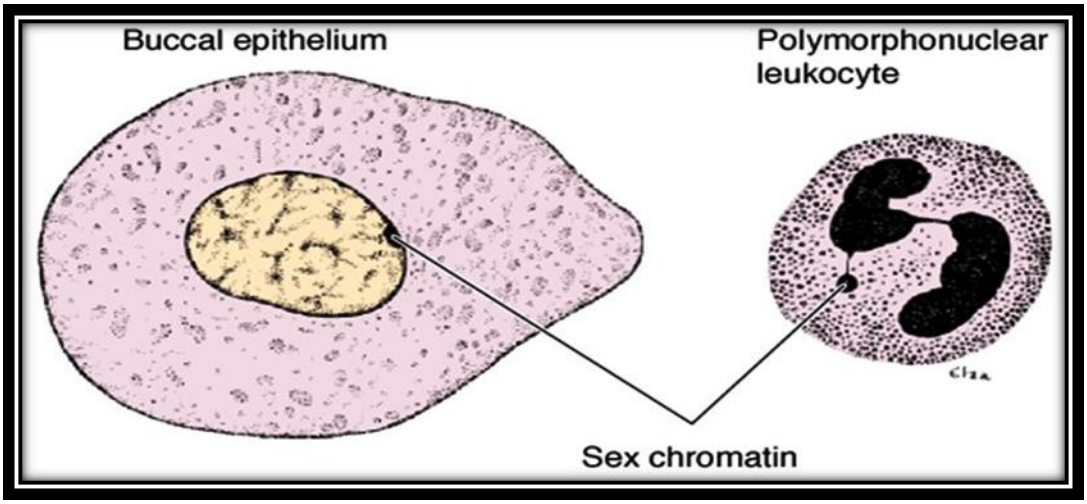



**FIGURE 2 - Barr Bodies in Neutrophils and Buccal Epithelium[16]**

Sex chromatin became part of human genetics in 1953, when Moore, Graham & Barr showed that it could be demonstrated in preparations of human skin biopsies. When chromosome studies became available from 1959 onwards, it became clear that there was a striking correlation between the presence or absence of sex chromatin and the number of X chromosomes in the nucleus. Sex chromatin indicates the presence of two X chromosomes, while the lack of sex chromatin is associated with a single X chromosome.[16]

Barr body can be observed with various nuclear stains like Feulgen, Carbol-fuchsin, Hematoxylin-eosin, Papanicolaou, Cresyl violet, Aceto-Orcein, and Fluorescence stains by Duffy in 1989. Sex determination by Barr body is rapid and easily implemented as it requires little equipment in contrast to techniques, such as PCR. Murakami H et al [17] in the year 2000 studied the sex determination using PCR on both permanent and primary tooth samples. The sensitivity of PCR for detection of Y chromosome - alphoid repeat sequence and X chromosome specific alphoid repeat sequence was 0.5 pg of genomic DNA and LAMP (Loop mediated isothermal amplification) method by Nogami et al.[18]

A Barr body represents inactive X chromosome in a female mammalian cell. Inactivation occurs by a process called lyonization.[19] According to Lyon hypothesis, in cells with multiple X chromosomes, all except one are inactivated in the early embryogenesis of mammals, except in marsupials.

## LYON'S HYPOTHESIS

STRUCTURE OF CHROMOSOME:

Chromosome can be defined as an organized structure, which consists of a single pair, long piece of Deoxyribo-Nucleic Acid (DNA) and its size varies from 1 to 30 micrometer in length and 0.2 to 20 micrometers in diameter. They are composed of proteins which includes Protamines, Histones and Nucleic acids.[20]

Mary Lyon, postulated a hypothesis that one of the two X – chromosomes in a female is functional, and the other gets inactivated during early embryogenesis. Either the maternal or the paternal X – chromosome may be activated in any given cell. This process of sex chromatin formation is called as Lyonization.[21] This phenomenon of inactivation of all X chromosomes except one is apparently a random process and usually does not have any phenotypic expression. X- inactivation is random, fixed, occurs early in development. Therefore, an x – linked trait may be expressed by some cells and not by others. [22,23]

As a rule, in human cells of either gender as the amount of Barr bodies that could be differentiated at interphase is one less than the amount of X chromosomes.[23]

Fig (3)

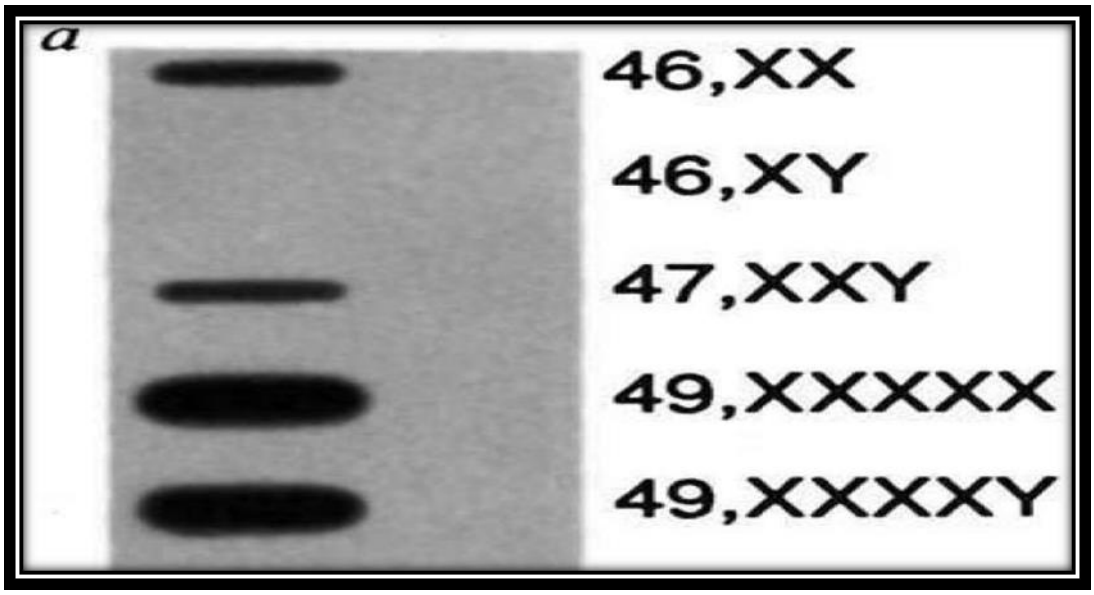



**FIGURE 3 - Chromosomal Patterns in Different Syndromes; Normal Male and Female [23]**

X-chromosome contains X Inactivation Center (XIC) which is located in long arm.

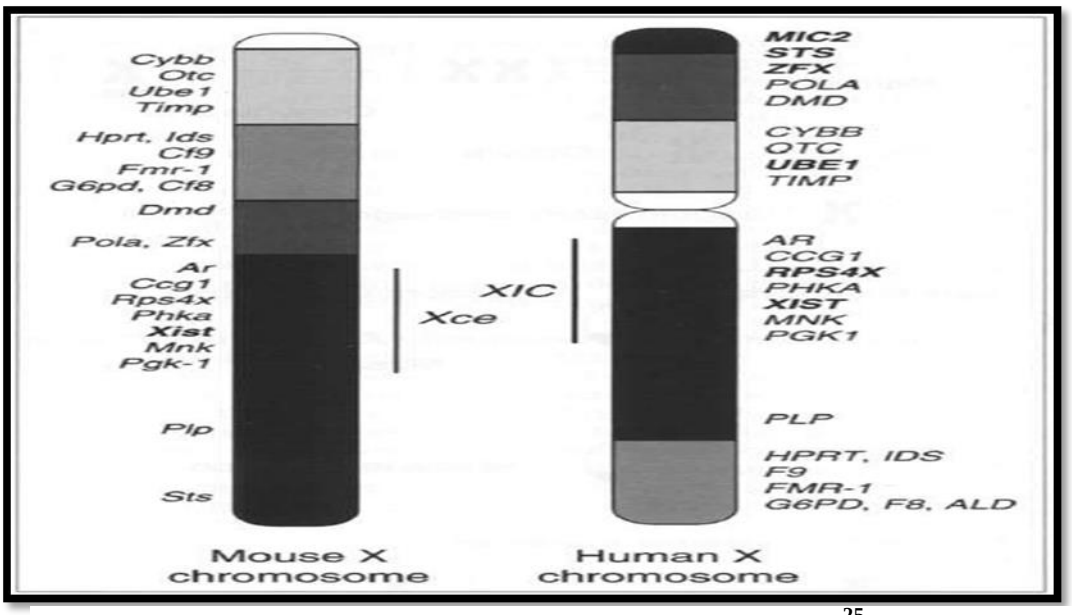



**FIGURE 4 - XIC Gene In Human X Chromosome.[25]**

XIC contains an unusual gene called inactive X (*Xi*)-specific/ transcripts (*XIST*). *XIST* expresses a noncoding functional 17 (kb) RNA molecule. *XIST* is expressed only when more than one X-chromosome is found in the same cell.[24,25] Fig (4)

In humans, usually females have one and males have no Barr bodies in each cell.

X inactivation center (XIC) plays a major role in the inactivation of excess X chromosome. It comprises of 12 genes, among which 7 code for proteins, 5 for untranslated RNAs, of which two genes (*Xist* and *Tsix*) - play a major role in the phenomenon of inactivating excess X chromosomes.[26]

XIC also has a role in chromosomal counting: making sure that the process of inactivation occurs only when two or more X-chromosomes are present. [27]

The functions of *Xist* and *Tsix* are presumably opposite. The suppression of *Tsix* on the subsequent inactive X chromosome resulted in increased levels of *Xist* around the inactivation centre and *Tsix* levels are well maintained on the future active X, thus, the levels of *Xist* remain low. In the random phenomenon the choice of chromosome to be activated or inactivated is not fixed. Whereas, in non-random this choice is pre-determined and with the available literature, researchers opined that maternally inherited X chromosome may be inactivated always.[20,28]

"It is thought that this constitutes the mechanism of choice and allows downstream processes to establish the compact state of the Barr body. These changes include histone modifications, such as histone H3 methylation and histone H2Aubiquitination, as well as direct modification of the DNA itself, via the methylation of CpG sites. These changes help inactivate gene expression on the inactive X chromosome and to bring about its compaction to form the Barr body".[25,29]

Nuclear sex helps in determining the genetic sex of an individual. It can be demonstrated by various techniques like the study of Davidson body in the polymorphonuclear leukocytes, karyotyping, fluorescent body (Y chromatin),

polymerase chain reaction and Barr bodies (X chromatin) which have been evolving in this modern era.[25,27]

Karyotyping: It is a study which determines the chromosome number and pattern of both autosomes and allosomes in a eukaryote cell using special staining techniques. This helps in determining the genetic sex. This technique is costly and not possible to do in all institutions.[3,25]

Fluorescent body (Y chromatin): Identification of nuclear fluorescent bodies in the interphase of a cell establishes the individual as male. However, it has lot of physiological and pathological variations limiting its routine use in sex determination. [(25)]Fluorescent bodies resembling Y bodies have been demonstrated in 5% of female in buccal mucosal samples. Rarely in some males it was very small fluorescent body reported as not seen and in some males there were two fluorescent bodies and in some females with tumors these bodies were identified.[30]

Polymerase chain reaction (PCR): PCR is a biomolecular technique, which can be used to amplify DNA sequences on chromosome. Amplifying the XY homologous gene, "Amelogenin" by PCR technique is one of the method in determining sex, this was explained by Butler in the year 2003. Amelogenin gene has two components one on each sex chromosome on the p22 region. Amelogenin gene mapping is used for identification of sex from the human tissue specimens ( biological samples ). Like karyotyping it is not feasible to do in all institutes and is more expensive.[31]

## BARR BODY – ITS SIGNIFICANCE

Barr body represents **an inactive X chromosome** which appears as a darkly-staining body attached to the nuclear membrane. The other stained bodies with in the nucleus are nucleolus and they constitute repetitive rDNA genes.[32]

Barr body evaluation was used in the Olympics 1968 to investigate sportsmen apparently attempting to represent as females to have benefit in the competitions. It was useful in detecting some of the sportsmen with testicular feminization syndrome (TFS), a condition where a genetic male (XY) grows phenotypically as female, because of impaired function of androgen receptors. In TFS women, Barr body evaluation will be negative and there by identify them as male.[33,34]

Barr body frequency varies not only with gender but also in different phases of Menstrual cycle, Pregnancy, Oral contraception, Postpartum period and in Menopause.[35,36] Whereas reactivated X -chromosome observed in physiological stress and in few malignant conditions like cervical and breast cancer.[37,38]

## STRUCTURE OF BARR BODY

Barr body is a darkly stained structure, 0.8 – 1.1micrometer in largest dimension, plano-convex or triangular in appearance, eccentrically located in the nucleus on the inside of the nuclear membrane Fig (5). However, the appearance of Barr body has variance. They are seen as biconvex, or spherical, or rectangular when visualized in an ordinary microscope. and when seen under electron microscope they appear as alphabetical letters like V,W,S or X.[19,25,39]

Evaluation of Barr bodies for sex determination is advantageous as it is easier than other techniques. Ordinary compound microscope with simple staining techniques will be sufficient and tissue sample for the study can be obtained from buccal mucosa atraumatically.[40]

Aggarwal et al[41], Anoop UR et al[54], Mittal et al[3] and various authors demonstrated Barr bodies from buccal mucosa.

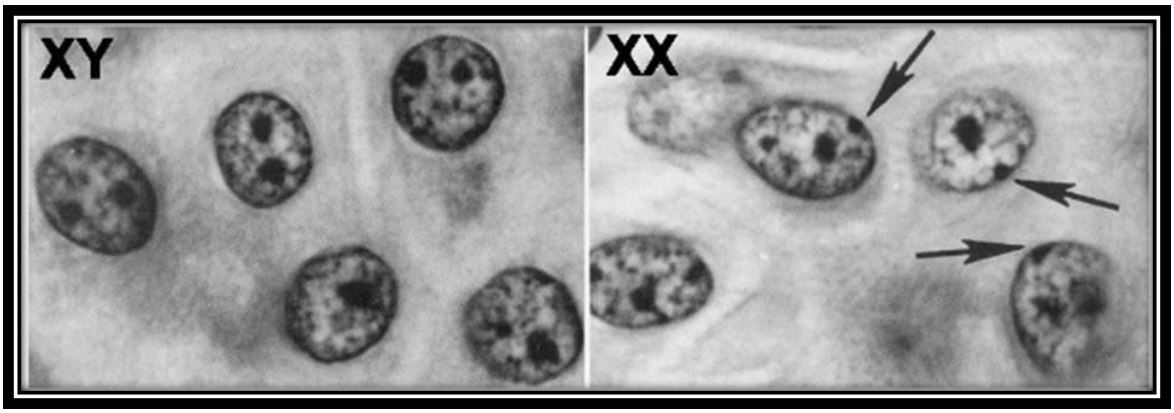



**FIGURE 5- Demonstration of Barr Bodies in Female Somatic Cells. Arrow - Barr Body[19]**

## EXFOLIATIVE ORAL CYTOLOGY

Cytology provides rapid diagnosis by minimally invasive technique. Exfoliative cytology as a method of diagnosis was first introduced by Papanicolaou in 1943. Exfoliative cells from oral epithelium have been widely used in cytology to detect abnormal nuclear and cellular morphology depicting precancerous and cancerous changes. Buccal mucosa due to more surface area is widely affected when exposed to insults in oral cavity resulting in epithelial changes.[42]

Exfoliative oral cytology has undergone significant advances and sequential improvisation related to screening of oral cancers and evaluation of oral precancerous lesions. Gold standard in diagnosing oral lesions is histopathological examination of the excised biopsy tissue but exfoliative cytology technique provides a range of diagnosis of preneoplastic, cancerous, infective and inflammatory disorders. Exfoliative oral cytological smears can play an important role in diagnosing lesions which are clinically not obvious or suspicious for malignancy and might obviate the need of invasive biopsy procedure.[43]

Heterogenous oral mucosa can be separated into masticatory mucosa, lining and specialized types. Masticatory type of mucosa covers the hard palate and gingivae and in places is bound directly to bone forming a mucoperiosteal membrane. Pink color is due to keratin layer. Lining mucosa covers the ventral tongue, floor of mouth, soft palate, buccal, labial and vestibular surface of oral cavity. Transparent lining epithelium is non-keratinizing, appearing red in color due to underlining blood vessels in lamina propria. Specialized mucosa lines the dorsum of the tongue covered by filiform papillae at specific locations.[43]

## EVOLUTION OF ORAL CYTOLOGY

Oral exfoliative cytology modifications have started with Gladstone in the year 1951 who used sponge technique in improving the quantity of cell obtained by oral exfoliative cytology. Schneider (1952) and Cawson (1960) have modified staining methods. King (1963) has used frosted glass slides. Staats and Goldsby in 1963 have compared metal and wooden spatulas for obtaining oral exfoliative material. Sandler in 1964 has improvised the technique further and used sharp curette to remove keratotic layer. Dumbach *et al* (1981) included deeper layers by use of curette.[44]

Oral exfoliative cytology involves scraping of the oral cavity randomly or from visible lesions of oral cavity. Collection devices such as Cytobrush, Orca-brush etc are used to procure cells from superficial and intermediate layers. Scraped material is spread over the slide followed by immediate fixation. These fixed smears are evaluated for cellular abnormalities after staining.[45]

Buccal smears are also used in Forensic Medicine, Criminology and Civil Law in cases of legitimacy, divorce, paternity, affiliation, marriage, education, impotence, right to disposal of property, intersex condition, cases of concealed sex and identification of the sex of individual whether living or dead. Sex determination can be determined by just using a single specimen of buccal smear.[46]

Buccal mucosa is easily approachable and most widely used for sex determination and clinical studies.[47] Buccal smears are also exploited to confirm the expected performance of a new lot of stain. Buccal smears after fixation are stained separately with hematoxylin and combination of OG and EA stains followed by drying and mounting. These sets of buccal smears are evaluated separately for nuclear staining by hematoxylin and cytoplasmic differentiation by OG and EA. Preparing

buccal smears in this way provide the true color of each major dyes without any possible interference.[48]

Buccal smears can be obtained by simple procedure as follows:

1. Explain the procedure in brief to the study subject and take an informed consent.
2. Instruct the study subject to vigorously rinse mouth with water several times before the test. It cleanses the area of excessive organism.
3. Scrape the subject's oral cavity or oral lesion with a spatula. If the scrape is for genetic assessment, it is taken from lateral Buccal mucosa just above the dentate line along the anterior two-thirds of the Buccal mucosa. If the scrape is for pemphigus, the lesion should be scraped, where the normal and the affected mucosa meet.
4. First scrape material is discarded.
5. Repeated scrapping of mucosa gently from deeper layers will obtain healthy epithelial cells.
6. Scraped material is gently spread over the labelled slide in single layer and fixed immediately with spray or liquid fixative which ensures accurate results.
7. Fixed smears are stained and evaluated. [45]
8. Instruct the subject to rinse his/her mouth after scrapping. Promote good oral hygiene.

