CERVICAL CYTOMORPHOLOGIC PRESENTATIONS: ASSOCIATION WITH PRE-CERVICAL CANCER DETERMINANTS IN WOMEN FROM NAKURU COUNTY

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DECLARATION

This thesis is my original work and has not been presented for a degree or other award in any other University.

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This thesis is dedicated to my son, Matthew Juan, and the entire Muitta family for their unwavering and consistent support during the study undertakings and Thesis preparation.

I love you all "*Asanteni sana*".

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ABBREVIATIONS AND ACRONYMS

ADC	Adenocarcinoma
AGC	Atypia of Glandular Cells
AIS	Adenocarcinoma In Situ
AUB	Abnormal uterine bleeding
CIN	Cervical Intraepithelial Neoplasia
CIS	Carcinoma In Situ
HPF	High Power Field
HR HPV	High risk Human Papilloma Virus
HSIL	High grade Squamous Intraepithelial Lesion
ICC	Invasive Cervical cancer
LBC	Liquid Based cytology
LSIL	Low grade Squamous Intraepithelial Lesion
MCH	Maternal Child Health
MTRH	Moi Teaching and Referral Hospital
PAP	Papanicalaou
RT	Reproductive Tract
RTI	Reproductive Tract Infections
NCRH	Nakuru County Referral Hospital
SCC	Squamous Cell Carcinoma
SIL	Squamous Intraepithelial Lesion
STI	Sexually Transmitted Infections
TAH	Total Abdominal Hysterectomy
TBS	The Bethesda System

DEFINITION OF OPERATIONAL TERMS

Anthropometry	The measurement of the proportions of the human body such as weight, height, MUAC (mean upper arm circumference).
Atypia/Atypical	These are variations in terms of cellular abnormalities away from normal morphology within a cell which can be confined in the nucleus or cytoplasm for example: AGC-Atypia of glandular cells, ASC-Atypical squamous cells, ASCUS-Atypical squamous cells of undetermined significance, LSIL-low grade squamous intraepithelial lesion, HSIL-high grade squamous intraepithelial lesion, AGCUS-Atypical glandular cells of undetermined significance.
Carcinoma	A carcinoma is any malignant cancer that arises from epithelial cells.
Conventional cytology	Traditional cytology techniques in which preparations of scrapped or excavated cellular material is directly smeared on a glass slide without any emulsification in medium or diluent and immediately fixed before staining.
Cyanophilic- Basophilic	A staining reaction in cells characterized by blue-green hue of the cells cytoplasmic pH largely under ovarian hormonal influence.
Cytomorphology	The study of morphological cellular components such as nucleic and cytoplasmic cellular components.
Cytopathic effect	Morphological variations in cells brought on by

	microorganisms (viruses and obligate intracellular organisms).
Dependent variables	Attributes and characteristics derived from pre cancer diagnosis (Grades of LSIL, HSIL and AGC/AIS).
Dyskaryosis	Cellular abnormality of cells particularly within the nucleus such as irregular nuclear membrane, prominence of chromatin granules, bi-nucleation etc.
Dysplasia	A histologic pre cancer definition pertaining to the polarity and normal maturation of the superficial layer of epithelium (superficial, intermediate and parabasal layers) but characterized with presence of atypical cells in the superficial layer and absence of basal layer abnormality involvement.
Eosinophillic-Acidophilic	A staining reaction in cells characterized by the variation of pale- yellow to pink hue imparted by Eosin azure stain treatment within the cytoplasmic pH largely under ovarian hormonal influence.
Hyperchromasia	A staining reaction in cells characterized by intense blue hue of haematoxylin stain reaction within the nucleus.
Hypochromasia	A staining reaction in cells characterized by weak blue hue of haematoxylin stain reaction within the nucleus.
Unhealthy diet	Consumed diets particularly factory processed in which artificial preservatives, enhancers and additives have been added.
Independent variables	Attributes and characteristics that are perceived to cause

	pre cancer genesis. Also used interchangeably in this current study as determinants, risk factors and predictors.
Invasive	A histologic cancer definition pertaining to basal layer abnormality involvement in addition to the superficial epithelium (superficial, intermediated and parabasal layers), when there is a breach in the basement membrane. If the invasion extends 3 mm or less, it is referred to as micro-invasive disease. If invasion is greater than 3mm, it is frankly invasive cancer.
Koilocytes	Epithelial cells infected with viral agents hence characterized by abnormal features (cytopathic effects) of irregular nucleic membrane margin, multi-nucleation, increased nuclear-to-cytoplasm volume ratio, hyperchromasia and prominence of nuclear coarse chromatin granules.
Mitotic figures	These are features identified within the nucleus of cells during microscopy such as chromosomal chromatids, centromere, and spindle fiber remnants.
Neoplasia	A histologic cancer definition pertaining to new growth of abnormally maturing cells within the epithelium. Varying grades are diagnosed depending on the magnitude and proportions of abnormal maturing cells within the epithelium layer.
Orangeophilic	A staining reaction characterized by varying orange hue shades imparted by OG6 stain affinity to keratin deposits

within cellular cytoplasm.

Healthy diet: Consumed diets naturally obtained from the environment devoid of synthetic manipulation.

Paraneoplasia Clinical syndromes or disorders that are triggered by an altered immune system response to neoplasia. They involve non-metastatic systemic effects that accompany malignant disease e.g. fever, bleeding, wasting etc.

Perinuclear haloing A cytopathic change in viral infected epithelia characterized by an increased clear perimeter space within the cytoplasm.

Reactive These are changes characterized by infiltration of inflammatory processes components such as an increase in number of leucocytes due to infection e.g. bacterial infection.

VIA/VILI Visual inspection using Acetic acid (VIA) and Lugols iodine (VILI). Visual ectocervix abnormality test. VIA-Aceto white patches-abnormality associated with abnormal protein in the cervical epithelium VILI-banana yellow associated with abnormally immature differentiated cells (rapid cell turnover).