**APPLICATIONS OF ORAL EXFOLIATIVE CYTOLOGY:**

- To determine sex chromatin for genetic counselling and as a source of DNA.
- As an aid in the study of diabetes mellitus, smoking, alcoholism, etc.
- Timely detection of oral cancer.
- In the evaluation of certain microbial diseases and dermatological lesions (pemphigus vulgaris).
- Assessment of certain nutritional deficiencies example - Iron deficiency, B12 and folic acid deficiency.
- Forensic dentistry evaluations.
- Predicting the cellular response of tumor to irradiation.

**The oral epithelial cells in the absence of disease:**

The normal epithelium of the oral mucosa sheds superficial and intermediate layer squamous cells. These cells are found singly or in clusters and similar to those seen in the saliva and sputum samples. Figure 6(A) & 6(B)

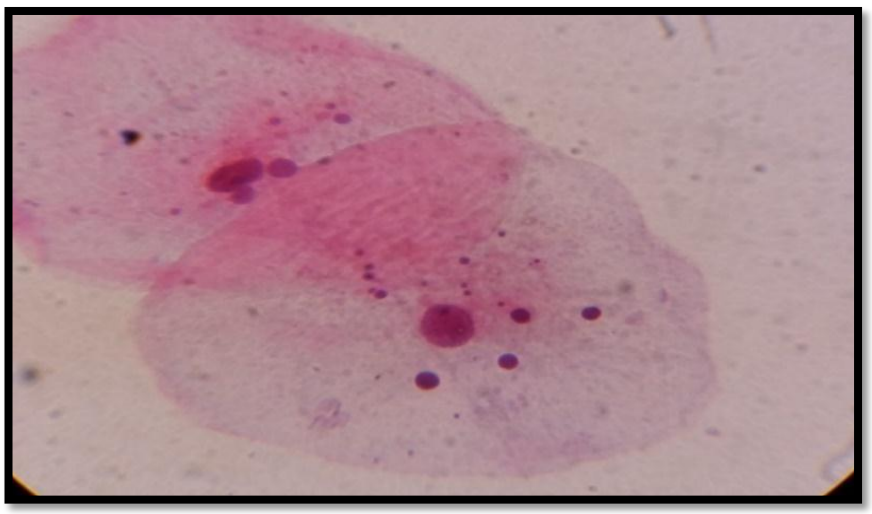


Figure: 6 (A) Normal squamous epithelial cells.[45]

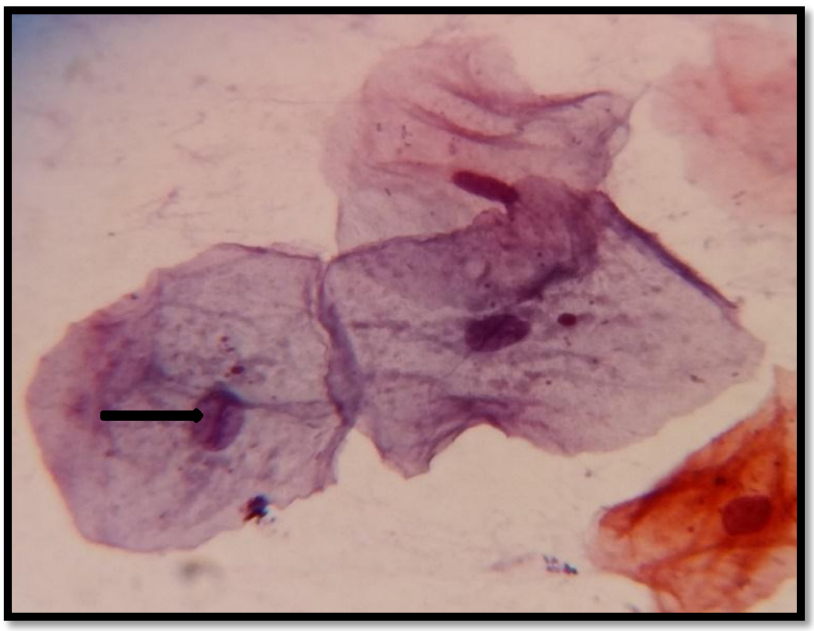


Figure: 6 (B) Normal squamous epithelial cells from oral scrapings.[45]

The cell shows a nuclear bar with lateral extensions (caterpillar nuclei) similar to Antischkow cells. (Depicted by arrow in microphotograph figure 6 (B))

The study of exfoliative cytology is concerned with the analysis of the cells which abrade from the body surfaces. The normal cellular physiological turnover leads to the shedding of the superficial squamous epithelial cells.[49]

The underlying deeper cells like the intermediate cells and the parabasal cells are adherent to one another normally. In pathological conditions, these cells lose their cohesiveness and the deeper layer sheds along with superficial cells. These cells are scraped off for cytological evaluation quantitatively and qualitatively. However, the usage of the cytologic smear depends on the proper preparation and evaluation by a cytologist.

It was observed that the cells show a nuclear bar with lateral extensions which is nothing but the longitudinal condensation of the nuclear chromatin, similar to those seen in Antischkow cells in the myocardium of rheumatic heart disease patients which are most commonly seen in cells from the floor of the mouth. This change is

related to the “nuclear creases” but its significance is not known. Similar findings are noted in the mesothelial cells of the pericardial surface.

Variable degrees of keratinization of the cells are noted from fully keratinized orange colored anucleated cells to smaller parabasal cells.[50,51]

**Other cells:**

Mucus-producing columnar epithelial cells and lymphocytes can be present, arising from the nasal mucosa, from salivary gland ductal epithelium and peritonsillar areas respectively.

**Oral flora:**

The oral flora most commonly in persons with poor oral hygiene are comprised of saprophytic fungi and bacteria amongs which the Protozoan, *Entamoeba gingivalis* is a common finding. An unusual organism called *Simonsiella* species which is also nonpathogenic is observed in persons with rich dietary intake of proteins and fats.

**Buccal cells in genetic counselling and as a source of DNA**

The exfoliative cytological analysis of the oral squamous cells for the detection of the sex chromatin (Barr bodies) is one of the cost effective and feasible procedure. These can be seen as half-moon shaped chromatin concentrations at the nuclear membrane. Theoretically the nonpyknotic squamous cells in all genetic females, having open vesicular nuclei should contain a Barr body.

However, in practice it can be identified in fewer than half of these cells on light microscopy of oral epithelial cells stained with Papanicolaou’s stain, but one should keep in mind that the peripherally placed chromocenters and focal thickening of the nuclear membrane may mimic Barr bodies and the visible sex chromatin varies with the menstrual cycle.

The finding of about half a dozen or more cells with a clear-cut single sex chromatin body is diagnostic of the XX female chromosomal constitution. However, an excess of Barr bodies (randomly more than two in a cell) indicates an excess of X chromosomes ("superfemales," with cells containing 47 chromosomes with XXX). Malignant cells occasionally also contain two or more Barr bodies, reflecting aneuploidy,[49-53] and it can be confirmed by enhanced glucose-6-phosphate dehydrogenase activity.

## STAINS USED IN THE IDENTIFICATION OF SEX CHROMATIN

**Various nuclear stains were used**

1. **Leishman stain** –

Blood smear spread on the glass slide was air dried and the smear was covered by undiluted Leishman's stain. Eight to ten drops of stain was added and left for 2 min 30 sec and twice the volume of buffered water was added and kept for 15 minutes. After fifteen minutes stain was washed with clean water and observed under oil immersion. Neutrophils were screened for Davidson bodies (Barr bodies).[54,57]

2. **Bierbrich Scarlet–Fast Green** –

The nuclei stains pale green; the sex chromatin stains pink to red and is seen as a V-shaped or triangular condensation with the base attached to the nuclear membrane. A narrow halo is usually noted around the unattached side. A thin, single or multiple strand of chromatin material is often observed running from the sex chromatin body toward the center of the nucleus.[55,63]

3. **Cresyl Violet** (Moore and Barr)-

The nuclei stain pale pink, and the sex chromatin stains deep pink to violet.[56]

4. **Guards stain -**

It was the first truly differential staining technique described by Guard that could be used for distinguishing the sex chromatin body from other chromatin clumps, this stain leaves a red sex chromatin body in a green cell. This stain for the first time revealed the presence of a previously uncounted intranuclear sex chromatin body.[56,57]

Instead, the method was time-consuming and difficult, in that it required frequent microscopic checking of the smears during differentiation in the fast-green stain during a 2-hr. period. It was, however significant improvement over previous staining techniques.

5. **Feulgen –**

It is a staining technique discovered by Robert Feulgen, mainly used in histology and cytology to identify chromosomal material or DNA material. It darkly stains the nucleus so used as nuclear stain. It depends mainly on acid hydrolysis of DNA, therefore fixing agents with strong acids should be prevented. It is a semi quantitative technique and it requires Schiff's reagent.[58,59]

DNA material stains – magenta and cytoplasm stains green color.

6. **Thionin**

It is a sensitive DNA stain that will make Barr bodies visible with fixation in modified Davidsons solution and buffering at different pH.[60] Sex chromatin stains dark blue or black and appear near the nuclear envelope.

7. **Hematoxylin and Eosin –**

Modified H and E technique was followed to stain the buccal mucosal smears. The scrapes were fixed in 95% alcohol. They were hydrated using decreasing grades of alcohol (100%, 90%, 80%) followed by water. The scrapes were stained with Mayers hematoxylin for 12 min, decolorized using 1% acid alcohol followed by

bluing in tap water for 5 min. They were counter stained using eosin for 2 min and then dehydrated using increasing grades of alcohol. The scrapes were cleared, mounted, and slides were screened under oil immersion.[54,57] Barr bodies appear as a pale pink to dark blue structure on the nuclear membrane.

**8. Alkaline Methylene blue -**

Buccal mucosal scrapes were collected and stained with alkaline methylene blue. Stain was added drop by drop over the smear which was left for 3 minutes and then was drained, washed and seen without mounting.[54,57] Barr bodies appears as dark blue structure on the nuclear membrane in the oil immersion.

**9. Acriflavine Schiff**

The slides treated with freshly prepared fluorescent Schiff-type reagents for 20 min, washed twice in acid alcohol for 1 min, and dehydrated in absolute alcohol.

Acriflavine stained sex chromatin appeared as bright greenish yellow spot. The microscope used in their study was fluorescence microscope ( Lawrence and Mayo ).[61,62]

Tibin K B *et al* [62] proposed that frequencies of Barr body were distinguishable between the male and females in buccal mucosal scrapes. The study was done to demonstrate Barr bodies were better stained with Acriflavine stain than Papanicolaou stain.

**10. Acridine Orange -**

"In this technique the fixed smears were passed through descending grades of alcohol, then rinsed for a few seconds in 1% acetic acid and washed in two changes of distilled water for about 1 minute, followed by staining in 0.01% acridine orange for 3 minutes, destaining in the phosphate buffer solution for 1 minute, differentiated in 0.1M calcium chloride solution for 30 sec to 1 minute. Excess calcium chloride was

removed by washing with phosphate buffer solution and mounting was done with cover slip using a drop of phosphate buffer solution". [2]

For this stain confocal microscope was used and that model used in their study was Zeiss 510LSM module laser scanning confocal microscope. The absorption range was 488nm, blue and the emission range was 505nm, green. Details of exfoliative cells were studied using 40X objective.

D. Shyam Prasad Reddy *et al* [2] proposed an innovative idea using a confocal microscope in exfoliative cytology to determine the sex of the individual from buccal mucosal scrapings. The exfoliative cells after being stained with acridine orange were observed for Barr bodies under a confocal microscope and the percentage of Barr-body-positive cells was determined.

## 11. PAPANICOLAOU STAIN

Papanicolaou stain (PAP stain) Is an important stain in the practice of cytopathology, named after George N. Papanicolaou in 1928, the father of cytopathology. It is a polychromatic stain containing multiple dyes to differentially stain various components of the cells. This method may display many variations of cellular morphology showing degree of cellular maturity and metabolic activity in the smear preparation of various gynecological specimen materials containing exfoliative cells and material from fine needle aspirations and buccal smears.(63,64)

Papanicolaou stain includes both acidic and basic dyes. Acidic dye stains the basic components of the cell and basic dye stains the acidic components of the cell. The polychromatic PAP stain involves **five dyes in three solutions**.

**Hematoxylin**: Natural dye hematoxylin is the nuclear stain which stains cell nuclei blue. It has affinity for chromatin, attaching to sulphate groups on the D.N.A. molecule. Harris' hematoxylin is the most commonly used cytologically.

**Orange Green 6**: This is the first acidic counterstain (cytoplasmic stain) which stains matured and keratinized cells. The target structures are stained orange in different intensities.

**Eosin Azure**: This is the second counterstain which is a polychrome mixture of - **Eosin Y -** gives a pink color to cytoplasm of mature squamous cells, nucleoli, cilia and red blood cells. Staining solutions commonly used in cytology are EA36 and EA50.

**Light green SF** stains cytoplasm blue in metabolically active cells like parabasal and intermediate squamous cells.

**Bismarck brown Y** – It stains nothing and it can be skipped.

**COMPOSITION AND PREPARATION OF REAGENTS**

Harris' hematoxylin:

Five grams of Hematoxylin, Fifty milliliters of Ethanol, 100 grams of Potassium alum, 2-5 grams of Mercuric oxide, 1000ml of distilled water and forty milliliters of Glacial acetic acid.

Orange G 6:

Phosphotungstic acid ( 0 -15 grams ), Nine fifty millilitres of Alcohol and Fifty milliliters of 10% aqueous Orange G.

EA 50 :

Ten milliliters of 0.04 M light green SF, Twenty milliliters of 0.3M eosin Y, Two grams of Phosphotungstic acid, seven hundred fifty milliliters of alcohol, two hundred fifty milliliters of Methanol and 20 milliliters of Glacial acetic acid.

Before staining, all stains should be filtered to avoid stain precipitates and contamination.

## PROCEDURE FOR PAPANICOLAOU STAINING

Both **progressive** and **regressive** nuclear staining techniques can be used in Papanicolaou stain. Before staining, immediately fix the prepared smears with 95% ethanol for minimum 30 min.

**Procedure of Progressive PAPANICOLAOU Staining Method -**

In the progressive method, the intensity of nuclear stain is controlled by first staining with hematoxylin and then immersing the slide into a blueing agent to attain the required intensity. Sott's tap water ( pH 8.02) is commonly used blueing agent.

**Procedure of Regressive PAPANICOLAOU Staining Method -**

In Regressive method, the nucleus is first over stained with non acidified hematoxylin and then excess stain is removed with dilute hydrochloric acid. The intensity is controlled by immersing the slide in running tap water. Timing is important, as over destaining may cause the nucleus to become hypochromatic.[63,64]

**INTERPRETATION –**

The nuclei should appear blue or black, cytoplasm appears blue or green and keratinizing cells appear pink or orange.

Tushar Mittal *et al* [(3)]conducted a study to determine the sex of the individual from buccal mucosal scrapings. Cells were observed for Barr bodies under oil immersion with a compound microscope and the percentage of Barr-body-positive cells was determined. Inferences from the study showed that the presence of Barr bodies in buccal mucosal cells can be demonstrated with a fair degree of accuracy using Papanicolaou staining. The sex of the individual could also be determined accurately, as two non-overlapping ranges for the percentage of Barr-body positive cells had been obtained for men and women.

Due to elaborate and complexity of the stain and many reagents rapid PAP stain was developed by reducing the staining time by different authors ( kline, Tao and Sato )with varying time like 4min, 5min and 90 seconds. However, the quality was not satisfactory as they have shown sub optimal cell morphology.

To overcome this, Yang and Alvarez developed Modified Ultra Fast PAP by reducing the staining time to 90 seconds and providing excellent cell morphology. It is a hybrid of Romanowsky and PAP stain. As it is newly developed stain and its costs on the higher side.

## 12.ACETO-ORCEIN (AO)

Orcein has two forms natural and synthetic, the natural form is obtained from lichens, **Rocellatinctoria and Lecanoraparella species**. Natural form is predominantly used for nuclear staining.[65]

The aceto-orcein stain was prepared in the manner recommended by Ritter. Forty – five milliliters of glacial acetic acid were warmed in an Erlenmeyer flask, then removed from the flame, and 1.0 gm of orcein added, with vigorous shaking. Fifty – five milliliters of distilled water at room temperature was immediately added and the flask stoppered and cooled with occasional shaking in running tap water. The Aceto-orcein was then filtered and maintained in a stoppered bottle, prefiltering as necessary. This filtrate was not stable and has to be made fresh before use or it can be refrigerated. The preparation can be used with or without lactic acid.[65,66]

Stock Solution - Synthetic orcein 1 gm, glacial acetic acid, 45 ml, boiled , cooled and filtered.

Simple AO solution -Dilute 45 parts stock solution and 55 parts distilled water and filtered. Periodic filtration should be done.

Lacto-aceto-orcein -Dilute stock solution with 70% lactic acid in 1:1 ratio and filtered. [63,67]

**Background**

Aceto-orcein squashes of meiotic chromosomes provided the first definitive information on the karyotype and chromosome morphology of Neurospora (McClintock 1945, Singleton 1953).[68] Orcein staining still provides the clearest definition of chromomere morphology at pachytene. The nucleolus is stained only lightly, allowing underlying chromosomes of the pachytene karyotype to be visualized. The fine detail revealed by this technique was superior to that obtained using hematoxylin or other procedures. The method has not been widely used, however, because it requires exceptional patience and skill.[67] **Preparations are short lived and cannot be made permanent**.

Orcein from some sources does not give good preparations. The cytoplasm was highly vacuolated in contrast to preparations made with hematoxylin according to a protocol in which chloroform clears the cytoplasm.

Despite these drawbacks, the method was used effectively for critical basic studies of meiosis and ascus development, meiotic behavior of chromosome rearrangements, and for assigning genetic linkage groups to cytologically defined chromosomes (Singleton 1952, Barry 1969, Perkins and Barry 1977).