ABSTRACT

Cervical cancer disease is among leading global cancers in women and it causes reproductive tract ill health. The disease is preceded by pre cancer status identified by detection of abnormal cells in smears from the cervical wall. High risk genotype16 and 18 human papilloma virus is implicated. Other predisposing determinants include *Chlamydia trachomatis* infections, lifestyle and nutritional factors for example healthy diet inadequacies, and chronic reproductive tract ill health. The link between these determinants and pre cervical cancer grades has only partially been examined with no studies reported among women from Nakuru County. This descriptive cross-sectional study was conducted at Nakuru County referral hospital to examine the relationship of determined pre cervical cancer grades in regards to cervical epithelial cytomorphologic features with outcomes of select microbial STI, lifestyle and reproductive health characteristics among women participants. A total of 142 women participants, ≥ 20years of age, were purposively enrolled into the study. Manifested clinical signs, lifestyle and diet practices were collected using a questionnaire. Anthropometric physical measurements were recorded. Serum extracted from whole blood was screened for *Treponema pallidum* and HIV antibodies. In addition endocervical swabs were used for *Neisseria gonorrhea*, *Chlamydia trachomatis* antigen and HPV oncoprotein detection while endocervical scrape smears were examined for cyto-morphological profiling and categorization of enrolled subjects using the Bethesda 2014 classification into four pre cervical cancer study groups of: 1) LSIL; n=35; 2) HSIL; n=59; 3) AGC/AIS; n=8 and 4) controls; (No evidence of cellular lesion) n=40.Cytomorphologic findings indicated that in all participants,~67% subjects manifested koilocytic cells, while ~28% had high grade cellular atypia in their smears. Select STI screened indicated that ≥11% subjects were positive for HIV1/2 and at least 10% for HR HPV 16/18. Collectively at least ~6% of test subjects tested positively for *Treponema pallidum*, *Neisseria gonorrhoea* and *Chlamydia trachomatis*. Additionally, multivariate logistic modeling indicated that HPV16/18 was associated with likelihood of having LSIL, HSIL and AGC/AIS pre cancer grade (P<0.0001; β>3.600; OR>2.0; 95% CI). Lifestyle and nutritional assessment illustrated that anthropometric median values ≤99 cm for bust girth and ≤86cm for waist circumference were associated with higher risks of presenting with HSIL and AGC/AIS grades (P<0.04; β>1.681; OR>5.0; 95% CI), while history of consuming unhealthy diet was associated with higher odds of presenting with LSIL (P=0.012; β, -1.433; OR; 4.190; 95% CI;). Reproductive health evaluation revealed that history of lower abdominal pain and vaginal bleeding was associated with higher chance of presenting with LSIL (P=0.003; β=1.758; OR=5.800; 95% CI); HSIL (P=0.001; β=2.183; OR=8.873; 95% CI) or AGC/AIS (P<0.0001; β=25.347; OR=1.019; 95% CI). These results confirm that a high koilocytic atypia magnitude in examined smears is a true pointer of pre cervical cancer genesis. Moreover, HR HPV16/18 infection, upper trunk and abdominal wasting gauged from low median scopes of bust and waist, consumption of unhealthy diet and clinical history of protracted symptomatic manifestation of abdominal pain and vaginal bleeding are important predictors of the development of pre cervical cancer in women from Nakuru County. Therefore, maximum atypia detection should further be enhanced through employment of LBC. Screening of HR HPV infections, integration of anthropometric measure, adopting nutritional counseling and reproductive tract sign monitoring and care in the MCH would reduce risk of developing pre cervical cancer signs.

CHAPTER ONE

INTRODUCTION

1.1 Background

Cervical cancer is the third most common cancer in women worldwide with an estimated 530,000 new cases annually (Torre *et al.*, 2015)**.** It is the second most common cancer among women in South-Central Asia, in the Far East in countries like Malaysia and in sub Saharan Africa, including Central and East Africa, where in Kenya it accounts for 8,600 deaths annually, per every 100,000 Kenyan women. 2454 cervical cancer cases are diagnosed annually in Kenya (Ferlay *et al.*, 2012). The problem is compounded by the lack of prompt screening service and inadequate provision of funds for pre cancer preventive and curative services from the national government especially to the County government (Abwao *et al.*, 1998; Memiah *et al.*, 2012; NCCPP 2012-2015; Muitta *et al.*, 2015).

Cervical cancer originates in epithelial cells localized within the transformation zone of cervical wall (Rubin, 2001) and is preceded by a pre cancer status characterized by cells manifesting peculiar morphological manifestations away from normal cervical epithelial cells. Cytomorphologic examination of cervical epithelial cells scrapped from the cervix and smeared on glass slides is employed for the identification of abnormality (atypia) which may range from Koilocytes to cells of higher degrees of abnormality depending on the severity of nuclear relative to cytoplasmic lesion. Identification of inclusions such as commensal bacterium, fungal and parasitic infections and inflammatory cells in smears is usually a pointer of diagnostic significance. Oncogenic transformation of these cells has been linked to exposure to viral infections such as high risk human papilloma viruses coupled with other secondary factors associated with lifestyle and nutritional practices such as alcohol

and tobacco usage and poor diet (Burd, 2003; Boardman *et al.*, 2012;Cancer research UK, 2015). High risk HPV subtypes 16 and 18 are associated with rapid progression of pre cervical cancer towards high grade of cervical intraepithelial lesion (Temmerman *et al*., 1999; Plummer *et al*., 2007 and Ai *et al*.,2015) while other high risk types of HPV namely 31, 33, 35, 45, 52, 56, 58, 70 and Type X including Herpes simplex virus (HSV) are implicated in pre-cancerous lesions manifested by the identification of atypical squamous cells of undetermined significance (ASCUS) in processed cervical smears (Evans *et al.*, 2006; De Vuyst *et al.*, 2008).

HPV infection is most common in sexually active young women, 18-30 years of age where most women in this age group are diagnosed with low grade pre cervical cancer (Burd, 2003). There is a sharp decrease in prevalence after 30 years of age. High grade cervical cancer prevalence however is more common in women >35 years, suggesting infection is advanced -age related, since it occurs at a younger age with a slow progression to a severer grade of cervical cancer proceeding (Burd, 2003 and Boardman *et al.*, 2012).

Persistence of infection is more common in high-risk oncogenic HPV types and is an important determinant in the development of pre cervical cancer (Burd, 2003).Widespread STI such as *Treponema pallidum, Neisseria gonorrhoea* and *Chlamydia trachomatis* may potentially promote cervical neoplasia (Kiviat *et al.*, 1990; Parsonnet, 1999 and YufangZhu et al., 2016). Routine screening of these STI is not included in the NCCPP program hence it is important to examine their role in cervical cancer development which has not been previously determined in patients attending Nakuru County referral hospital. Reproductive health practices such as long-term use of oral contraceptives particularly in 30-45 years age group are associated with increased onset risk and mortality from cervical cancer (Cancer

Research UK, 2015). Oral and injectable hormonal contraceptive use is frequent in women of reproductive age, putting them at risk for pre cervical cancer occurrence later in their advanced and post-menopausal ages (Cancer Research UK, 2015). Multiplicity of sexual partners even in discordant couples has been linked to risk of contracting oncogenic organisms such as HR-HPV, Chlamydia and HIV (Burd, 2003; Shepherd *et al.,* 2011; Boardman *et al.,* 2012).

1.2 Statement of the problem

Cervical cancer disease in Kenya accounts for some 8,600 deaths annually per every 100,000 Kenyan women. These statistics are documented in WHO and IARC global updates (Appendix VI; VII; Ferlay *et al.*, 2012; IARC, 2015). Mortality rate due to the disease are on the rise in Kenya attributed to poor screening facilities, the lack of timeliness in screening for early signs of the disease in the cervix and the lack of access to reproductive health centers especially in the rural areas (Ngugi *et al.,* 2012; Muitta *et al.,* 2015). Severe and mildly dyskaryotic atypical cells such as koilocytes may be missed or may not be sufficiently distinct owing to mimicry as a result of infection from non-oncogenic microbial organisms including irradiation effects that may modify cervical epithelial cell morphology (Lee, 1997). Patients with borderline or mildly dyskaryotic cyto-morphology including cytopathic effects may actually have a higher grade of pre cervical cancer. This however requires secondary confirmation through biopsy test only available in specialised histo-pathology laboratories at exorbitant fee (Pattern, 1978; Nayar *et al.,* 2015). Moreover implementation of liquid based cytology (LBC) has not been introduced in Nakuru County referral hospital cytology laboratory. LBC greatly enhances cervical cellular harvest thereby greatly increasing the chances of atypical cell detection (Gupta *et al*., 2016).

Additionally, the screening of oncogenic STI such as HR HPV conducted through DNA technology is currently only available in the private and research laboratories not only in Nakuru County but in the whole of Kenya. (De Vuyst *et al.*, 2005; Arbyn *et al.*, 2008; Were, 2010). HR HPV infection persistence causes cervical oncogenesis. The role of STI microbes like *Chlamydia trachomatis* in the onset of cervical cancer is known (Kiviat *et al.*, 1990; Viikki, 2000; Antilla *et al*., 2001; STD Trends USA, 2015) but no such studies have been done in Nakuru region where cervical cancer prevalence is on the rise as suggested by pilot cancer registry studies conducted by Rajab *et al.*, 2014.

Incidence of cervical cancer associated with various socio-economic factors ranging from lifestyle practices such poor nutritional status and reproductive ill-health all of which may potentially offset pre-cervical cancer have not previously been examined in Nakuru county, although are documented to play a role in cervical oncogenesis (Temmerman *et al.*, 1999; Durowade *et al*., 2012; Lesmes-Fabian *et al.*, 2013 and Cancer Research UK, 2015). Health wellness assessment in women attending Maternal Child Heath clinic (MCH) through anthropometry and reproductive tract symptomatic sign monitoring is not conducted. Furthermore nutritional counseling in MCH may lack the necessary urgency and vibrancy in the promotion of a culture that practices healthy lifestyles in the promotion of holistic body wellness. Addressing correct atypia recognition mechanisms in cervical smears including the use and application of sensitive molecular detection (DNA based) STI screens are interventions that can be applied in the prevention of pre cervical cancer development.

1.3 Justification

Invasive cervical cancer disease management presents with poor prognosis but pre-cancerous states are treatable and preventable when correctly detected through early identification of epithelial atypia during cyto morphological examination. Findings on putative predictors such as microbial STI revealed that HR 16/18 HPV detection and prevention that was significantly associated with the pre cancer grades can be integrated into the NCCPP program as the basis for the promotion and advocacy of HPV vaccine administration to prevent exposure. The establishment of lifestyle practices such as consumption of unhealthy non-nutritional diets leading to gradual weight and body mass loss and noted reproductive health characteristics of protracted symptomatic clinical signs for example lower abdominal pain coupled with abnormal vaginal bleeding can be established during women clinic visits by conducting thorough clinical history examination.

Early and speedy detection of pre cancer predictors in order to curb development of epithelial abnormality change will aid to curb pre cervical cancer genesis. With passionate confidence, national SDG targets as crafted from the global international community (SDG, 2015), will be met and addressed with the hope that accomplishment of better health care provision in cervical cancer management can be achieved. Optimal utilization of available laboratory resources will ensue, thereby ensuring high standards of pre cervical cancer cytological diagnosis as well as minimize diagnostic pitfall due to atypia mimicry. Better practices in the aversion of disease development will optimistically aid in diminishing cancer stigma, pain, suffering, infirmity in invasive disease and eventual reduction of women mortality rate.

1.4 Research questions

1. What are the cervical epithelial cytomorphologic presentations in cervical smears from women with varying degrees of pre cervical cancer grades at Nakuru County referral hospital?

2. What are the occurrences of putative STI in women with determined pre cervical cancer grades at Nakuru County referral hospital?

3. What are the lifestyle and nutritional practices in women presenting with different grades of pre cervical cancer at Nakuru County referral hospital?

4. What are the associations of reproductive health history and practices among women presenting with varying pre cervical cancer grades at Nakuru County referral hospital?

1.5 Hypothesis

1. An increase in cervical epithelial cytomorphologic atypia severity increases with pre cervical cancer grade outcome in cervical smears of women at Nakuru County referral hospital.

2. Putative HR HPV STI infection exposure is more rampant as compared to bacterial STI in women presenting with varying precancerous grades at Nakuru County referral hospital.

3. Unhealthy lifestyle and nutritional practices contribute to an increase in pre cancer grade outcome in women at Nakuru County referral hospital.

4. Clinical history presentations of reproductive ill health and practices are associated with risk of pre cervical cancer development among women with varying degrees of pre cervical cancer grades at Nakuru County referral hospital.