The stains: 2% acetocarmine and orcein are chromatin-specific dyes. They bind permanently to the nucleoprotein component of chromatin. Thats why chromatin can be visualized by treating it with the two dyes. The dyes are very active and small quantities of them can be very effective to observe chromatin material. The binding of the 2 dyes is gradual and improves with time; and can be facilitated by applying gentle heating of the glass slide over a flame. Both orcein and acetocarmine can do

the same "wonders". They work well on most biological samples. They stain both euchromatin and heterochromatin alike; but a stain like Giemsa binds euchromatin and heterochromatin differentially. The differential staining by Giemsa is what produces the bands of stain on chromosomal structures. Though carmine is pinkish while orcein is purplish. It is important to note that both can be dissolved in acetic acid, acitic and propionic acid or propionic acid.[69]

**AO staining**

AO staining does not require the addition of iron ions. The staining procedure is similar to the aceto-carmine method. Fixed material is transferred for an appropriate time to 1% aceto-orcein and then analyzed by the squash technique.

Simple aceto-orcein stains the nucleus and cytoplasm more rapidly than lacto-aceto-orcein, but both preparations are temporary. With both stains, contamination of bacteria is not prominent. The orcein smear can be readily scanned in oil-immersion objective, which gives a large field and depth of focus.[67]

Orcein has several advantages for nuclear sexing. These include ease of preparation, rapidity of sexing, and the revelation of fine details of nuclear structure which are enhanced with squash technique and helps in revelation of fine nuclear detail and helps in better detection of Barr body.[1,67]

Datar U *et al* [1] proposed that Papanicolaou stain(PAP) and Acetoorcein (AO) are comparatively less studied staining techniques for Barr body identification and they did a prospective study to evaluate the efficacy of these stains in Barr body identification from buccal mucosa scrapings and concluded that AO was more effective for delineation of nuclear details, shorter staining duration and overall better sex determination efficacy in relation to PAP.

Archana T *el* [70] proposed to study quickness and reliability of 3 special nuclear stains in sex chromatin identification. Sex determination using Barr bodies in buccal scrapes is a simple method, which can be employed as a significant adjunct to other approaches of sex determination.

Anoop UR *et al* [71] proposed that Barr bodies can be easily identified with ordinary stains. He also concluded that it helped in identifying the sex of an individual when used judiciously.

Amrithaa Priyadharscini *et al* [39] proposed in a review of the importance of Barr bodies in sex determination that they can be obtained from various sites in the oral cavity such as pulpal tissue, buccal smears, teeth heated to high temperatures etc. Thus the identification of Barr bodies proved to be an effective tool for gender determination in forensic investigations.

Singh S *et al* [54] opined that sex identification plays a crucial role in many situations to clarify medico legal issues. In these situations often very minimal samples are available in the form of buccal smears (epithelial cells) or scrapes and it is one of the simplest method for sex determination. Samples stained with alkaline methylene blue and Hematoxylin and eosin and both these stains showed positivity for Barr bodies with an efficacy of 88% by alkaline methylene blue and 80% by hematoxylin and eosin stain. They compared peripheral smears stained with Leishman stain which showed 100% positivity.

Tschentscher F *et al* [31] proposed sex determination by human DNA samples by pyrosequencing of short PCR products and they used Amelogenin gene which is routinely applied method in forensic work and clinical testing.

# 4. MATERIALS AND METHODS

**Source of Data:**

Apparently normal healthy individuals pursuing M.B.B.S in this institute comprised the study subjects. A total of 207 students from three different batches were included in the study and the study period was from 1st October 2016 to 30th June 2018.

**Inclusion Criteria:**

- Healthy subjects of age group between 18 – 25 years were included in the study.

**Exclusion Criteria**

- Individuals with Dental braces and Retainers.

Buccal mucosal smears were collected from the study subjects in the form of small groups ranging from 10-15 students at a time. Student was asked to rinse the mouth with water before the procedure. A wooden spatula was used to collect the material by drawing the spatula along the buccal surface of cheek. The collected cellular material was then transferred onto two clean glass slides and smears were prepared. The slides were immediately fixed using 95% ethyl alcohol.

**METHOD OF STAINING:**

Four hundred fourteen smears were collected, these wet fixed smears were divided into two groups. The study group smears were stained with Aceto-orcein and other control group smears were stained using Papanicolaou stain.

**Aceto-Orcein staining Method :**

Stock solution of Aceto-Orcein was prepared dissolving 1gm of orcein in 45ml of glacial acetic acid. The solution was boiled, cooled and filtered. The working solution was prepared by diluting 45 parts of stock solution in 55 parts of distilled water with periodic filtration.

Aceto-orcein Squash technique employed was as follows: One drop of working solution was placed in center of a smeared slide and a cover slip was placed over the drop. One or more layers of filter paper were placed over cover slip and firm pressure was exerted by drawing the thumb across the coverslip in a single direction, taking care to prevent the gliding of coverslip. The margins were sealed with DPX**.**

**Papanicolaou staining method –**

**Reagents utilized were**

1. Harris Hematoxylin
2. 95%Alcohol
3. 70%Alcohol
4. 50%Alcohol
5. OG6
6. EA 36
7. 1% Acid alcohol

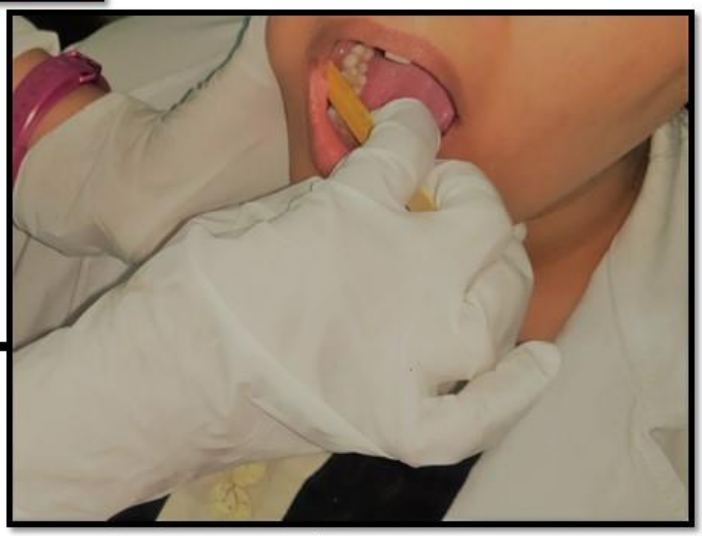
**Technique:**

- Washed with water.
- Stained with Harris Hematoxylin – 5 minutes.
- Washed with water.
- Dipped in 1% Acid alcohol.
- Bluing for 10 minutes
- Dehydrated in 70% alcohol - 2min
- Dehydrated in 95% alcohol - 2min
- Dehydrated in 95% alcohol - 2min
- Stained in OG 6 - 2min.
- Rinsed in 95% alcohol - 2min
- Rinsed in 95% alcohol - 2min
- Stained in EA 36 - 3 min
- Rinsed in 95% alcohol -1min
- Dried
- Cleared in Xylene
- Mounted

**FIGURE 7 – BUCCAL SMEAR – COLLECTION AND PREPARATION**

Wooden spatulas used to take the smears

Collection of the sample from the medical student



Two unstained smears on the clean glass slide

Labomed Lx 400 microscope used to evaluate the smears

**FIGURE 8 – REAGENTS USED FOR AO & PAP STAINING TECHNIQUES**



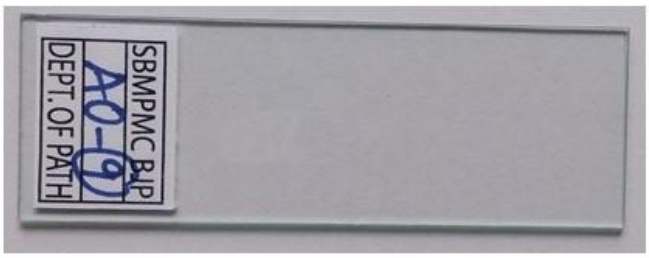




A. Distilled water
B. Coplin Jar
C&D. Aceto-Orcein stain Reagents
Orcein Powder; Glacial Acetic acid


E. Ethanol
F. PAP Stain Reagents
Left to Right – OG 6;
EA -36
Harris Haematoxylin.

**FIGURE 9 – AO SMEAR PREPARATION – SQUASH TECHNIQUE**

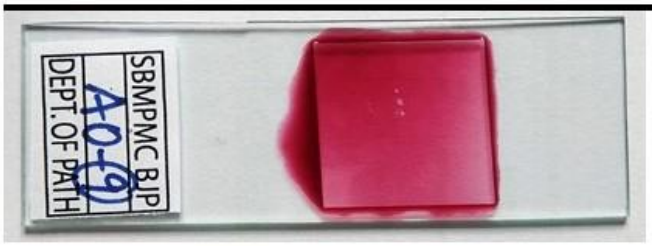



Labelled clean glass slide


scraping transferred onto the slide

★ Change in the back ground to highlight the collected sample.


One drop of working solution over the scraped material




Coverslip was placed over the stain


Procedure of Squash technique, which enlarges the nucleus


Excess stain was blotted and DPX applied

Smears with at least 100 cells with well preserved cytomorphological features were included in the study, whereas the smears with less than 100 cells were excluded. Under oil immersion, in 100 cells, the Barr body count was calculated in both the groups. ≤5% were recorded as male and those with >5% were recorded as female.

Criteria followed for Barr body Evaluation –

1. Cells with a fine vesicular or granular nucleus and well delineated nuclear border were considered.
2. To diagnose/ label as a Barr body, it should be present over the nuclear membrane, and appear in profile as a bar,or semidisc, or triangle,with the flat side against the nuclear membrane.
3. The length of the chromatin body should exceed 1 μm.
4. Cells contaminated with bacteria and cells with centrally located Barr-like bodies were excluded.

   Doubtful cells were considered as negative

**Table 1 - Cytomorphological parameters evaluated for estimating the efficacy of the two stains were as follows**

| Features | Aceto-Orcein | Papanicolaou |
|---|---|---|
| Nuclear staining (Chromatin pattern) | ☐ Smudgy<br>☐ Moderately Crisp<br>☐ Crisp | ☐ Smudgy<br>☐ Moderately Crisp<br>☐ Crisp |
| Background | ☐ Hemorrhagic<br>☐ Clean<br>☐ Bacilli | ☐ Hemorrhagic<br>☐ Clean<br>☐ Bacilli |
| Cell morphology | ☐ Not preserved<br>☐ Moderately preserved<br>☐ Well preserved and crisp | ☐ Not preserved<br>☐ Moderately preserved<br>☐ Well preserved and crisp |
| Overall staining | ☐ Bad<br>☐ Moderately good<br>☐ Good | ☐ Bad<br>☐ Moderately good<br>☐ Good |
| Percentage of Barr Bodies | | |

**SAMPLE SIZE**

Sensitivity of Aceto-orcein and PAP ( 98% and 90% ) [1,2] considering 95% confidence level and 80% power, **the calculated sample size was Two Hundred Seven ( 207 ).**

**STATISTICAL ANALYSIS**

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square ($\chi^2$)/Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data. The difference of the means of analysis variables between two independent groups was tested by unpaired t test. Bivariate correlation analysis using Spearman correlation coefficient was used to test the strength and direction of relationships between the ordinal levels of variables. Analysis for Sensitivity- specificity was done to check relative efficiency. If the p-value was < 0.05, then the results were considered to be statistically significant, otherwise it was considered as not statistically significant. Data was analyzed using SPSS software v.23.0. and Microsoft Excel office.

Predictive value of a positive test result or positive predictive value ( PPV )

PPV = True positive/( true positive + false positive )

Predictive value of a negative test result or negative predictive value ( NPV )

NPV = True negative/( true negative + false negative )

# 5. RESULTS

A total of 207 students were included in the study group. Amongst which 97 (46.9%) were male and 110 (53.1%) were female, with a male to female ratio of 1:1.13. Which is represented in figure 10. Mean age of females was 20.8 years and 21.1 years in case of males with an age range of 20 – 25 years, a detailed tabulation of which is made in Table 2 and Fig 11.

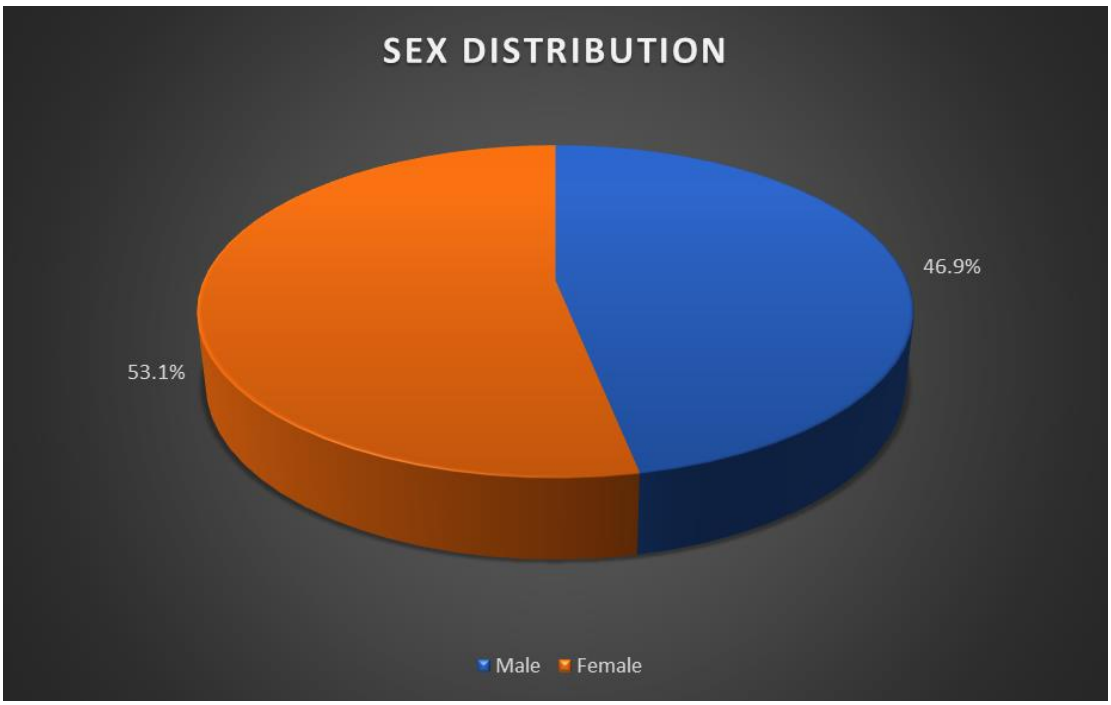



**FIGURE 10 - Distribution of Sex among both Study Groups**

| Table 2 – Mean age between study groups and sex | | | | |
|---|---|---|---|---|
| SMEARS | AGE(YEARS) | | | |
| | MALE | | FEMALE | |
| | Mean | SD | Mean | SD |
| ACETO ORCEIN | 21.1 | 1.5 | 20.8 | 1.2 |
| PAPANICOLAOU | 21.1 | 1.5 | 20.8 | 1.2 |

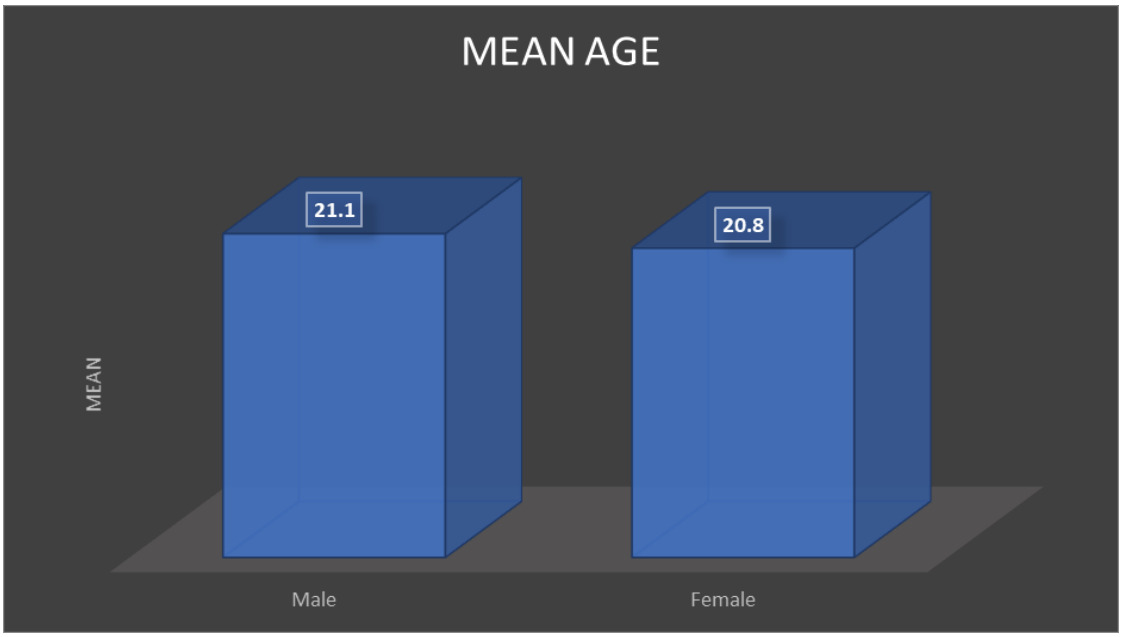



**FIGURE 11 - Mean Age between Study Groups and Sex**

A total of 414 buccal smears were prepared from study subjects and stained with Aceto-Orcein and Papanicolaou staining methods as mentioned earlier. These smears were evaluated for the parameters like nuclear stain, cell morphology, background, overall staining and percentage of Barr bodies. The detailed analysis of these parameters are as follows :

**DISTRIBUTION OF NUCLEAR STAIN BETWEEN STUDY GROUPS**

In case of Aceto-Orcein stained smears, crisp nuclear staining was noted in 206 smears whereas, 192 smears were found to have crisp nuclear staining in Papanicolaou method. Both the methods offered excellent nuclear staining in all the smears with absence of smudging of the nucleus. The detailed analysis of this is tabulated in the Table 3 and graphically represented in Figure 12.