1.6 Objectives

General objective

To identify risk factor predictors that offset pre cervical cancer states and their association with different grades of pre cervical cancer among women at Nakuru County referral hospital.

Specific objectives

1. To determine the types of cervical epithelial cytomorphologic presentations in cervical smears from women with varying pre cervical cancer grades at Nakuru County referral hospital.
2. To detect select microbial STI namely HR HPV16/18, HIV1/2, *Treponema pallidum, Neisseriae gonorrhoea and Chlamydia trachomatis* among women presenting with varying pre cervical cancer grades at Nakuru County referral hospital.
3. To assess lifestyle and nutritional practices with varying pre cervical cancer grades in women at Nakuru County referral hospital.
4. To examine the association of reproductive health history and practices with varying pre cervical cancer grades in women at Nakuru County referral hospital.

1.7 Significance of the study

The identification of determinants considered from the evaluation of specific objectives comprising of detected atypical cellular forms in examined smears including screening of implicated microbial STI namely HR HPV16/18 can be applied as effective diagnostic and preventive tools in the circumvention of pre cervical cancer state. Moreover lifestyle attributes of nutritional inadequacies and noted low medium values in addition to analysis of reproductive health characteristics and practices are essentials that can be integrated through anthropometric measure, adoption of nutritional counseling and reproductive tract sign monitoring and care in the MCH. They can also be applied as monitor tools aiding in the surveillance of progression from pre cervical cancer to advanced cancer. These characteristics can be

assimilated in existing pre cervical cancer diagnostic measures as standard protocol and operational procedures within the Nakuru pre cervical cancer management policy. It is anticipated that in the near future, reduced burden and mortality of women in Nakuru from cervical cancer disease can be realized. Additionally, it is expected that the stigma, pain and anguish associated with the disease can entirely be eliminated in women thereby preserving their overall holistic health. Government expenditure for the management and treatment of cancer can be reduced and reserves from extra revenue saved instead diverted to address and accomplish pre cervical cancer prevention and still be sufficient to meet other developmental agendas of equally high priority.

1.8 Conceptual framework

This was designed with the aim to broaden the general view that a speculative relationship exists between known pre cervical cancer risk factors (independent variables) such as HRHPV, intervening factors and pre cervical cancer state of LSIL, HSIL and AGC/AIS (dependent variable). The model considered data from previous conducted studies, illustrating this intricate network (Viiki *et al.*, 2000). The study also compared the causal relationship between socio-demographic attributes (age, parity, educational level) including lifestyle, nutrition or dietary practices as well as reproductive health characteristics and practices. Their interactions with exposure to obligate intracellular sexually transmitted microbe infections (Kiviat *et al.*, 1990; Parsonnet, 1999; Burd, 2003 and Memiah *et al.*, 2012) and occupational practices i.e. exposure to pesticide farming chemicals, toxic industrial emission, including radiation (Ana, 2009; Bolognesi, 2011; Lesmes-Fabian, 2013) was also considered (Figure 1.1).