**Table 2 - Distribution of Nuclear stain between study groups**

| Nuclear stain | ACETO ORCEIN | | PAPANICOLAOU | | p value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| SMUDGY | 0 | 0.0 | 0 | 0.0 | - |
| MODERATELY CRISP | 1 | 0.5 | 16 | 7.7 | <0.001* |
| CRISP | 206 | 99.5 | 192 | 92.8 | <0.001* |
| Total | 207 | 100.0 | 207 | 100.0 | |

Note: * significant at 5% level of significance ($p<0.05$)

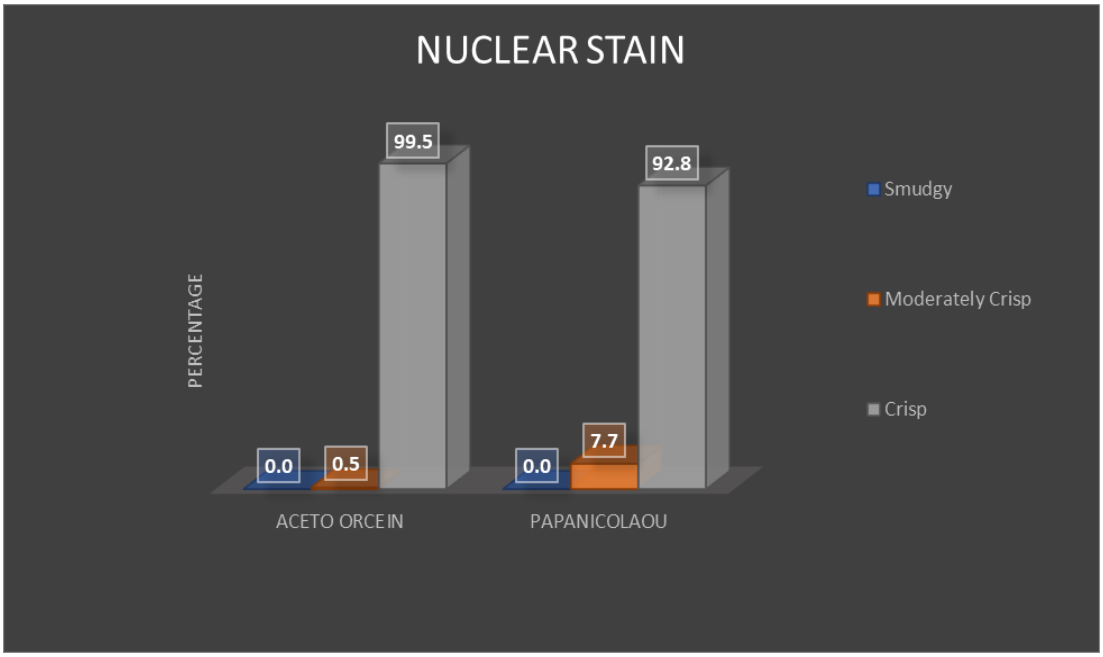



**FIGURE 12 - Distribution of Nuclear Stain Between Study Groups**

## CELL MORPHOLOGY

Ninety nine percent of the AO smears had shown well preserved and crisp cell morphology. But in the case of PAP smears only 95.7% cases had well preserved and crisp cell morphology. These results were found to have a p value of 0.010 which was statistically significant. The further stratification of the data was represented in (Table 4 & Figure 13). Both these techniques were capable of maintaining cell morphology, so they had not shown any unpreserved cell morphology.

| Table 3 - Distribution of cell morphology between study groups | | | | | |
|---|---|---|---|---|---|
| Cell morphology | ACETO ORCEIN | | PAPANICOLAOU | | p value |
| | N | % | N | % | |
| Not preserved | 0 | 0.0 | 0 | 0.0 | - |
| Moderately Preserved | 1 | 0.5 | 9 | 4.3 | 0.010* |
| Well Preserved and crisp | 206 | 99.5 | 198 | 95.7 | 0.010* |
| Total | 207 | 100.0 | 207 | 100.0 | |

Note: * significant at 5% level of significance (p<0.05)

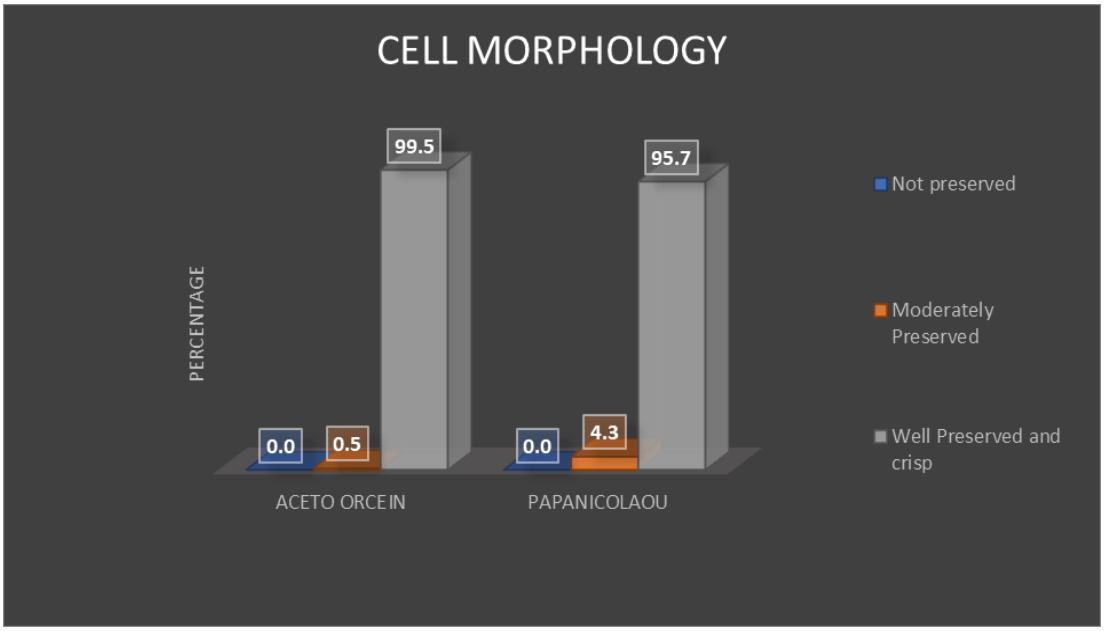



**FIGURE 13 - Distribution of Cell Morphology Between Study Groups**

## BACKGROUND STAINING OF BOTH STAINS

Almost all cases of AO stained smears showed clean background. In comparison to AO stain nearly 10% of cases had shown bacilli in the PAP stain which was statistically significant with a p value <0.001. Detailed representation is done in (Table 5 & Figure 14)

**Table 4 - Distribution of background between study groups**

| Background | ACETO ORCEIN | | PAPANICOLAOU | | p value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Hemorrhagic | 0 | 0.0 | 0 | 0.0 | - |
| Clean | 204 | 98.5 | 184 | 88.9 | <0.001* |
| Bacilli | 03 | 1.5 | 23 | 11.1 | <0.001* |
| Total | 207 | 100.0 | 207 | 100.0 | |

Note: * significant at 5% level of significance (p<0.05)

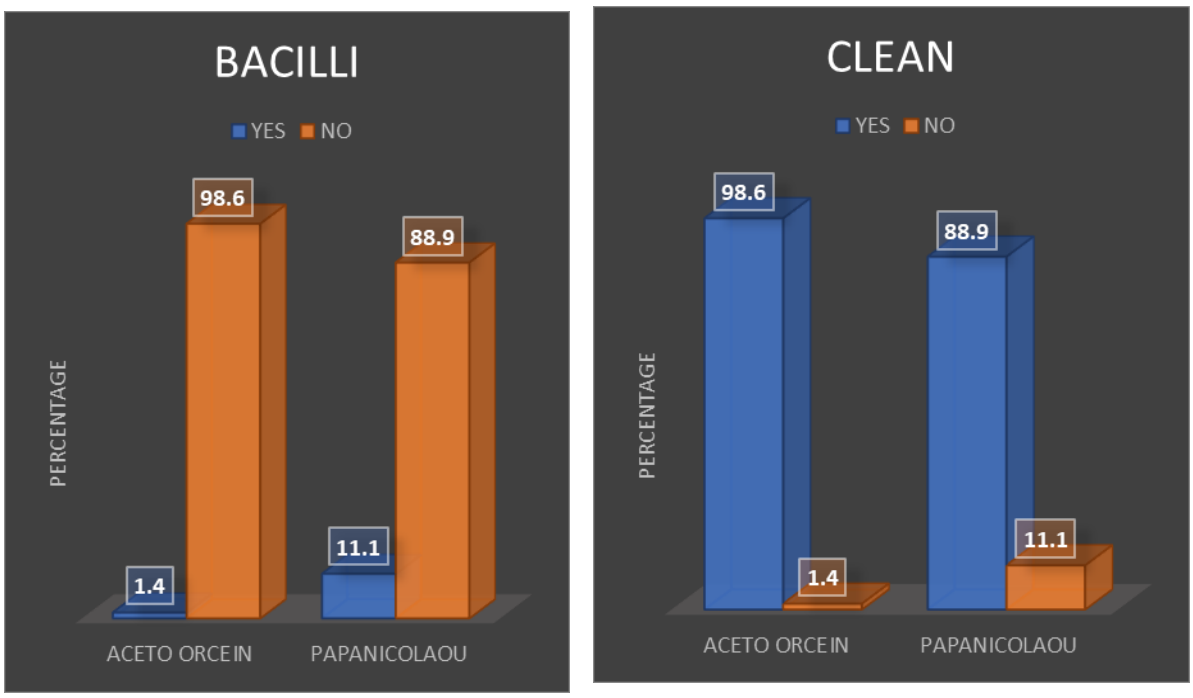



**FIGURE 14 - Distribution of Background between Study Groups**

## OVERALL STAINING CHARACTERISTICS

Overall staining of both the stains i.e., Papanicolaou ( PAP ) and Aceto-Orcein (AO) were good. ( Table 6 )

After considering parameters like nuclear, cytoplasmic and background staining characteristics, the overall staining was found to be comparable in both the groups.

**Table 5 - Distribution of overall staining between study groups**

| Overall staining | ACETO ORCEIN | | PAPANICOLAOU | | p value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Good | 207 | 100.0 | 207 | 100.0 | - |
| Moderately Good | 0 | 0.0 | 0 | 0.0 | - |
| Bad | 0 | 0.0 | 0 | 0.0 | - |
| Total | 207 | 100.0 | 207 | 100.0 | |

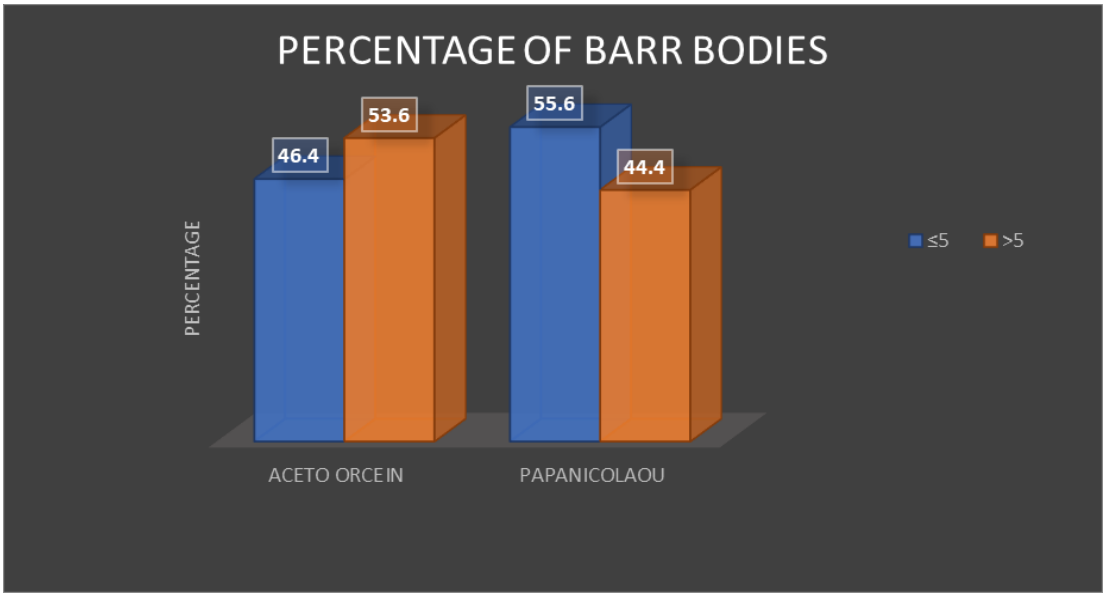
## PERCENTAGE OF BARR BODIES

After considering cut off of **Five** Bar bodies in hundred cells, the smears were categorized as males and females. As per the said criteria 96 were males and 111 were females in AO smears whereas in PAP stained smears, 115 and 92 were categorized as males and females respectively. However, these results were not statistically significant as the p value was 0.062. ( Represented in Table 7 and graphically in Fig 15)

**Table 6 - Distribution of percentage of Barr bodies between study groups**

| Percentage of Barr bodies | ACETO ORCEIN | | PAPANICOLAOU | | p value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| ≤5 | 96 | 46.4 | 115 | 55.6 | 0.062 |
| >5 | 111 | 53.6 | 92 | 44.4 | |
| Total | 207 | 100.0 | 207 | 100.0 | |

**FIGURE 15 - Distribution of Percentage of Barr Bodies between Study Groups**



## PERCENTAGE OF BARR BODIES IN TWO DIFFERENT GROUPS

Comparing this data with the sex of the study subjects was done. The accuracy of AO smears was found to be 96.9% and 98.2% in case of males and females respectively. In case of PAP smears the comparison yielded accuracy rates of 90.7% and 92% in males and females respectively. ( Detailed representation is done in Table 8 & Fig 16, Tab 9& Fig 17 )

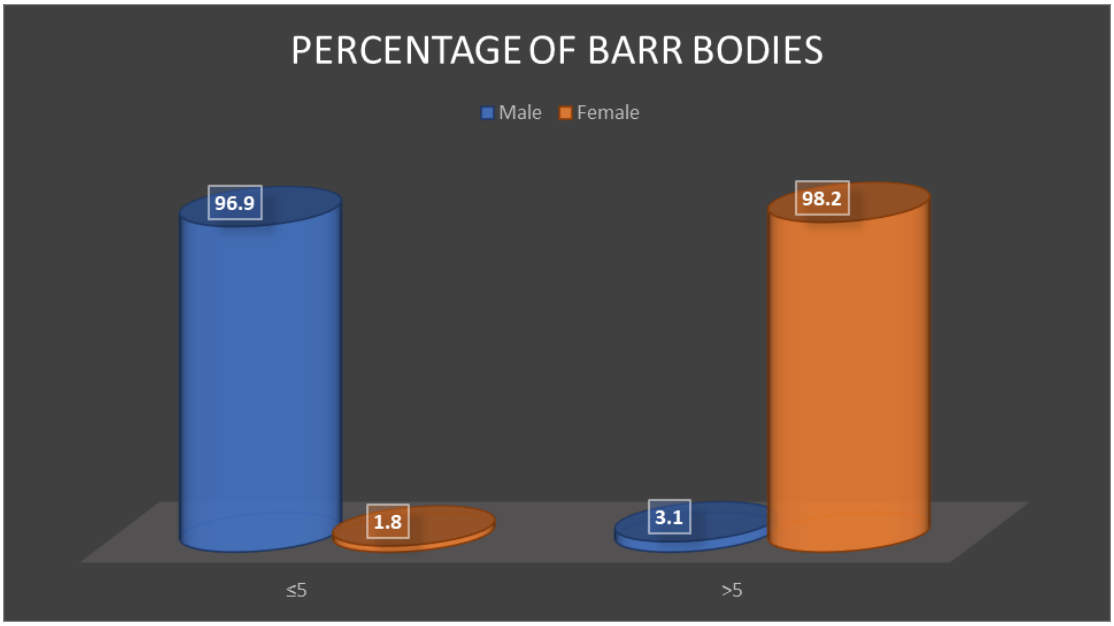
## ACETO ORCEIN

**Table 7 - Percentage of Barr bodies among Aceto Orcein group**

| Percentage of Barr bodies | SEX | | | | p value |
|---|---|---|---|---|---|
| | Male | | Female | | |
| | N | % | N | % | |
| ≤5 | 94 | 96.9 | 2 | 1.8 | <0.001* |
| >5 | 3 | 3.1 | 108 | 98.2 | |
| Total | 97 | 100.0 | 110 | 100.0 | |

Note: * significant at 5% level of significance (p<0.05)

**FIGURE 16 - Percentage of Barr bodies among Aceto-Orcein**



## PAPANICOLAOU

| Table 8 - Percentage of Barr bodies among Papanicolaou group | | | | | |
|---|---|---|---|---|---|
| Percentage of Barr bodies | SEX | | | | p value |
| | Male | | Female | | |
| | N | % | N | % | |
| ≤5 | 97 | 90.7 | 18 | 18.0 | <0.001* |
| >5 | 0 | 0.0 | 92 | 92.0 | |
| Total | 97 | 90.7 | 110 | 110.0 | |

Note: * significant at 5% level of significance (p<0.05)

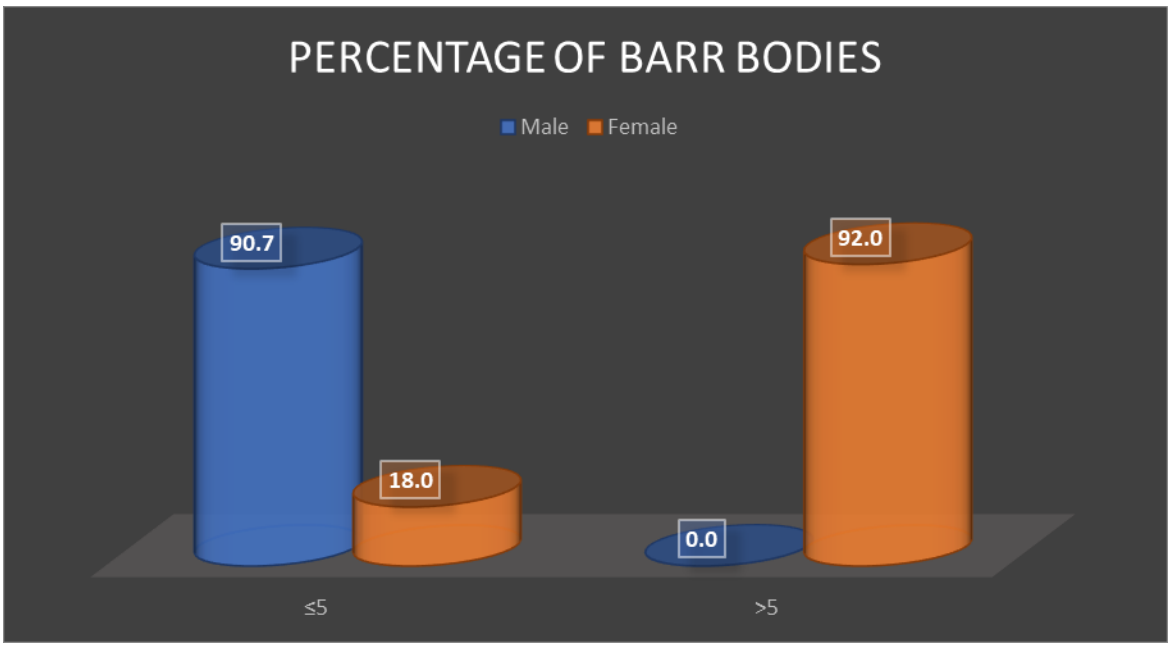



**FIGURE 17 - Percentage of Barr Bodies among Papanicolaou**

## MEAN BARR BODIES BETWEEN STUDY GROUPS AND SEX

On an average mean Barr bodies were found to be higher in AO smears among both males and females when compared with PAP stained smears which was found to be statistically significant (p value < 0.001).The details along with standard deviation is mentioned in the Table 9 and graphically represented in figure 13.