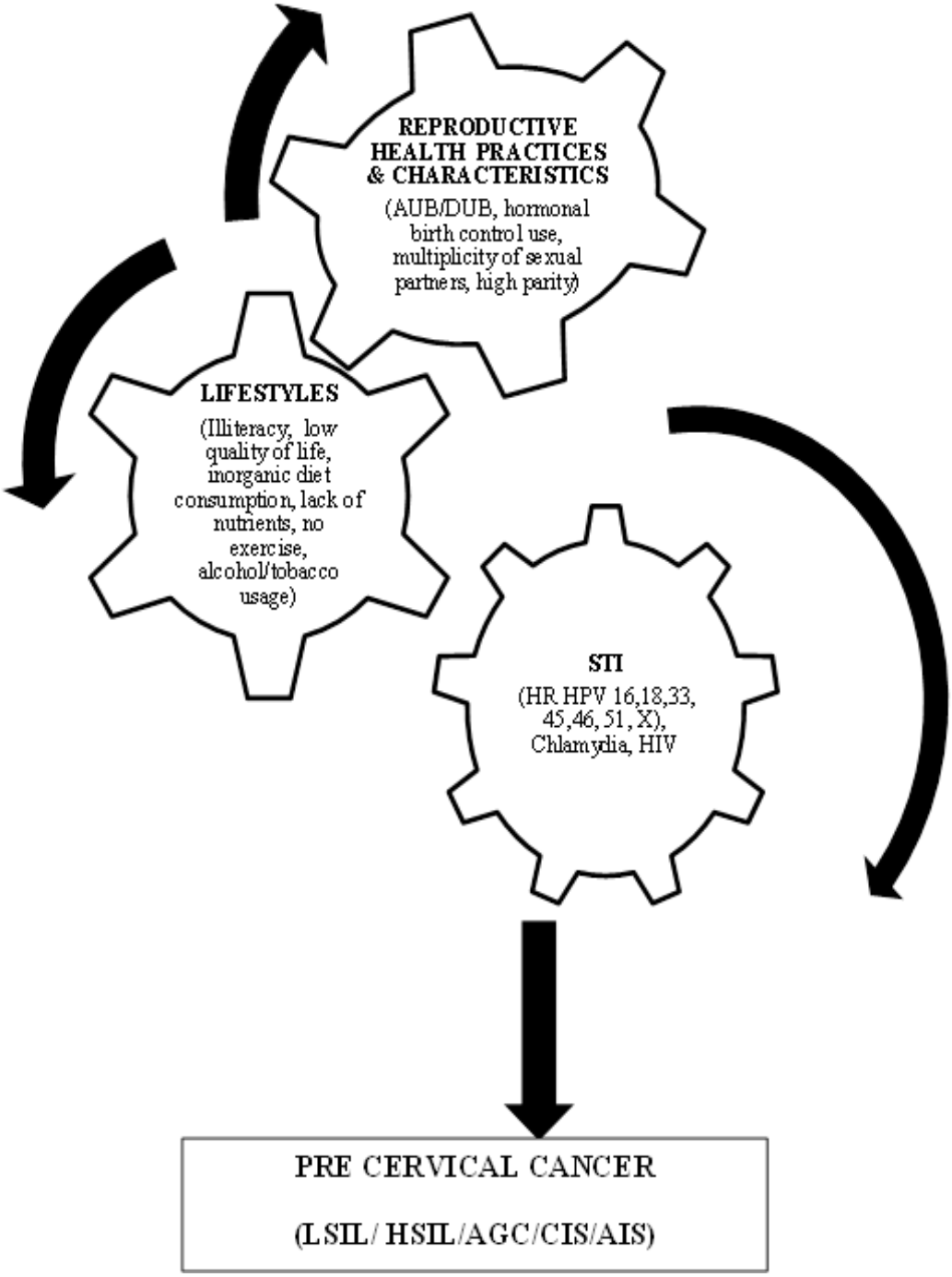

Figure 1.1: Conceptual framework of pre cervical cancer determinants

Relationship network designed using information on the inter-relationships between risk factors of pre cervical cancer such as HR HPV STI, lifestyle, unhealthy diets, reproductive health practices, demographic and socio-economic factors. (Kiviat, 1990; Parsonnet, 1999; Viikki, 2000; Antilla, 2001; Samoff, 2005;Del Prado-Lu, 2007;STD Trends In The United States, 2015; Cancer Research UK, 2015; Lesmes-Fabian, 2013).

CHAPTER TWO

LITERATURE REVIEW

2.1 Reproductive tract anatomy relation with cervical cancer

The reproductive tract (RT) anatomical region in women affected by cervical cancer disease is referred to as the cervix. The cervix is the part of the uterus connected to the upper vagina. In the cervical anatomy, its origins are initially detected in cells that lie on the surface of the cervix known as squamous cells (Rubin, 2001). The size and shape of the cervix varies widely with age, the hormonal state and parity in different women, where in the latter it appears bulkier and its lower-most opening is wider with a more gaping slit-like shape, unlike as seen in nulliparous women. The visible portion that projects into the vagina is known as the *portio vaginalis* whose passage way dilates during childbirth, to allow the baby during birth to traverse the birth canal (Anderson and Symmers, 2014; Ferris, 2004).

The ecto-cervix is the outer region of the cervix consisting of multiple layers of squamous epithelia made of an outer and inner superficial squamous epithelial cell layer. The endocervix is the invisible region consists of the intermediate parabasal and basal layers of both intermediate and parabasal epithelial squamous cells and basal columnar squamous epithelia (Figure 2.1). Two important regions associated with cervical epithelia growth, development proliferation and maturation are the Squamo-Columnar Junction-SCJ and Transformation Zone-TZ. These are active differentiation and cellular conversion sites of differentiating cervical cells located in the endocervix region. The SCJ is a highly variable zone seen as two separate regions, an original and a new junction. The junction is a clear distinct zone contrasted by the presence of a layer of squamous epithelial cells and an underlying columnar epithelial cell layer. Its localization within the cervical wall depends on a woman’s age, parity, hormonal

status, use of contraceptives and pregnancy amongst a host of other factors. During the pre-puberty years, the junction is seen in close proximity to the external OS of the cervix. Under the influence of oestrogen hormone, the cervix matures, enlarges and elongates during puberty, resulting to the eversion of the columnar epithelial layer, hence a new SCJ junction forms from the columnar reserve cells (Figure 2.2).The physiological replacement of everted columnar cells on the ecto-cervix, by newly forming squamous epithelia from the sub columnar reserve cells results to squamous metaplasia. This replacement occurs within the transformation zone (TZ) of the SCJ. The identification of the SCJ and the TZ is crucial in the diagnosis of cervical carcinogenesis (Anderson and Symmers 2014; Nucci, 2009).

Ovarian hormones such as oestrogen have a crucial role in the overall epithelium layer maturation influencing the development of epithelial cells within the epithelium. The layer consists of superficial and intermediate epithelial cells in its upper portion and underlying these cells are parabasal cells in the lower parabasal and basal layers. Oestrogen is needed not only for glycogen deposition and utility but for promotion of epithelial cell maturity. In contrast, progesterone is involved in the desquamation of the upper layer which is then replaced by newly generated cells converted from columnar cells of the parabasal layer in processes that occur within the TZ (Atia, 2010, Gray *et al.*, 2010; Boardman e*t al.*, 2012). It is also crucial in the maintenance of an undifferentiated state within cells of the parabasal layer.

Cytologic smear examinations of epithelium layer cellular content reveals eosinophilic pigmentation of superficial cells and or assortment of cyanophilic and or occasionally basophilic pigmentation of intermediate cells including parabasal cells. These staining characteristics are acquired from subjecting cells to differential staining technique such as the Pap smear staining protocol technique where glycogen

deposition and utilization mechanisms are regulated within the cell. Depending on the maturity stage of the particular epithelial cell largely under the influence of ovarian hormones namely oestrogen and progesterone, different epithelial cells will retain a particular dye (Hughes, 2000).

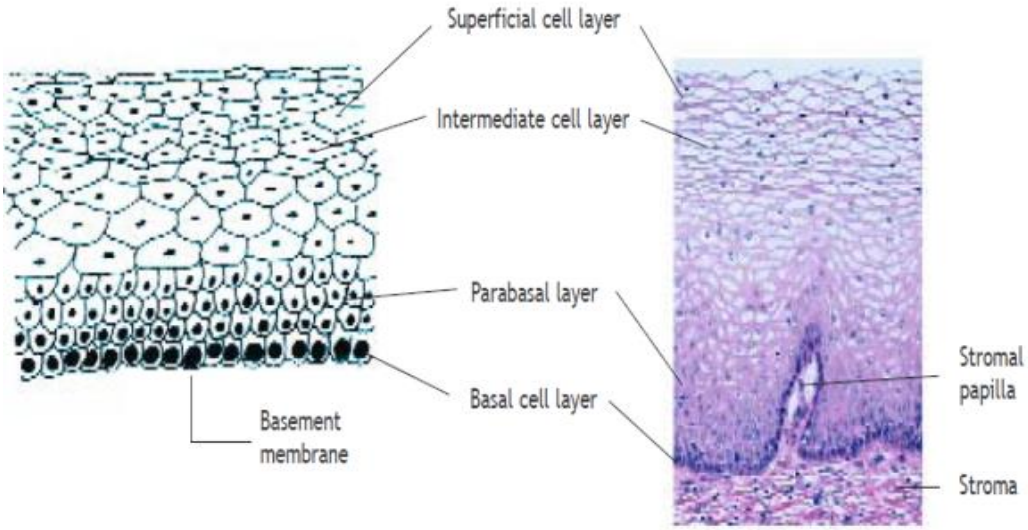

Figure 2.1: Cervical epithelium layers: Schematic and biopsy tissue derived

Source: De May, 2007.

Figure 2.2: Schematic and histologic images of epithelium basement layer

Source: De May, 2007.

2.2 Epidemiology, burden and challenge of cervical cancer disease

Every year cervical cancer affects an estimated 500,000 women worldwide. Women in the developing world account for 85 percent of cervical cancer deaths every year (Ferlay *et al.*, 2012). Records available from on-going hospital-based cancer registry projects in local hospitals indicate that the cervical cancer situation in Kenya currently stands at an estimated 2454 annual number of cervical cancer cases in women while the annual number of deaths due to cervical cancer is 1676. It is projected that by the year 2025, the number of new cervical cancer cases annually will reach 4261 (Rajab *et al.*, 2014; NCCPP, 2012-2015; SCCA, 2015). According to reports by GLOBOCAN a leading International organization that publishes epidemiological trends, although incidence rates for different types of cancers combined are nearly twice as high in more developed than in less developed countries in both males and females, mortality rates are only 8% to 15% in these countries in comparison to the least developed countries (Ferlay *et al.*, 2012 and Torre *et al.*, 2015). This disparity reflects regional differences in the mix of cancers, which is affected by risk factors and detection practices, and/or the availability of treatment (Torre *et al.*, 2015).

Currently no specific data on prevalence of pre-cervical cancer risk factors in Nakuru County exists consistent with global and regional data (Boardman *et al.*, 2012; Memiah *et al.*, 2012; Ali-Risasi *et al.*, 2015). Studies conducted in Kenya mainly focus on risk factors and predictors causing cervical cancer disease and not on pre-cervical cancer onset. This study also highlights the prediction of lifestyle attributes gauged through anthropometric analysis and clinical RT symptomatic signs analysis beyond what is currently studied and documented on the role played by HRHPV and HIV co- infections in Kenya.

Risk factors associated with cancer of the cervix include infections (Vaccarella *et al.*, 2013).Cancer of the cervix as defined by its pathogenesis is a type of cancer that strictly arises from epithelial cells, and classified by its histopathological characteristics, in which a single normal body cell undergoes a genetic transformation into a cancer cell. This cell and its descendants, produce the population of cells that we recognize as a tumor (Abrams, 2012). The two major types of cancers that arise in the cervix are squamous cell cancers (SCC) that affects the parabasal squamous epithelium and the adenocarcinomas that affect the inner layer (endothelial) glandular lining of the endocervical canal (Rubin, 2001). Squamous cell cervical cancer comprises about 80% of all cervical cancers. The second most common form, adenocarcinoma, has also dramatically risen since the 1970's (Cancer Research UK, 2015). About 3% to 5% of cervical cancers have characteristics of both squamous and adenocarcinomas and are called adeno-squamous carcinomas (Rubin, 2001). Squamous cell carcinoma (SCC or SqCC) is a cancer of epithelial cells known as the squamous cells. These cells consist of the main part of the epidermis of the skin. Confined to the superficial milieu, they also shield the inner muscular walls of visceral organs such as the stomach as well as line hollow conduits and canals such as blood vessels.

All squamous cell carcinoma lesions are thought to begin via the repeated, uncontrolled division of cancer stem cells of epithelial lineage or characteristics. The uncontrolled multiplication of cervical epithelium results to SCC. Abnormal squamous cells display particular cytological or tissue architectural characteristics of squamous cell differentiation during mitosis. In excavated cervical smears, occasionally cells from surrounding reproductive tract tissues may be found which

may be diagnostic for other types of invasive SCC and adenocarcinoma such as SCC of the vulva and vagina and adenocarcinoma of the cervix and endometrium (Boras *et al.*, 1999; Hughes, 2000; Patricia *et al*., 2012 and Atilgan *et al*., 2012). Cervical cancers arise within the squamo-columnar junction between the columnar epithelium of the endocervix and the squamous epithelium of the ecto-cervix. At this site, there are continuous metaplastic changes whose activity is greatly increased in high risk HPV infection exposure (Burd, 2003). Accumulation of these cancer cells causes a focus of abnormal cells locally confined within the specific tissue in which the progenitor cell resides, a condition referred to as squamous cell carcinoma *in situ*. This is diagnosed when the tumor has not yet penetrated the basement membrane or other delimiting structure, prior to invasion to adjacent tissues.

2.3 Pre cervical cancer genesis and progression

Despite having both humoral and innate protective mechanisms, most cancers that occur in the body may be due to ingestion, body contact or inhalation of carcinogens or microbes. These agents may either promote carcinogenesis through inflammation or directly interfere with cellular division life cycle.

Exposure source ranges from what is consumed, what is inhaled and what may have gained entry into the body either as infection or radiation including what may have been inherited through an abnormal gene resulting to the expression of abnormal protein products. Cancer affects the cellular machinery of division resulting to unregulated growth. Cells referred to as tumour cells have the potentiality of affecting neighbouring cells which will have similar characteristics as themselves. They invade surrounding tissues causing lesions and at times forming a mass. Cancerous cells may be transported in body fluids to affect other tissue and organs a situation termed as metastasis (Boardman *et al.*, 2012).

HR HPV infection exposure in metaplastic cells of the TZ promotes generation of abnormal epithelial cell clones through HPV nucleic acid integration within the cell. The persistence of HPV infections in women enhances a slow but steady clonal expansion of these groups of abnormal cells driven by particular risk factors that boost physiological changes in infected cells localized within the TZ (Sell *et al.*, 1980). It can speculatively be assumed that viral infections would naturally clear off by themselves if not edged on by risk factors that alter the body's defense capability that is a weakened or low immunity. HIV infections, chronic ill health including poor quality of life are amongst a host of factors that may promote persistence of HR HPV infections (Burd, 2003; Memiah *et al.*, 2012).

2.4 Stages of pre cervical cancer and invasive cancer disease

1. Stage 0: Precancerous lesion involves only the cells on the surface of the cervix.

2. Stage I: Low grade cancer is confined to the superficial cells of cervix, and may be evident only under microscopic evaluation (stage I A) or apparent by visible or physical examination (stage I B).