**Table 9 - Mean Barr bodies between study groups and sex**

| SMEAR | Barr bodies | | | | p value |
|---|---|---|---|---|---|
| | MALE | | FEMALE | | |
| | Mean | SD | Mean | SD | |
| ACETO ORCEIN | 1.6 | 1.1 | 14.9 | 2.2 | <0.001* |
| PAPANICOLAOU | 0.5 | 0.6 | 7.5 | 1.6 | <0.001* |

Note: * significant at 5% level of significance (p<0.05)

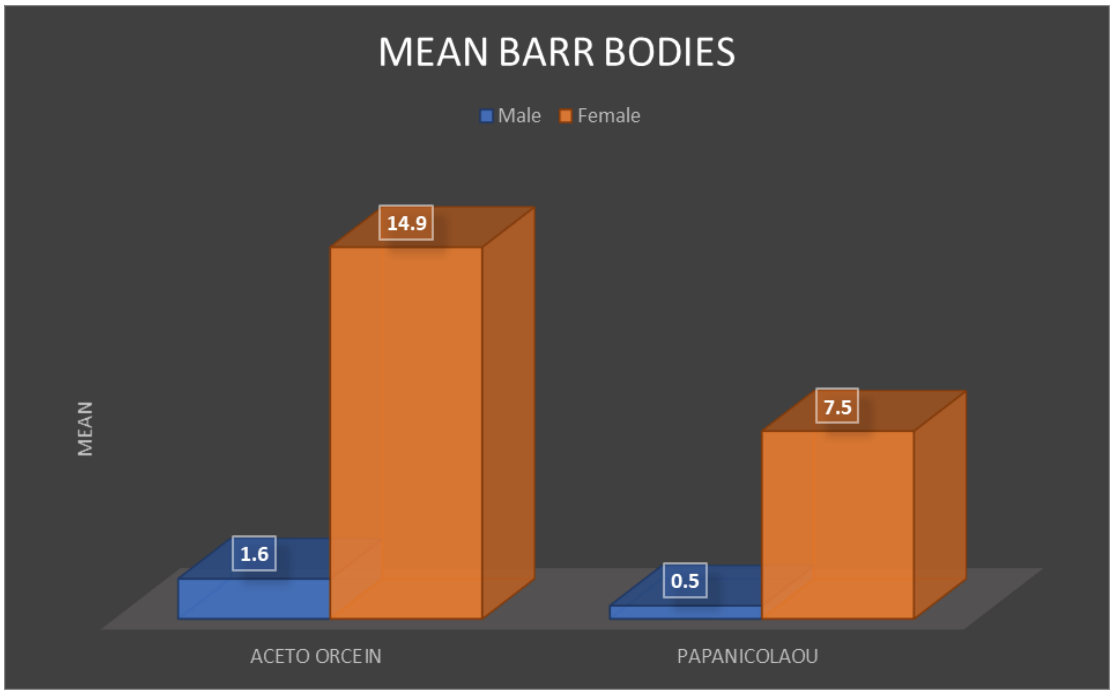



**FIGURE 18 - Mean Barr Bodies between Study Groups and Sex**

## MEAN BARR BODIES BETWEEN STUDY GROUPS

Mean percentage of Barr bodies, Percentage was almost double the number in the smears stained with Aceto-orcein ( 8.7 ) when compared with Papanicolaou stain ( 4.3 ) which was found to be statistically significant with a pvalue <0.001. Detailed representation was done in tabular form in Table 10 and pictorially in figure 14.

**Table 10 - Mean Barr bodies between study groups**

| | ACETO ORCEIN | | PAPANICOLAOU | | p value |
|---|---|---|---|---|---|
| PERCENTAGE OF BARR BODIES | Mean | SD | Mean | SD | |
| | 8.7 | 6.9 | 4.3 | 3.7 | <0.001* |

Note: * significant at 5% level of significance (p<0.05)

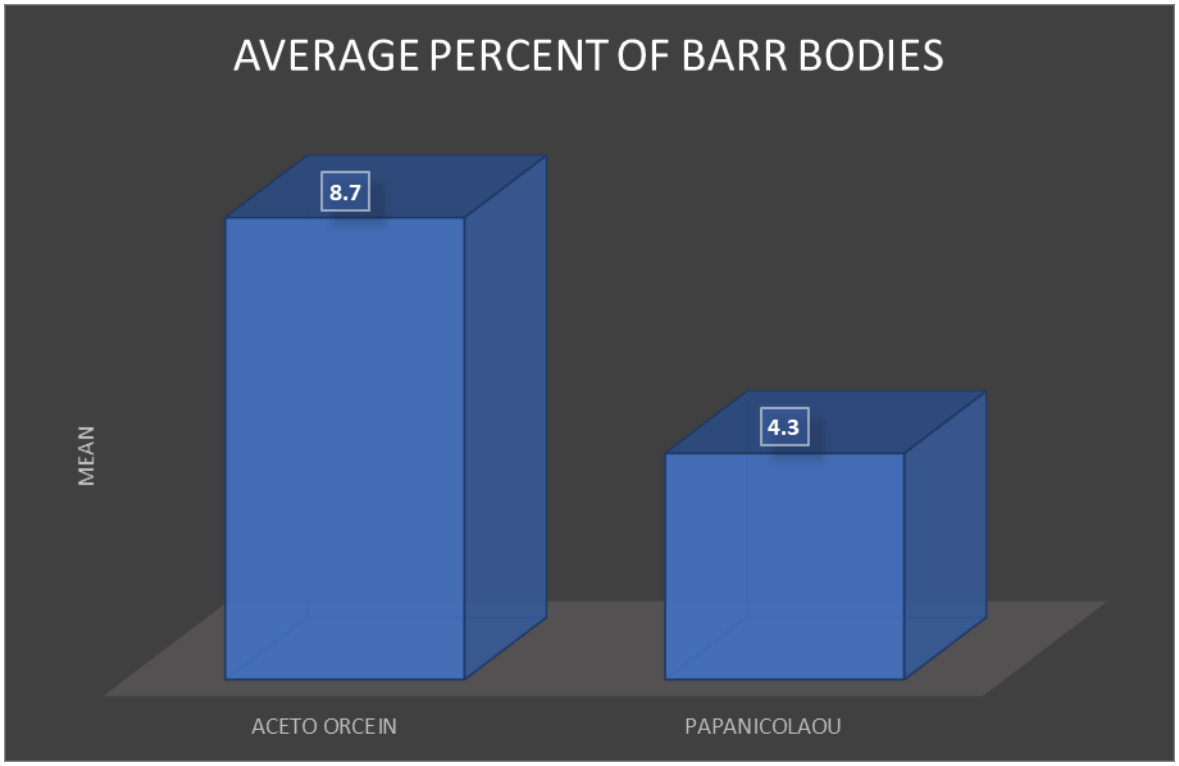



**FIGURE 19 - Mean Barr Bodies between Study Groups**

## SPEARMAN'S CORRELATION

| Table 11 - Spearman's correlation coefficient between sex and percentage of Barr bodies | | | | |
|---|---|---|---|---|
| Spearman's correlation coefficient | ACETO-ORCEIN | | PAPANICOLAOU | |
| | $r_s$value | p value | $r_s$ value | p value |
| | 1 | <0.001* | 0.908 | <0.001* |

Note: * significant at 5% level of significance ($p<0.05$)

The Spearman's rank-order correlation is the non-parametric version of the Pearson product-moment correlation. Spearman's correlation coefficient, (ρ, also signified by $r_s$) measures the strength and direction of association between two ranked variables.

The spearman's correlation coefficient $r_S$ is 1 for AO stain and it is 0.908 for Pap stain which is significant and the p value ( <0.001 ) is significant for both the stains in calculating percentage of Barr bodies and identifying sex.

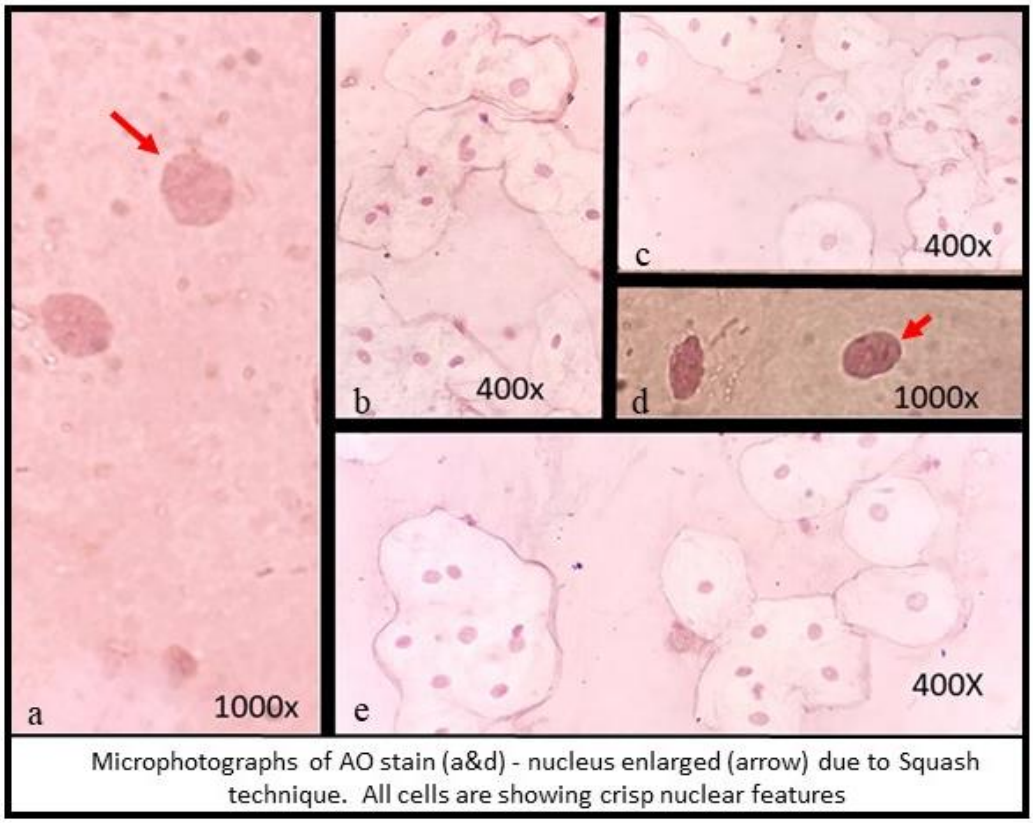
## DIAGNOSTIC EFFICACY OF METHODS IN DETECTING CORRECT SEX

The analysis of Sex determination by AO and PAP stained buccal smears has yielded a sensitivity and specificity of 98.18% and 96.91% whereas in Papanicolaou (PAP) sensitivity and specificity was 83.64% and 100% respectively. The accuracy of Aceto-Orcein and Papanicolaou in diagnostic efficacy in sex determination was 97.58% and 91.3% respectively. Detailed analysis of the efficacy of two staining methods is tabulated in table 13

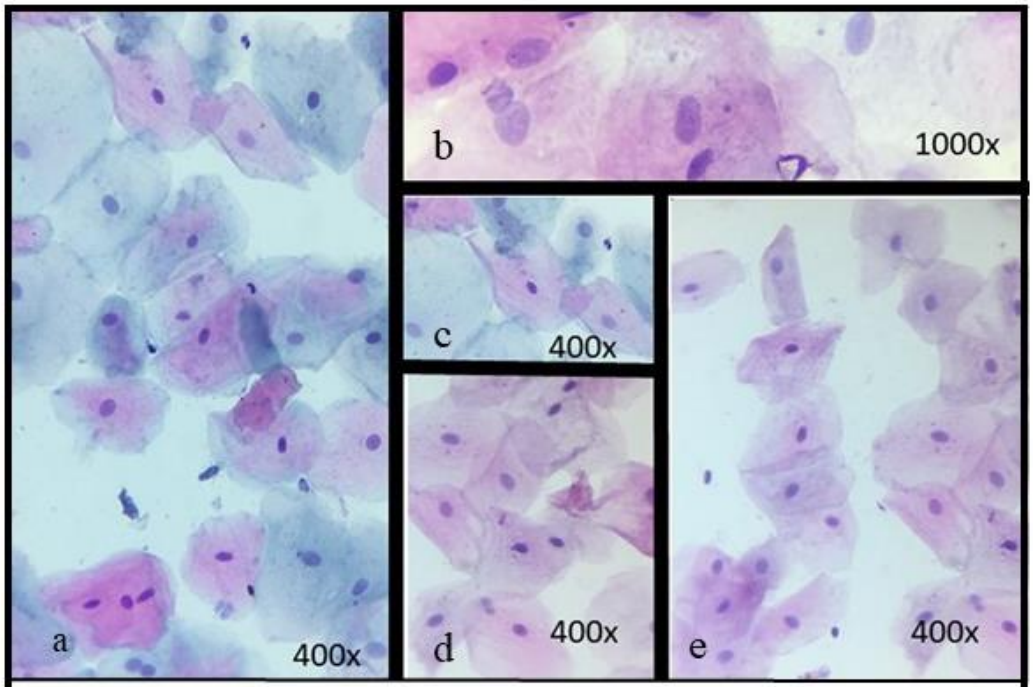
**Table 12 – Diagnostic Efficacy of methods in detecting Correct sex**

| | ACETO ORCEIN | PAPANICOLAOU |
|---|---|---|
| Sensitivity | 98.18% | 83.64% |
| Specificity | 96.91% | 100.00% |
| PPV | 97.30% | 100.00% |
| NPV | 97.92% | 84.35% |
| Accuracy | 97.58% | 91.30% |

**FIGURE 20 – COMPARISON OF NUCLEAR STAINING ( crisp & moderately crisp ) – AO & PAP STAINED BUCCAL SMEARS.**

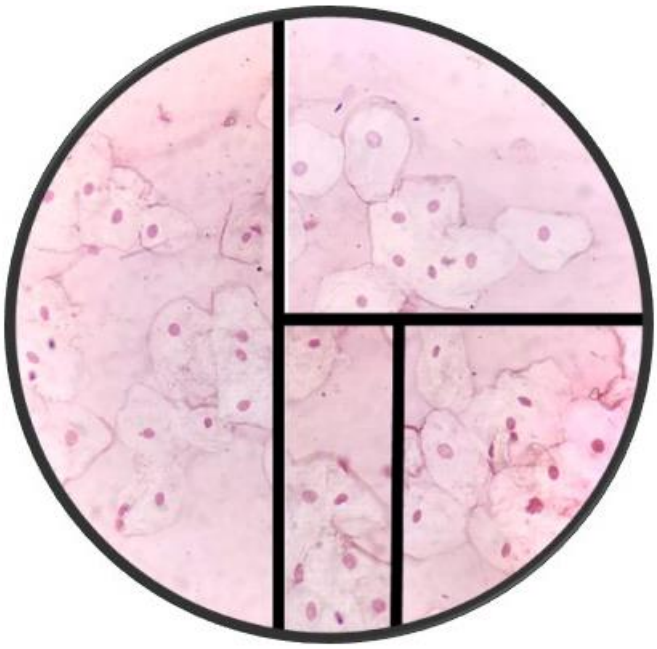



Microphotographs of AO stain (a&d) - nucleus enlarged (arrow) due to Squash technique. All cells are showing crisp nuclear features

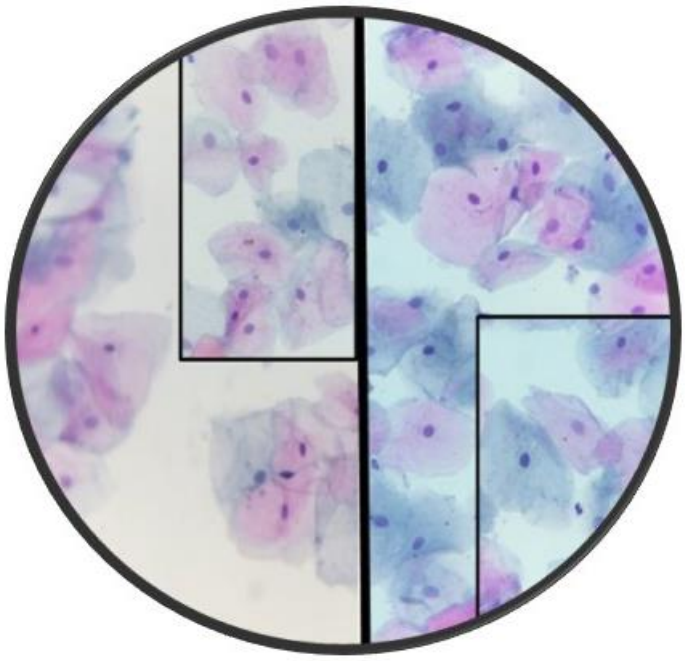



Microphotographs of PAP stain - nucleus appears smaller in comparison to that of AO stained smears. Most of the cells are showing crisp nuclear features

**FIGURE 21 – COMPARISON OF CYTOPLASMIC STAINING ( well preserved & crisp features ) – AO & PAP STAINED BUCCAL SMEARS**



Microphotograph – composite image from multiple smears of AO stain is showing Well preserved and Crisp cell morphology and clear cytoplasm



Microphotograph – composite image from multiple smears of PAP stain showing Well preserved and Crisp cell morphology but showing different colours of cytoplasm based on their maturity