3. Stage II: Low grade cancer has spread beyond the superficial cells of the cervix to involve the tissues surrounding the cervix (parametria) or the upper portion of the vagina.

4. Stage III: High grade cancer which has spread beyond the cervix to the lower vagina or to the sides of the pelvis, or causes a blockage of drainage from the kidney, a condition called hydronephrosis.

5. Stage IV: Cancer invades structures adjacent to the cervix such as the bladder or rectum or has spread to other parts of the body such as the liver or lungs.

6. Recurrent/Relapsed: Cervical cancer is still detected or has returned (recurred/relapsed) following an initial treatment with surgery, radiation therapy, and/or chemotherapy (Camphausen, 2008).

Once the lesion has grown and progressed to the point where it has breached, penetrated, and infiltrated adjacent structures, it is referred to as "invasive" squamous cell carcinoma or the more rare form of glandular cell cervical cancer (Adenocarcinoma). An invasive carcinoma cell is able to spread to other organs and cause a metastasis, or "secondary tumor" formation. SCC cells are not destructive or invasive but with sufficient mismanagement, in a period of time, they progress to the malignant states which are then difficult to manage as they invade and destroy surrounding tissues by forming metastases extended all over the body. This eventually causes death to the host through nutrient deprivation as cancerous cells put high demands on the host nutrients and oxygen for respiration. Metastasized cervical cancer is also a cause immunosuppression.

2.5 Epithelial cell abnormality in smears

Early diagnosis of cervical cancer disease is detected through the identification of abnormal cellular morphology in smears excavated from both the ecto-cervix and endocervix. Confirmatory pre cervical cancer and cervical cancer diagnosis post the detection of abnormal epithelial cells in smears is done from biopsy tissue excisions and examination from abnormal sections of endothelial, endocervical, ectocervical and glandular epithelium layer thickness whose variability degree can be microscopically distinguished according to cellular maturation and differentiation abnormality as per the FIGO classification (Percorelli *et al.*, 1999).

In cervical cytological preparations, epithelial features and their associated distortions are displayed when microscopic examinations of smears from the cervix are observed.

These abnormalities are graded as per their levels of severity manifested during microscopic examinations (Ferris, 2004). Cervical epithelial cell nomenclature applies a grading classification through the use of referenced systems accepted in the global cytologists' community. The Bethesda system is one such system widely used in many countries. This system classifies cervical cellular morphology largely into a three segment methodology (Nayar *et al.*, 2015), namely:

i) Negative for Intraepithelial Lesion or Malignancy (no cellular lesion).

ii) Epithelial cell abnormality (specified as squamous or glandular).

iii) Other malignant neoplasms (endometrial or vulval).

Within these segments cellular morphology dependent on proportions or magnitudes of abnormal cells are classified into benign (non-neoplastic), atypical squamous cells (ASC), atypical squamous cells of undetermined significance(ASCUS), low grade-atypical squamous cells of undetermined significance (ASCUS LOW), high grade-atypical squamous cells of undetermined significance (ASCUS HIGH), low grade squamous intraepithelial lesion (LGSIL), high grade squamous intraepithelial lesion (HGSIL), atypia of glandular cells (AGC), atypical glandular cells of undetermined significance (AGCUS), atypical glandular cells of undetermined significance-low grade(AGCUS LOW) and atypical glandular cells of undetermined significance-high grade (AGCUS HIGH), updated atypia categories by Barcelos *et al.*, 2011 and Nayar *et al.*, 2015.

Other systems used to grade cervical cell morphology include BSCC system (Denton *et al.*, 2008) and WHO system (Maeda *et al.*, 1997). Modern laboratories in developed nations also use automated devices and software applications which compare imagery through computer-assisted interpretation of cervical cytology. Certain key cellular characteristics are used to determine the specific interpretation a cytology report upon

detection of cellular abnormality. These include comparisons of nuclear enlargement and as such the volume of the nucleus relative to that of the cytoplasm is assessed and where sizes appear shifted these are noted. This is the nuclear versus cytoplasmic volume ratio (N/C). Other features include irregularity of nuclear membrane, intensely staining nucleus and cytoplasm (hyperchromasia) and the prominence and distribution of chromatin granulation is also assessed (Appendix VIII).

High risk HPV and HSV persistent infections initiate nuclear dyskaryotic changes referred to as cytopathic effects. Obligate intracellular bacterial organisms also elicit similar effects. Abnormal cellular morphology identification is characterized by nucleic changes away from normal during cytological screens. Nucleic abnormal characteristics within a normal cytoplasm are referred to as dyskaryosis. Epithelial cells having these effects are called koilocytes. Koilocytosis has not only been seen in superficial and intermediate epithelial layer, but has also been demonstrated in parabasal cells (columnar) within the parabasal layer of the epithelium (Eurocytology, 2016, see Figure 2.3). Koilocytosis has been synonymously referred to as cytopathic effects of which key cellular features include nuclear enlargement and as such the volume of the nucleus relative to that of the cytoplasm sizes appears shifted (Denton *et al.*, 2008; Nayar *et al.*, 2015; Eurocytology, 2016). Other features include irregular nuclear membrane, intensely staining nucleus and cytoplasm (hyperchromasia) and increased cytoplasmic space within cells perinuclear ‘haloing’ often referred to as cytoplasmic clearing (Nayar *et al.*, 2015). Many texts of cervical cytology have indicated that cytopathic effect is synonymous to low grade squamous cell lesions abbreviated as LSIL and most are caused by HRHPV viral nucleic acid integration (Hughes, 2000). The above described cellular features are very similar to those associated with irregular epithelial maturity and differentiation in intermediate and

high grade pre cancer of HSIL manifestation, of which some, but not all are enhanced by high risk HPV invasion (Lee *et al.*, 1997). HSIL must be distinguished from cytopathic effect in biopsy tissue examinations to assess the degree of tissue layer involvement and invasion (Boardman *et al.*, 2012).

2.5.1 Cervical epithelial cell abnormality management

Early cervical cancer identification stems from the capture of early cellular changes which sometimes can be very subtle and confusing even to the most experienced pathologist, cytologist or colposcopist. Cytological diagnosis does not automatically translate to homology in a preceding neither colposcopic nor histologic biopsy test screening. In essence, there exists the possibility of false positive or false negative occurrence. Cytologic screen examinations can only predict a situation of cervical cellular abnormality but must be confirmed by colposcopic examination and subsequent histologic biopsy screening.

In cervical ill health, diverse atypical cytomorphologic characteristics manifest depending on what is influencing cellular abnormality. Pre cervical cancer signs are managed using different regimens depending on its degree of severity (Casanova *et al.*, 2008). In cervicitis, which is evidenced by benign reactive changes of cervical epithelia, chiefly epithelial cytolysis will be present on cellular examination. Prevailing bacterial infection is managed with a course of prescribed antibiotics. Parasitic infections are managed using anti protozoan therapy such as flagyl, while fungal infections are treated using oral antifungal medications. Patients are further advised and counseled on improving dietary intake with emphasis on fruit and vegetable diet supplementation when viral cytopathic effects are evident in smear prep examinations. In both low grade and high grade pre cancer and even in invasive

cervical cancer disease (LSIL, HSIL, CIS, AGC and AIS), cytological reports must first be confirmed through colposcopy including biopsy tissue sampling of detected abnormal regions of the cervix on iodine and acetic acid application. Biopsy reports indicating CIN1 (mild dyskaryosis cytologically or LSIL) are referred for a course of cervical ablation and cryotherapy post the colposcopic examination. Repeat Pap smear after six months is also recommended. In CIN2 and CIN3 (reported as HSIL cytologically) which is considered as a high grade pre cancer however, lesions which are severer than those in CIN1 are managed through a course of LEEP or in some instances TAH is recommended depending on the woman's age especially if she has passed child bearing period. HSIL, CIS, AIS grades are considered precursor of SCC and adenocarcinoma (ADC) for which TAH is recommended. Radiotherapy and chemotherapy are applied in the management if metastasis has occurred, depending on the extent of spread (Casanova *et al.*, 2008).

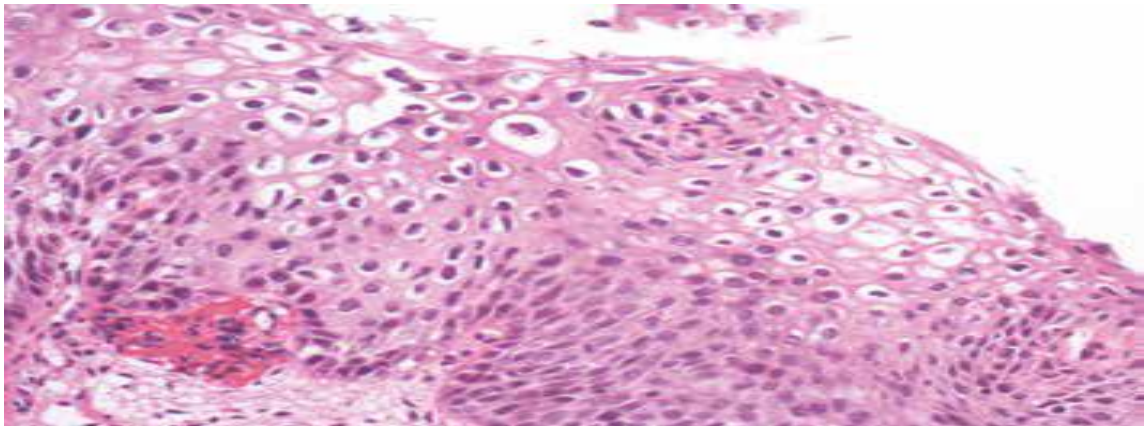

Figure 2.3: Biopsy tissue showing cellular abnormality of "perinuclear haloing"

Biopsy tissue showing parabasal and basal layer with HPV-associated changes. Koilocytes are prominent, characterized by perinuclear halos with cytoplasmic clearing, nuclear hyperchromasia, irregularity of the nuclear membrane, and variation in nuclear size. Source: Histopathology Reporting Hirschowitz *et al.*, 2012.

2.6 Causes of pre cervical cancer due to STI

Microbial viral and bacterial infections have been implicated in the onset of many cancers. High risk HPV strains such as HPV16 and HPV18 play a significant role in the genesis of malignancy of the cervix by integrating viral DNA in the cervical cells. The DNA integration process is preceded by the disruption of the E1/E2 protein regions of the acid which results to the activation of E6 and E7 proteins. The E6 gene expressing E6 protein is one of the earliest genes to be expressed soon after HPV infection and viral replication resulting to multiple HPV copies in host cells results. E6 binds to tumour suppressor genes particularly p53 inducing its integration, which results in the prevention of apoptosis, while E7 will suppress particular genes involved in cell cycle arrest such as cyclin dependent kinases and inhibitors (CDK) and particularly to retinoblastoma suppressor protein (Rb) by binding to the G0/G1-specific cycle check points promoting continuous unregulated cell growth. E5 protein binds with the platelet-derived growth factor ß receptor, promoting a sustained mitogenic signal that enhances unregulated cell growth by inhibiting cycline-derived growth arrest. These events are synergistic and lead to deregulation of cell division cycle with the convenient inhibition of apoptosis, which allows for the accumulation of mutations (Faridi *et al.*, 2011). However, the expression of the E6 and E7 oncoproteins alone is insufficient for cellular transformation, indicating the requirement for additional genetic alterations according to Burd, 2003.

Bacterial organisms which trigger inflammation promotes carcinogenesis through activation of inflammatory cells thereby enhancing the secretion of cytokines, chemokines and Nitrogen oxide species (NOS) particularly noxious isoforms such as nitric oxide synthase and reactive nitrogen species that damage putative tumour suppressor genes rendering them ineffective in triggering the cell to undergo

apoptosis (Nath, *et al*., 2010). Additionally they induce secretion of T cell regulatory molecules (TRegs) which cause immunosuppression as a protective mechanism that protects the host from mounting a hyperactive immune response secondary to a chronic infection/inflammation leading to vulnerability of the host to genesis of neoplasm.

Research indicates that early onset of cervical cancer begins as a result of persistent or recurring high risk HPV infection (WHO/ICO, 2010 and Faridi *et al.*, 2011). According to the American Cancer Society reports, infection with HR HPV is the main risk factor for