**FIGURE 22 – COMPARISON OF OVERALL STAINING WITH REGARDS TO CYTOMORPHOLOGICAL FEATURES OF AO & PAP STAINED BUCCAL SMEARS**

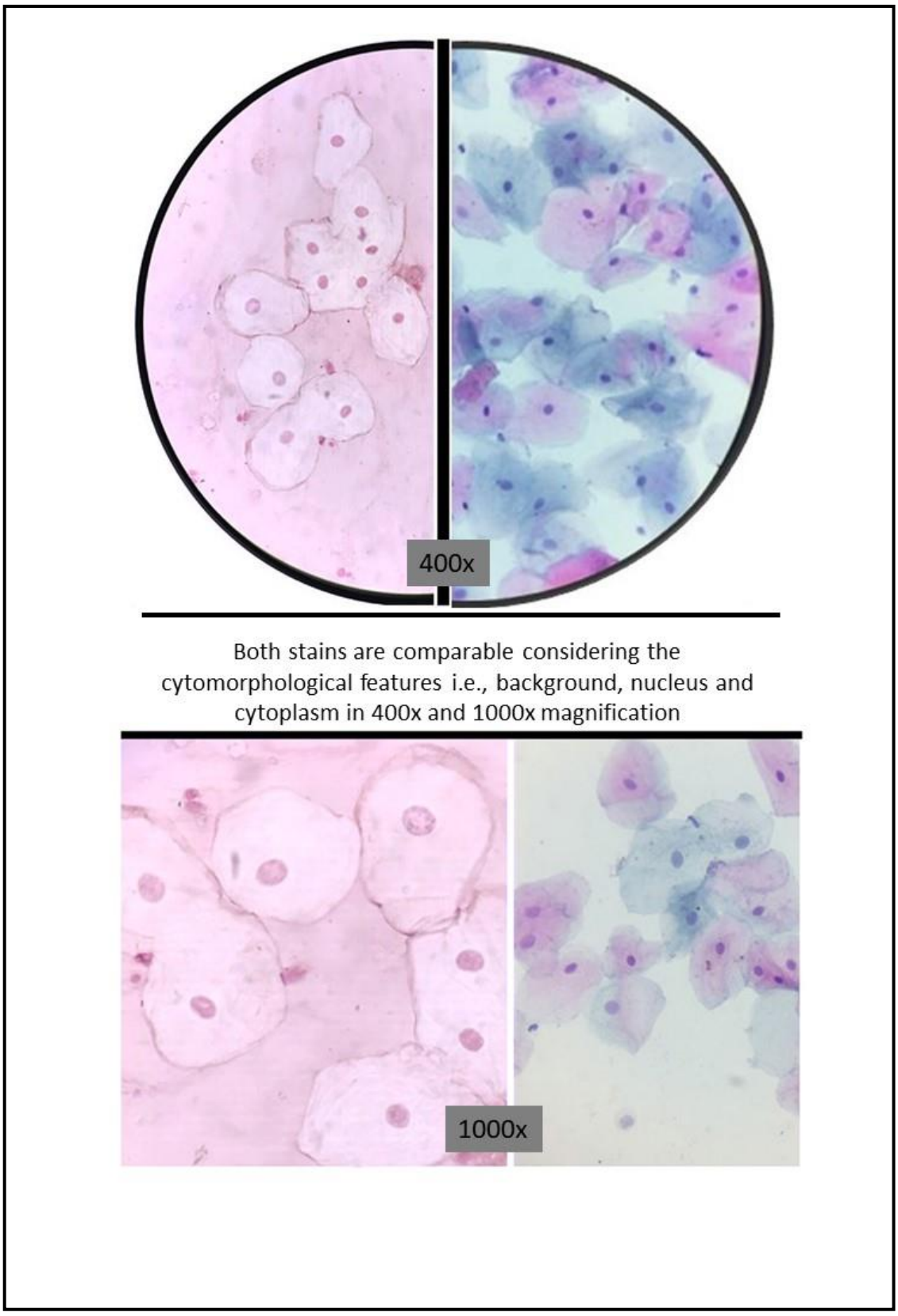
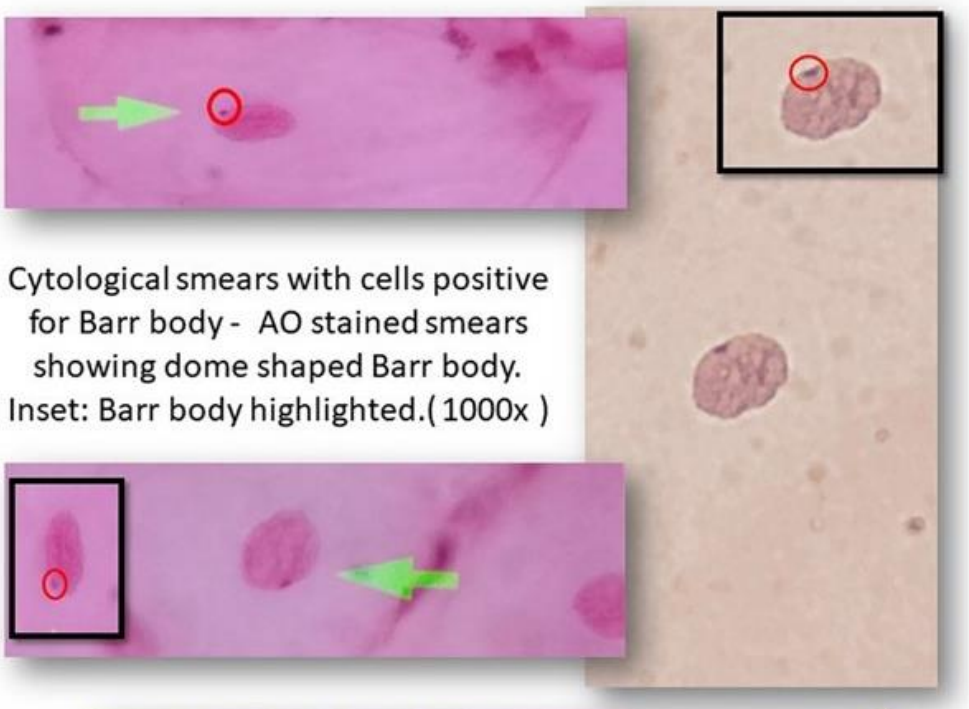
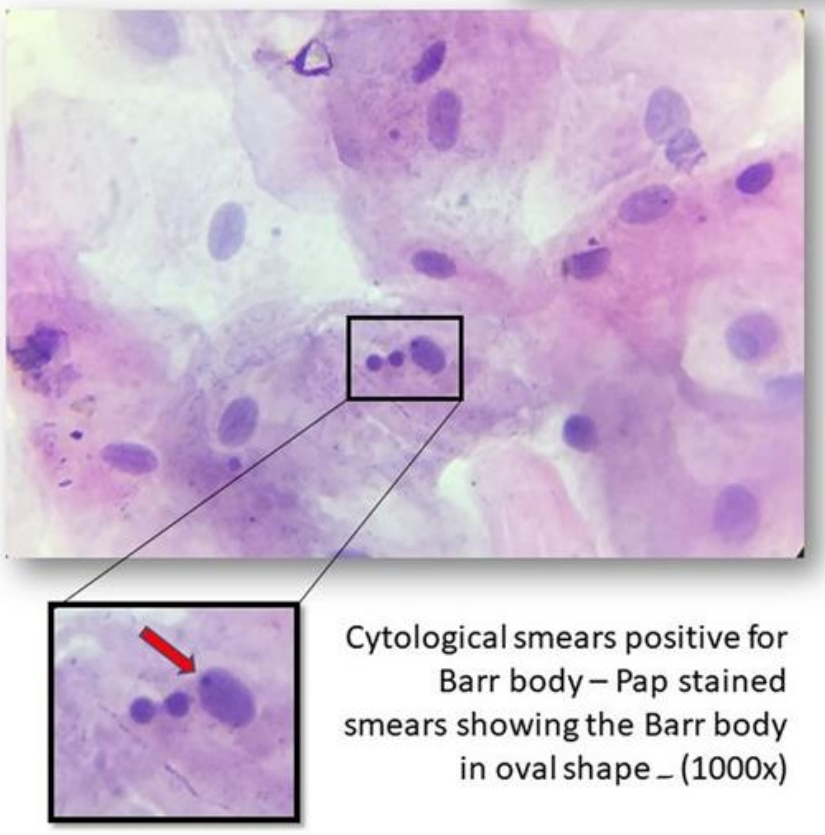


**FIGURE 23 – BUCCAL SMEAR – BARR BODY EVALUATION IN AO & PAP STAINED BUCCAL SMEARS**

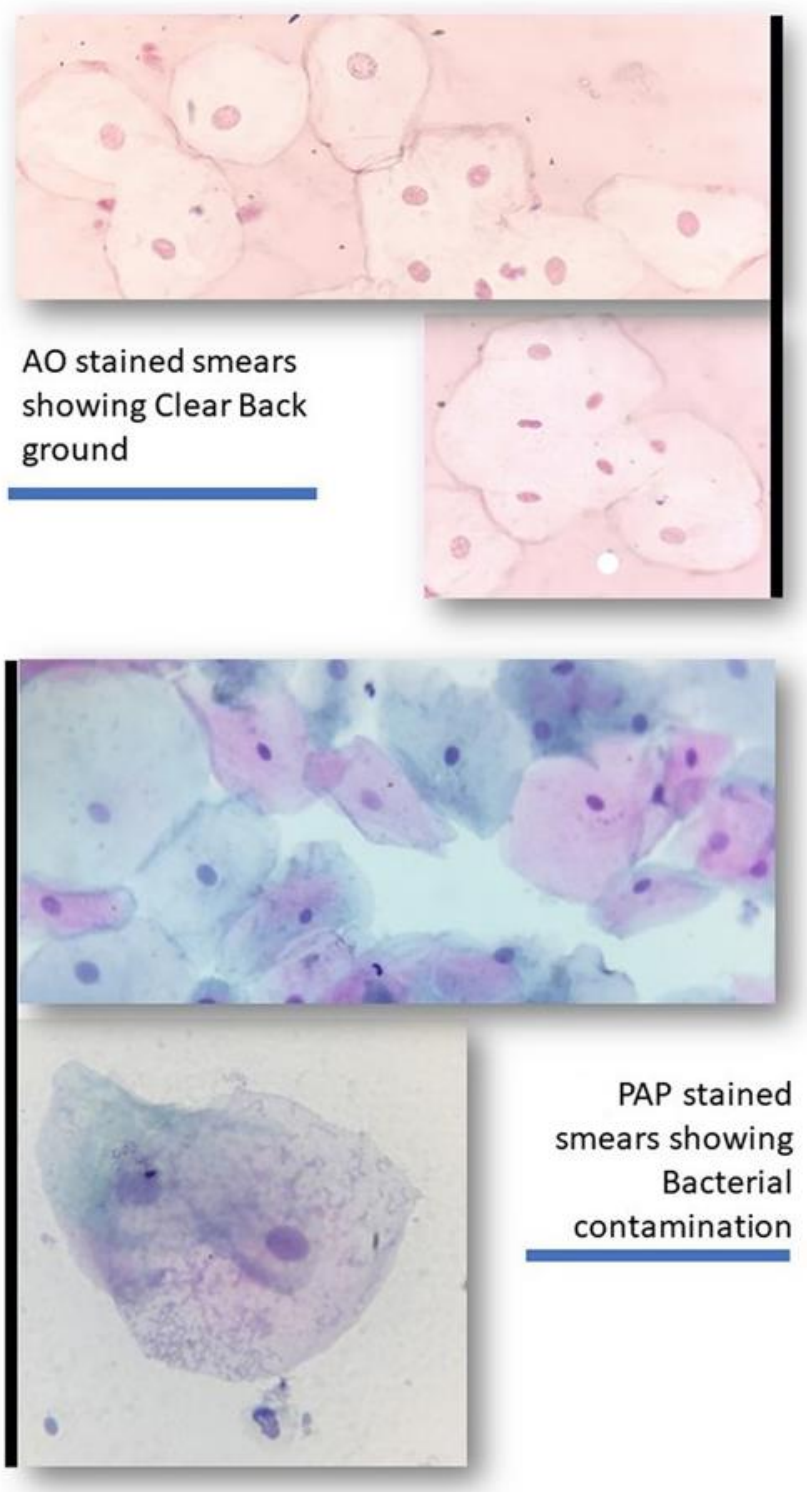


Cytological smears with cells positive for Barr body - AO stained smears showing dome shaped Barr body. Inset: Barr body highlighted.( 1000x )



Cytological smears positive for Barr body – Pap stained smears showing the Barr body in oval shape _ (1000x)

**FIGURE 24 – COMPARISON OF BACKGROUND STAINING OF AO & PAP STAINED BUCCAL SMEARS**



# 6. DISCUSSION

Even in this advanced era of molecular testing, few tests have withstood the test of the time. One such test which is simple, rapid and repeatable with better accuracy is the buccal smear examination for sex determination which is important in cases of ambiguous genitalia, hermaphroditism, epicene and intersexuals. Further investigation of these cases forms the most common request for screening by buccal smear for Barr body evaluation.

Buccal mucosa forms an easy source of exfoliative cells with minimal invasiveness which can be performed even in field settings. The simplicity of this procedure will be of huge help in case of natural calamities, mass transit accidents, terroristic attacks and disasters

This procedure has undergone various modifications and improvements over the years. However, PAP stain is one of the preferred stain for Barr body analysis in buccal smears. Many authors have evaluated the utility of various other stains to reduce the time taken and to have better detection of Barr bodies with chromatin specific stains. Amongst these methodologies, Aceto-Orcein has both the advantages in the form of chromatin specificity and rapid staining in a matter of seconds further aiding in reducing the time taken.

To evaluate the utility of the AO stain in normal individuals, in this study the medical students comprised of the study group where comparison has been done with PAP stained buccal smears of the same individual. Counting of the 100 cells while arriving at the mean Barr body percentage is a routine practice. Among 207 apparently healthy individuals AO stained buccal smears had twice the detection capability of the Barr bodies with a mean Barr body percentage of 14.9% in females and 1.6% in males. Whereas, PAP stained smears were found to have a mean Barr

body percentage of 7.5% in females and 0.5% in males. These results were statistically significant with a p value < 0.001. The better performance of AO smears could be due to the chromatin specificity of Aceto-Orcein and the absence of cytoplasmic staining which yielded a better contrast in evaluation of the nucleus.

## PRESENCE OF BARR BODIES IN MALES

Following the general physiological principle of X – inactivation as stated by Lyon hypothesis Barr bodies in males should be absent. However, this is not the case as found by many studies conducted in varied study populations with observations of presence of Barr bodies ranging upto 5% in males. Various authors demonstrated the presence of Barr body in males in buccal mucosal smears as well as in other structures.

Sanderson[67] as early as in 1960 has explained the discovery of slim heterochromatic crescent suggestive of sex chromatin in male nuclei in oligophrenic subjects and in 1961, Nagamori and Takeda[72] in plucked hairs without epithelial root sheath and various authors with different stains and samples have also found Barr bodies in males ranging upto 8%.

Khanna *et al.*[73] used dental pulp for the determination of the Barr body. They used three different stains like Haematoxylin and Eosin, Feulgen and Acridine Orange to count the Barr bodies which were < 3% in male samples and in female samples they identified it as positive with all the stains.

Nagamori *et al.*[74] in their study titled as "Sex determination from buccal mucosa and hair root by the combined treatment of Quinacrine staining and the fluorescent Feulgen reaction using a single specimen" also demonstrated Barr body positive cells in male subjects.

"The positivity for Barr bodies in males is due to the inheritance of males to carry primary sex organs of both the sexes. Y chromosome is one of the smallest chromosome and it size varies in size from 6 million base pairs to 5 million, and this is due to the repeated DNA pyro sequencing and gene silencing and this Y chromosome contains genetic material for the development of male features and this development mainly occurs due to the TDF (testis-determining factor). This factor is closely linked to a group of genes, called "sex determining region Y" (*SRY*) which is located in the short arm of the Y chromosome. The process of inactivation is a mystery, but it has been suggested that it is under the control of inactivation centre, located at Xq13. XIST, a gene which is transcribed from the inactive X, is necessary for initiation and propagation of X inactivation and does so by coating the inactive X. As inactive X is turned off by XIST allele, up to 21% of genes on Xp, and 3% on Xq may escape X inactivation." [23,62] Which was proved by Lyon MF in human genetics in 1998.

In case of females there is a concurring opinion that mean Barr body percentage is greater than 5%. So, Datar *et al.*[1] in their study titled as "Cytological assessment of Barr bodies using Aceto-Orcein and Papanicolaou stains in Buccal smear and their sex estimation efficacy in an Indian sample" had taken Barr bodies ≤5% as male and if the percentage of Barr bodies >5% were considered as female.

Following the above mentioned criteria, AO stained smears and PAP stained smears were categorized into males and females. This was compared with the gender of the study subject. The sensitivity of Aceto-Orcein was 98% which was quite higher when compared to PAP stain, where the sensitivity was 83.6% respectively. AO has better accuracy in comparison with PAP.

The percentage of Barr bodies in AO-stained smears ranged from 5-18 among females and from 0 to 8 in males, while with PAP it ranged from 4-12 in females and 0-2 in males where as in Datar[1] et al study in AO stain it ranged from 0-8 in males and 5-32 in females and in PAP it ranged from 4-20 in females and 0-5 in males.

| **Table 13 - Comparison of Statistical Parameters of Aceto Orcein stain of present study with Datar et al study** | | | |
|---|---|---|---|
| | | Present study | Datar *et al.*[1] |
| Mean Percentage of Barr bodies | Females | 14.9% | 12.4% |
| | Males | 1.6% | 2.3% |
| Sensitivity | | 98.18% | 98% |
| Specificity | | 96.91% | 98% |
| Accuracy | | 97.58% | 98.3% |

| **Table 14 - Comparison of statistical Parameters of Papanicolaou stain of present study with Datar et al study** | | | |
|---|---|---|---|
| | | Present study | Datar *et al.*[1] |
| Mean Percentage of Barr bodies | Females | 7.5% | 9.2% |
| | Males | 0.5% | 1.2% |
| Sensitivity | | 83.64% | 90% |
| Specificity | | 100% | 100% |
| Accuracy | | 91.30% | 95% |

Using AO technique there was a better chance of detection of Barr bodies. These results were also noticed with the study done by Datar *et al*[1] who have made a similar observation even by counting 50 cells for the calculation of mean Barr body percentage. In the present study, adopting the criteria for counting a minimum of 100 cells to arrive at the mean Barr body percentage has yielded a standard deviation of 2.2 and 1.6 in females and 1.1 and 0.6 in males showing an advantage over the counting of fifty cells as done by Datar *et al.*[1]

Mittal T *et al.*[3] in their study titled *"Sex Determination from Buccal Mucosal Scrapes"* have analyzed the smears using Papanicolaou stain. From their observations, percentage of Barr bodies in males ranged from 0-4%, where as in females it was 20 -78%. The range and the mean percent of Barr bodies among women in the present study are concurring with other studies.

Barr body evaluation using highly sophisticated microscopic techniques like confocal microscopy and utilisation of highly specific fluorescent stains ( D.Shyam Prasad Reddy et al[2] ) like Acridine Orange, Acriflavaine Schiff ( Tibin et al[62] ) the mean Barr body percentages documented by these authors were similar to the studies done using routine stains and Bright field microscopy. Thus proving that bright field microscopy and routine stains are on par with said advanced technologies.

Tibin K B *et al.*[62] in their study conducted to determine the efficacy of Papanicolaou and acriflavine Schiff in buccal smears also documented that the frequency of Barr bodies varied from 27 -70% in female samples and 0-8% in male samples.

Thus there is no need of the highly sophisticated instruments and fluorescent stains as similar values were noted with bright field microscopy and cost effective stains like PAP, Aceto-orcein, H & E, Feulgen and Guard.

Aceto-orcein has been chosen amongst the chromatin specific stain as the other alternatives like Feulgen and guard which are time consuming, tedious and difficult to standardise.

Hermann and Davis[75] in 1955 evaluated a total of 100 oral smears for Barr bodies and stated 0-2% incidence of Barr body positive in males and 10% to 32% in females.

Manjula bai *et al.*[76] (1997) in their study on different ethnic groups like Indian, Malaysian and Chinese subjects did not report any Barr body positive cells in men.

Shruthi Singh *et al.*[54] conducted a study to disclose Barr bodies in buccal scrapes for sex determination using 100 buccal scrapes fixed in 95% formalin and stained with H&E and special stain alkaline methylene blue. Both these stains are time consuming when compared with Aceto-Orcein.

Suazo *et al.*[77] (2010) conducted a study on histological sections of human dental pulp using H and E stain and found out that the mean of Barr body - positive cells was 20.4 in female samples. There was no Barr body - positive cells in preparations of male subjects.

Teplitz R. L.[12] (1965) conducted a study to demonstrate Barr bodies in cone cells of human retina and concluded that each retinal cone cell of the female contains a sex-chromatin body not present in comparable material from the male

Vernino *et al.*[11] (1960) conducted a study to demonstrate Barr bodies in the nuclei of osteoblasts, osteocytes and periosteal cells of female mammals.

In the present study, three male buccal smears stained with Aceto-Orcein showed >5% of Barr bodies which could be due to the staining , fixation and ethnicity. Few authors have noted Barr bodies upto 8% in males.[1,74]

In the Present study, two females showed <5% of Barr bodies , this could be due to the above mentioned causes as we conducted our study in three terms of medical students where they came from different geographical locations in India.

Interestingly, Mittal *et al.*[3] explained that the mean and range of Barr bodies differs with people who are from Caucasoid origin and Nigerian population. Obi and Ikerionwu also explained there is relationship between race and Barr bodies.

Barr bodies in an individual varies not only with the sex of the person but also with phases of menstrual cycle and in menopause.[1,35]

Hagy *et al.*[78] as early as 1972 explained that during the proliferative phase the percentage of Barr bodies was higher when compared to the secretory phase. The probable explanation offered was that the hormonal regulation during the different phases of menstrual cycle and Glucose-6-Phosphate dehydrogenase activity.[35,37,38]

Sastry et al in 1985 explained that variation of sex chromatin percentage in menstrual cycle and menopause. They concluded that absence of Barr bodies in menopause females, and varying percentage of Barr bodies in menstruating females, can be found.

Mean percentage Barr bodies were also low during pregnancy, as well as in women on oral contraceptives and in breast, cervical and oesophageal cancers,[37,38,78,79] and teratomas. Frequency was much more decreased when there was vascular invasion and when tumours had high malignant potential.[37,38]

Low frequency of Barr body was also observed in new born females and their mothers on the 1$^{st}$ postpartum day, increasing gradually on the 2$^{nd}$ and 3$^{rd}$ day, which stabilized on the 5$^{th}$ day and finally became similar in both mothers and the children.[36,80,81] Reactivation of X chromosome was observed whenever the body was under physiological stress.[38]

To further evaluate the AO stained smears apart from analysing the mean Barr body percentage, cytomorphological features like nuclear staining (chromatin pattern),

back ground, cell morphology and overall staining were also studied in both AO and PAP stained smears

The distinct advantage of AO smears over PAP stained smears was that the commensal organisms in the buccal mucosa were stained only in 1.5% of cases, which was 11% in the PAP stained smears. This enabled us in having better nuclear morphological appreciation with minimal overlapping and thereby reducing the screening time.

PAP, being the complex stain it is, with two counter stains for the cytoplasm had a clean background in case of 88.9% smears, whereas, AO smears had excellent contrast with clear demarcation of nucleus which enhanced the interpretation of buccal smears. In 99.5% of cases the absence of cytoplasmic staining in AO smears offered ease in counting the minimum number of cells required i.e., 100 cells, which was taken as a criteria in this study.

After considering the nuclear and cytoplasmic features in the form of overall staining both the stains had better morphology and were comparable. Nuclear staining, cytoplasmic staining and nuclear membrane integrity parameters were described by Archana *et al*[70] in their study who compared stains like carbol fuchsin, Papanicolaou and Acridine Orange. They compared light and deep staining in nucleus and cytoplasm and nuclear membrane integrity.

Based on these observations buccal smear Barr body evaluation for sex determination has excellent sensitivity, specificity and accuracy which are essential for a screening test. Addition of Aceto-Orcein into the methodology retains this accuracy and reduces the over all analysis time, significantly. Cases which have been not identified conclusively by Barr body examination needs to be evaluated further by –

blood samples for Davidson bodies, Karyotyping, FISH and Polymerase chain reaction.

Aceto-Orcein with its many advantages is an excellent choice. However the smears stained by following this procedure loses the staining characteristics over time. This is not an issue in case of analysis when rapid screening and reporting of results is done. Whereas, it will be a disadvantage if the smear has to be archived for review on a later date.

Barr bodies in buccal smears can also be determined with PAP stain , which has an equitable degree in determining sex chromatin. Because of its prolonged staining technique, cyto morphological features and various reagents Aceto-Orcein stood ahead in determining Barr body evaluation in clinical settings. Although, in few borderline subjects confirmatory tests like DNA profiling should be done, which is usually performed in subjects who lack the positive confirmation by gender determination as well as in discrepancy of genetic profile cases. Human dental pulp, which is an excellent source of DNA can be used for these purposes. One more advanced technique is with Amelogenin gene which can be done by pyrosequencing of short PCR products helps in conclusively identifying the genotypic gender of a person.

# 7. CONCLUSION

- Sex determination can be done rapidly by determining the percentage of Barr bodies in buccal scrapes by Aceto-Orcein stain and PAP stain.
- Aceto-Orcein staining by squash technique is a rapid, economic and simple method providing 95-98% accuracy.
- It can be employed as a significant adjunct to other approaches of sex determination, even at a crime site.
- It can reduce staining time with accurate results, in rural areas and during natural calamities. It can be done with simple equipment and in a cost-effective way, whereas technically other methods of sex determination are not possible due to lack of sophisticated infrastructure in these settings.
- Yet, with all that said, in borderline cases, it is essential to utilize the more intricate methods of sex determination.

# 8. SUMMARY

Sex determination by obtaining the epithelial cells from buccal mucosa is an useful test in many conditions. The sex of the individual can easily be identified by determining the percentage of Barr body positive cells. The presence of Barr bodies in buccal mucosal cells could be determined with a fair degree of accuracy using the Papanicolaou staining technique.

However, bacilli staining, time taken for staining led to the continued search for a better option and improving the technique further. Aceto-Orcein staining is an extremely easy technique, economic and a rapid scrutiny for Barr body is facilitated by squashing which enlarges the nucleus and aids in evaluation.

In the present study, 414 smears were prepared from medical students attending Department of Pathology in BLDE ( Deemed to be university) Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura. Among the smear samples one was stained with Aceto-Orcein and the other with Papanicolaou. Evaluation was done under oil immersion by counting 100 cells obtaining percentage of Barr body.

A prefixed criteria was used to evaluate the percentage of Barr bodies. Presence of Barr bodies ≤5% were taken as masculine gender and if the percentage of Barr bodies >5% were taken as feminine gender. Mean percentage of Barr bodies with Aceto-Orcein ( 8.7% ) staining is double the number when compared to Papanicolaou ( 4.3% ) staining which are statistically significant with a p value < 0.001.

Mean percentage of Barr bodies were consistently higher in females ( 14.9% with Aceto-Orcein and 7.5% with Papanicolaou stain ) than in males ( 1.6% with Aceto-Orcein and 0.5% with PAP stain ) which are statistically significant with a p value < 0.001.

Buccal smears were also evaluated for 4 parameters such as nuclear staining, cell morphology, back ground staining , over all staining.

Out of 414 smears, AO Stained 206 ( 99.5% ) smears showed acceptable nuclear staining (crisp) . PAP stained 192 ( 92.8% ) smears showed acceptable nuclear staining and 16 (7.7%) smears showed moderately crisp nuclear staining. Both the stains were comparable, they did not show any smudgy nuclear staining and these findings were statistically significant.

Two hundred and six AO stained smears showed acceptable cell morphology (well preserved and crisp). whereas, 198 (95.7%) cases of PAP stained smears showed acceptable cell morphology and 09 (4.3%) smears showed moderately preserved cell morphology. Both the stains were comparable. These observations were statistically significant with p value < 0.001.

Among 414 smears, 204( 98.5%) AO smears and 184 ( 88.9% ) PAP smears showed clean background and 03 ( 1.5% ) AO stained smears and 23 ( 11.1% ) PAP stained smears showed bacilli in the back ground with statistically significant p value <0.001.

Overall staining of both the stains i.e., Papanicolaou ( PAP ) and Aceto-Orcein (AO) were good. After considering parameters like nuclear, cytoplasmic and back ground staining characteristics the overall staining was found to be comparable.

The sensitivity and accuracy of Aceto-Orcein was 98.18% and 97.58% respectively which was higher when compared to Papanicolaou stain with sensitivity and accuracy of 83.64% and 91.30% respectively, whereas specificity of Aceto-Orcein is 96.91% and with PAP stain it was 100%.

It is just as accurate as other methods but much more rapid, and has the additional advantage that fine nuclear details and structure were more readily revealed by squashing and flattening of the freshly obtained cells.

As with every other screening procedure, in certain border line cases it needs to be paired with confirmatory tests like molecular methods by DNA analysis, Karyotyping, FISH and PCR for a conclusive diagnosis.

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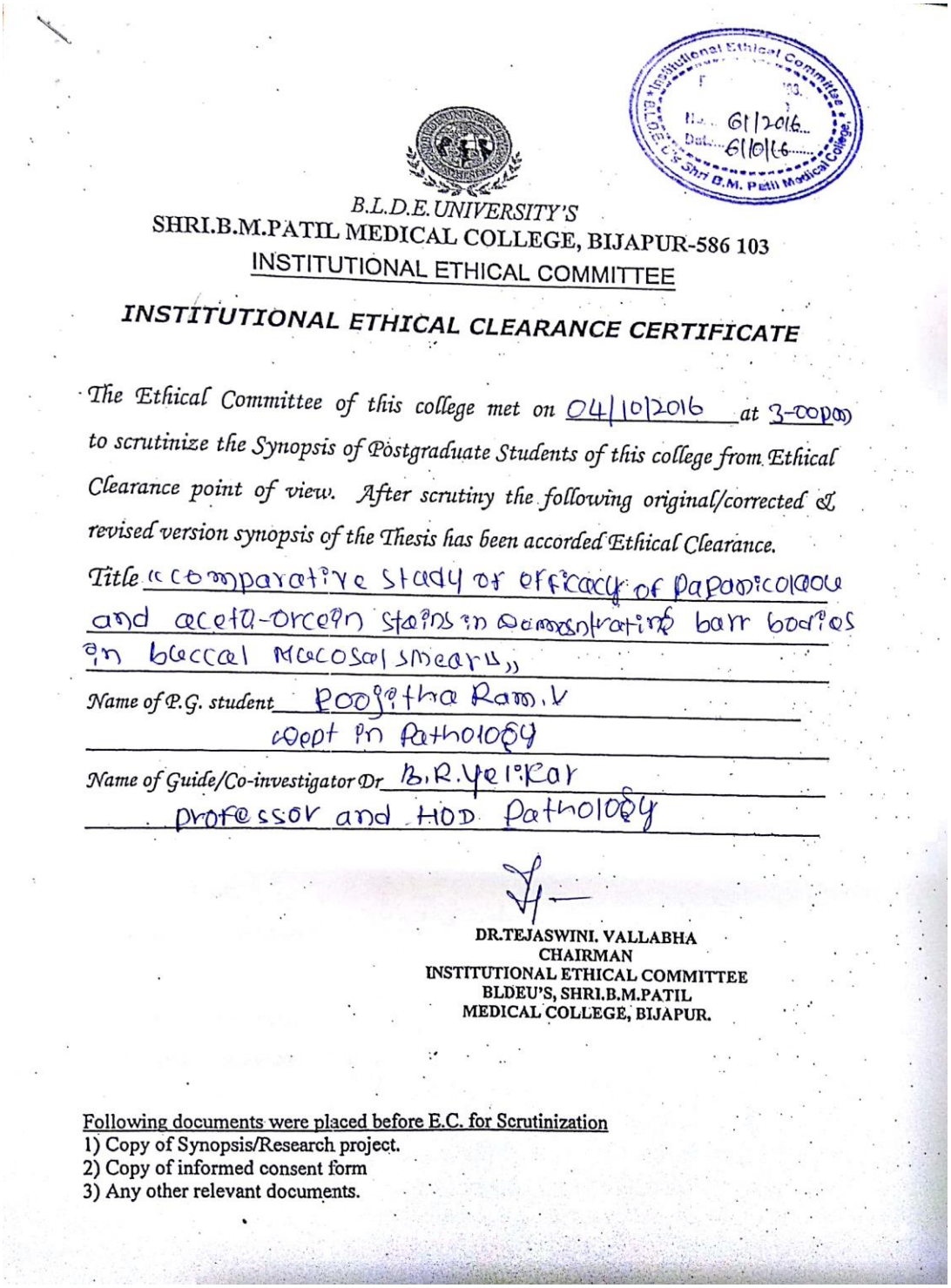
# ANNEXURE-I

# ETHICAL CLEARANCE



Institutional Ethical Committee
No. 61/2016
Date 6/10/16
Shri B.M. Patil Medical College

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

**INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE**

The Ethical Committee of this college met on 04/10/2016 at 3-00pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Comparative Study of efficacy of Papanicolaou and aceto-orcein stains in demonstrating barr bodies in buccal Mucosal smears"

Name of P.G. student Roopitha Ram.V
Dept in Pathology

Name of Guide/Co-investigator Dr B.R.Yelikar
Professor and HOD Pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

## ANNEXURE-II

# BLDE (DEEMED TO BE UNIVERSITY) SHRI B M PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTER VIJAYAPUR, KARNATAKA

**INFORMED CONSENT FOR PARTICIPATION IN**

**DISSERTATION/RESEARCH**

I, the undersigned,__, S/O D/O W/O____, aged _____years, ordinarily resident of____do hereby state/declare that Dr Poojitha Ram. V of Shri B.M Patil medical college Hospital has examined me thoroughly on at_____(place) and informed me that he/she is conducting dissertation/research titled "Comparative study of efficacy of Papanicolaou and Aceto-Orcein stains in demonstrating Barr bodies in buccal mucosal smears" under the guidance of Dr B.R. Yelikar, requesting my participation in the study. Doctor has also informed that the observation/results of test will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the procedure related complications most of them are treatable but are not anticipated.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of study related to diagnosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can

terminate me from the study at any time from the study.

After understanding the nature of dissertation or research, diagnosis made, I the undersigned Shri/Smt ---------- under my full conscious state of mind agree to participate in the said research/ dissertation.

Signature of Study subject:

Signature of doctor:

Witness: 1.

2.

Date:

Place

## ANNEXURE-III

## PROFORMA

**Case No:**

**Buccal Smear No:**

| | |
|---|---|
| **Name:** | |
| **Age(Yrs):** | |
| **Sex:** | |
| **Adequacy: For both smears (>100 well preserved cells - Adequate. <100 cells - Inadequate)** | **Adequate / Inadequate** |

## COMPARISON OF PARAMETERS

| Features | Aceto - Orcein | Papanicolaou |
|---|---|---|
| Nuclear staining (Chromatin pattern) | ☐ Smudgy<br>☐ Moderately Crisp<br>☐ Crisp | ☐ Smudgy<br>☐ Moderately Crisp<br>☐ Crisp |
| Background | ☐ Hemorrhagic<br>☐ Clean<br>☐ Bacilli | ☐ Hemorrhagic<br>☐ Clean<br>☐ Bacilli |
| Cell morphology | ☐ Not preserved<br>☐ Moderately preserved<br>☐ Well preserved and crisp | ☐ Not preserved<br>☐ Moderately preserved<br>☐ Well preserved and crisp |
| Overall staining | ☐ Bad<br>☐ Moderately good<br>☐ Good | ☐ Bad<br>☐ Moderately good<br>☐ Good |
| Percentage of Barr Bodies | | |

## KEY TO MASTER CHART

| | | |
|---|---|---|
| Sl.no | - | Serial Number |
| Grp | - | Aceto-Orcein or Papanicolaou stain |
| AO | - | Aceto-Orcein |
| PAP | - | Papanicolaou |
| Zero (0) | - | Absence of the feature |
| One (1) | - | Presence of the feature |

# MASTER CHART

## AO

| GRP | S. No | Name | Age | Sex | Adequate | Nuclear stain | | | Background | | | Cell morphology | | | Overall staining | | | Percentage of Barr bodies |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Smudgy | Moderately Crisp | Crisp | Hemorrhagic | Clean | Bacilli | Not preserved | Moderately Preserved | Well Preserved and crisp | Bad | Moderately Good | Good | |
| AO | 1 | B Priyanaka | 20 years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 11 |
| AO | 2 | Gulshan Kumar Gaurav | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 3 | M Ashok Prakhyath | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 4 | Praveen Kumar H | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 5 | A VSSR Chiranjeevi | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 6 | B V Vishnu Vardhan Reddy | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 7 | Chandrakant Myakeri | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 8 | C Venkat Sai Bharath | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 9 | Ramees S | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 3 |
| AO | 10 | Shahana Shetagar | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 11 | Siddharth Patil | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 4 |
| AO | 12 | Vinaj Amoji | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 13 | Afreen Aralimalti | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 13 |
| AO | 14 | Chandrashekhar S Halli | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 4 |
| AO | 15 | Deepak Hadimani | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 16 | Kottam Reddy Vishnu Teja | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 17 | M Renuka Patil | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 18 | Mohan Singh | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 19 | Sree Gayathri N | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 20 | Osha Raag Chowdhury | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 21 | Saurav Kumar | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 22 | Shahrukh Khan | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 23 | Venkatesh Narayan Sarwad | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 24 | Abhishek Kumar | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 25 | Abhishek Tyagi | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 26 | Ahaziah Nicolas U | 19years | Male | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 27 | Aishwarya Hunashikatti | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 28 | Akshay K | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 29 | Aman Agarwal | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 30 | Amit Desai | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 31 | Archana C Sajjan | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AO | 32 | Arpit Vatts | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 33 | Ashique Ummer K M | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 34 | Ashwath Narayan G | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 35 | Rodgi Chinmaya | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 36 | Daneshwari Kottalamath | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 37 | Deepa Vivekanand Shirolkar | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 38 | Deepika Patil | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 39 | Dhanraj S Hosamani | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 40 | D Gayathri | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 41 | Gandhi Suyash Gajanan | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 42 | Garlapati Vardhan Vishnu | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 43 | Geddam Avinash | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 44 | Girija Gudagunti | 21Years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 45 | Joyeta De | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 46 | Jyothi Parvathi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 19 |
| AO | 47 | Jyothi Gejji | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 48 | Sarayulasya Reddy Kandi | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 49 | Kosuri Kanaka Swarna Bharathi | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 50 | Krishna Reddy Singa Reddy | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 51 | Krishna Sai Davala | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 52 | Laxmi siddu Gadadani | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 53 | L Venkata sandeep Reddy | 21 years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 54 | M Sandesh Gouda | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 55 | Mandadi leelakrishna Prasad | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 56 | Matarn Meenakshi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 57 | Mounika | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 58 | Naravula Divyashree | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 59 | Nidhi Aravind Mangalwedhe | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 60 | Nikhil Taranath Sitimani | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 61 | Nishitha B | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 62 | Nushi srinath Reddy | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 63 | Pannuru Abhishek | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 64 | Parvat Reddy Rohit Reddy | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 65 | Poornima N Sajjan | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 13 |
| AO | 66 | Prachi Prathyush | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 67 | Prahalad M | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 68 | Prathiba Nirogi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 69 | Preeya Saluja | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 70 | Priyadarshini M Tambake | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AO | 71 | Radhey Kapil Joshi | 18years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 72 | Rayasam Meghana | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 73 | Renuka Ashok Majjigudda | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 74 | Rohan Suhash Nathwani | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 75 | Rubiya Khanam | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 76 | Rutwik Doddawad | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 77 | Sadafara Janvekar | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 78 | Saloni Gupta | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 79 | Samruddi Gaddad | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 80 | Sangan Prem Chandra Reddy | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 81 | Shamila P | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 82 | Sayandeep Kusalkanti Das | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 83 | Sharadhi S petkar | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 84 | Sharanya Manchala | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 85 | Shashank M Hiremath | 18Years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 86 | Shreya Arun Kulkarni | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 87 | Shreya Rani Patil | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 88 | Sneha ulhas Kumar Arakeri | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 89 | Soujanyana C Hala Kurki | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 90 | Sourabh S Patil | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 91 | Sukriti Marwala | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 13 |
| AO | 92 | Sumedha Singala | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 93 | Suprit Melali | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 94 | Tanya | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 95 | Varshini S | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 96 | Veerendra B Patil | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 97 | Vempalli Shruthi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 98 | Venkatt Meghana Bhimanadhan | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 99 | Venus Hobam | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 100 | Vikas Ashok Khot | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 101 | Vikram R | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 102 | Vrindha Pahuja | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 13 |
| AO | 103 | Franko K Abraham | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 104 | Gunmidipudi Hyndavi | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 105 | Anki Reddy Lakshmi PR | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 106 | Bingesh | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 107 | Kaluvakollu Padmini | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 108 | Koduri Himaja | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 109 | Sai Hemanth Thati | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AO | 110 | Swaroop Singh Bhati | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 111 | Kajal S Muddennavar | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 112 | M. Shamreen Banu | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 113 | Swetha Hadimai | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 114 | Aboorvanila M | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 115 | Aishwarya C Kotihal | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 116 | Akshata K Karemmavar | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 117 | Amruta Gangashetti | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 10 |
| AO | 118 | B.Aishwari Gowd | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 119 | Daneshwari Mannangi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 120 | Daphibapaka Khongjee | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 121 | Ghanta Varsha | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 122 | Karna Pendakur | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 20 |
| AO | 123 | Kolagani Himaja | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 124 | Megha C Immannavar | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 125 | Ramya Deepika Jatla | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 126 | Saradaka Shullai Lamare | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 127 | Srinidhi H Kulkarini | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 10 |
| AO | 128 | Srushti S Koti | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 129 | Sushma NandaReddy | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 11 |
| AO | 130 | Swetha Haddalageri | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 13 |
| AO | 131 | Telukutla Sowmya | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 132 | Vaishnavi Patil | 24years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 133 | Veluru Chandrika | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 13 |
| AO | 134 | Shivukumar Dinnnimani | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 135 | Shrinivas Nayak | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 136 | Siddharath Ramesh Desai | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 137 | Tushar Mankar | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 138 | Adarsh NK | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 139 | Aditya Patil | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 140 | Aravind Gowtham C M | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 141 | Megharaj I H | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 142 | Mohammad Waseem | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 143 | Murgesh S K | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 144 | Naralanka Adithya | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 145 | Naveen Kanakaraya | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 146 | Popuri Sai Krishnan | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 147 | Rahul Kumar Guptha | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 148 | Rohith S Hiremath | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AO | 149 | Sangamesh M | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 150 | Suhas S Hiremath | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 151 | Sunil Biradar | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 152 | Thaniparthi Saitej | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 153 | Shantalinga B Bhairagonda | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 154 | Hemant | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 155 | Nidhi Rani | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| AO | 156 | Priyanka | 23years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 157 | Alfy Alphonsa Babu | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 158 | Aditi | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 159 | Anupama Iranna Guchetti | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 160 | Soujanya | 23years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 161 | Abhishek | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| AO | 162 | Archana | 23years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 163 | Arpita Nesur | 24years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 164 | Arpita Sajjanar | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 165 | Arun Prashanth M K | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 166 | Aryan Jain | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 167 | Astha Agrawal | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 168 | Babar Ashlesha Ramesh | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 169 | Bhagyashree B.Gunnapur | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 170 | Chaithra PA | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 171 | Chereddy Venkata Raja A | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 172 | Deeksha Chhabra | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 173 | Dharmaraj H P | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 174 | Divya Basavaraj Jyothi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 175 | Ekaansh Karir | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 176 | Esha Mohapatra | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 177 | Faiz Husain | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 178 | Gagan B R | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 179 | Gaurav Arora | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 180 | Gayathri D.patil | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 181 | Gopika Nanda Kumar | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 182 | Greeshma Mathay | 23years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 183 | Harshak Vaibhav N | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 184 | Harshitha Rajput | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 185 | Harshitha Prasad | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 186 | Hemanth Kumar T H | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 187 | Imam Mohamad Junedi A | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AO | 188 | Inapurapu Himanshu Kumar | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 189 | Indira Chakravarthy | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 190 | Isha Arora | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 191 | Ishan garg | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 192 | Jini S | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 193 | Jitendra Kumar Mishra | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 194 | karumuri venkata Jayabala | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 195 | Keshav Vishesh | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| AO | 196 | Kiran Kumar T | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| AO | 197 | Konda Monish Reddy | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 198 | Krupa C M | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 199 | Ruchi Singh | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| AO | 200 | Kumari sheethal Singh | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 14 |
| AO | 201 | Lakshitha Narang | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 15 |
| AO | 202 | Likitha U M | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 17 |
| AO | 203 | Manpreet Kaur | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 16 |
| AO | 204 | Merlyn Joby | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 18 |
| AO | 205 | Rakesh Singh Gupta | 18years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| AO | 206 | Santhosh Mishra | 18 years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 5 |
| AO | 207 | Sunil simhaRaj | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |

# PAP

| GRP | S. No | Name | Age | Sex | Adequate | Nuclear stain | | | Background | | | Cell morphology | | | Overall staining | | | Percentage of Barr bodies |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Smudgy | Moderately Crisp | Crisp | Hemorrhagic | Clean | Bacilli | Not preserved | Moderately Preserved | Well Preserved and crisp | Bad | Moderately Good | Good | |
| PAP | 1 | B Priyanaka | 20 years | Female | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 2 | Gulshan Kumar Gaurav | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 3 | M Ashok Prakhyath | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 4 | Praveen Kumar H | 23years | Male | Adequate | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 5 | A VSSR Chiranjeevi | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 6 | B V Vishnu Vardhan Reddy | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| PAP | 7 | Chandrakant Myakeri | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 8 | C Venkat Sai Bharath | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 9 | Ramees S | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| PAP | 10 | Shahana Shetagar | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 11 | Siddharth Patil | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| PAP | 12 | Vinaj Amoji | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 13 | Afreen Aralimalti | 22years | Female | Adequate | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 14 | Chandrashekhar S Halli | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 15 | Deepak Hadimani | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 16 | Kottam Reddy Vishnu Teja | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 17 | M Renuka Patil | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 18 | Mohan Singh | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 19 | Sree Gayathri N | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 7 |
| PAP | 20 | Osha Raag Chowdhury | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 21 | Saurav Kumar | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 22 | Shahrukh Khan | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 23 | Venkatesh Narayan Sarwad | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 24 | Abhishek Kumar | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 25 | Abhishek Tyagi | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| PAP | 26 | Ahaziah Nicolas U | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 27 | Aishwarya Hunashikatti | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 28 | Akshay K | 21years | Male | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 29 | Aman Agarwal | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 30 | Amit Desai | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 31 | Archana C Sajjan | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 32 | Arpit Vatts | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| PAP | 33 | Ashique Ummer K M | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAP | 34 | Ashwath Narayan G | 20years | Male | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 35 | Rodgi Chinmaya | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 36 | Daneshwari Kottalamath | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 37 | Deepa Vivekanand Shirolkar | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 38 | Deepika Patil | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 6 |
| PAP | 39 | Dhanraj S Hosamani | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 40 | D Gayathri | 20years | Female | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 41 | Gandhi Suyash Gajanan | 20years | Male | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 42 | Garlapati Vardhan Vishnu | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 43 | Geddam Avinash | 20years | Male | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 44 | Girija Gudagunti | 21Years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 45 | Joyeta De | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 46 | Jyothi Parvathi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 8 |
| PAP | 47 | Jyothi Gejji | 20years | Female | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 8 |
| PAP | 48 | Sarayulasya Reddy Kandi | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 49 | Kosuri Kanaka Swarna Bharathi | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 50 | Krishna Reddy Singa Reddy | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 51 | Krishna Sai Davala | 21years | Female | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 52 | Laxmi siddu Gadadani | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 53 | L Venkata sandeep Reddy | 21 years | Male | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| PAP | 54 | M Sandesh Gouda | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 55 | Mandadi leelakrishna Prasad | 20years | Male | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 56 | Matarn Meenakshi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 57 | Mounika | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 58 | Naravula Divyashree | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 59 | Nidhi Aravind Mangalwedhe | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 8 |
| PAP | 60 | Nikhil Taranath Sitimani | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 61 | Nishitha B | 21years | Female | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 62 | Nushi srinath Reddy | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 63 | Pannuru Abhishek | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 64 | Parvat Reddy Rohit Reddy | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 65 | Poornima N Sajjan | 20years | Female | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 66 | Prachi Prathyush | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 67 | Prahalad M | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 68 | Prathiba Nirogi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 69 | Preeya Saluja | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 70 | Priyadarshini M Tambake | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 71 | Radhey Kapil Joshi | 18years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 72 | Rayasam Meghana | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAP | 73 | Renuka Ashok Majjigudda | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 74 | Rohan Suhash Nathwani | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 75 | Rubiya Khanam | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 76 | Rutwik Doddawad | 18years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 77 | Sadafara Janvekar | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 78 | Saloni Gupta | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 79 | Samruddi Gaddad | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 80 | Sangan Prem Chandra Reddy | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 81 | Shamila P | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 82 | Sayandeep Kusalkanti Das | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 83 | Sharadhi S petkar | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 84 | Sharanya Manchala | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 85 | Shashank M Hiremath | 18Years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 86 | Shreya Arun Kulkarni | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 87 | Shreya Rani Patil | 21years | Female | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 88 | Sneha ulhas Kumar Arakeri | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 89 | Soujanyana C Hala Kurki | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 90 | Sourabh S Patil | 19years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 91 | Sukriti Marwala | 20years | Female | Adequate | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 92 | Sumedha Singala | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 93 | Suprit Melali | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 94 | Tanya | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 95 | Varshini S | 19years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 96 | Veerendra B Patil | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 97 | Vempalli Shruthi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 98 | Venkatt Meghana Bhimanadhan | 21years | Female | Adequate | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 99 | Venus Hobam | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 5 |
| PAP | 100 | Vikas Ashok Khot | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 101 | Vikram R | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 102 | Vrindha Pahuja | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 103 | Franko K Abraham | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 104 | Gunmidipudi Hyndavi | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 105 | Anki Reddy Lakshmi PR | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 106 | Bingesh | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 107 | Kaluvakollu Padmini | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 10 |
| PAP | 108 | Koduri Himaja | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 109 | Sai Hemanth Thati | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 110 | Swaroop Singh Bhati | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 111 | Kajal S Muddennavar | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAP | 112 | M. Shamreen Banu | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| PAP | 113 | Swetha Hadimai | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 114 | Aboorvanila M | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 115 | Aishwarya C Kotihal | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 116 | Akshata K Karemmavar | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 117 | Amruta Gangashetti | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 5 |
| PAP | 118 | B.Aishwari Gowd | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 119 | Daneshwari Mannangi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 5 |
| PAP | 120 | Daphibapaka Khongjee | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 4 |
| PAP | 121 | Ghanta Varsha | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 122 | Karna Pendakur | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 12 |
| PAP | 123 | Kolagani Himaja | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 124 | Megha C Immannavar | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 125 | Ramya Deepika Jatla | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 126 | Saradaka Shullai Lamare | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 127 | Srinidhi H Kulkarini | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 |
| PAP | 128 | Srushti S Koti | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 5 |
| PAP | 129 | Sushma NandaReddy | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 5 |
| PAP | 130 | Swetha Haddalageri | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 131 | Telukutla Sowmya | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 5 |
| PAP | 132 | Vaishnavi Patil | 24years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 133 | Veluru Chandrika | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 134 | Shivukumar Dinnnimani | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 135 | Shrinivas Nayak | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 136 | Siddharath Ramesh Desai | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 137 | Tushar Mankar | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 138 | Adarsh NK | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 139 | Aditya Patil | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 140 | Aravind Gowtham C M | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 141 | Megharaj I H | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 142 | Mohammad Waseem | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 143 | Murgesh S K | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 144 | Naralanka Adithya | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 145 | Naveen Kanakaraya | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 146 | Popuri Sai Krishnan | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 147 | Rahul Kumar Guptha | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 148 | Rohith S Hiremath | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| PAP | 149 | Sangamesh M | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 150 | Suhas S Hiremath | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAP | 151 | Sunil Biradar | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 152 | Thaniparthi Saitej | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 153 | Shantalinga B Bhairagonda | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 154 | Hemant | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 155 | Nidhi Rani | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 156 | Priyanka | 23years | Female | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 10 |
| PAP | 157 | Alfy Alphonsa Babu | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 158 | Aditi | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 159 | Anupama Iranna Guchetti | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 10 |
| PAP | 160 | Soujanya | 23years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 161 | Abhishek | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 162 | Archana | 23years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 163 | Arpita Nesur | 24years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 5 |
| PAP | 164 | Arpita Sajjanar | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 165 | Arun Prashanth M K | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 166 | Aryan Jain | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 167 | Astha Agrawal | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 168 | Babar Ashlesha Ramesh | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| PAP | 169 | Bhagyashree B.Gunnapur | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 170 | Chaithra PA | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 171 | Chereddy Venkata Raja A | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 172 | Deeksha Chhabra | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 173 | Dharmaraj H P | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 174 | Divya Basavaraj Jyothi | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 175 | Ekaansh Karir | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 176 | Esha Mohapatra | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 177 | Faiz Husain | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 178 | Gagan B R | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 179 | Gaurav Arora | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 180 | Gayathri D.patil | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 9 |
| PAP | 181 | Gopika Nanda Kumar | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 6 |
| PAP | 182 | Greeshma Mathay | 23years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 10 |
| PAP | 183 | Harshak Vaibhav N | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 184 | Harshitha Rajput | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 185 | Harshitha Prasad | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 186 | Hemanth Kumar T H | 24years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 187 | Imam Mohamad Junedi A | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 188 | Inapurapu Himanshu Kumar | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 189 | Indira Chakravarthy | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAP | 190 | Isha Arora | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 191 | Ishan garg | 23years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 192 | Jini S | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 193 | Jitendra Kumar Mishra | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 194 | karumuri venkata Jayabala | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 195 | Keshav Vishesh | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 196 | Kiran Kumar T | 22years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 197 | Konda Monish Reddy | 21years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 198 | Krupa C M | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 199 | Ruchi Singh | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 200 | Kumari sheethal Singh | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 201 | Lakshitha Narang | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 202 | Likitha U M | 21years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 8 |
| PAP | 203 | Manpreet Kaur | 20years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 204 | Merlyn Joby | 22years | Female | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 7 |
| PAP | 205 | Rakesh Singh Gupta | 18years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| PAP | 206 | Santhosh Mishra | 18 years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| PAP | 207 | Sunil simhaRaj | 20years | Male | Adequate | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |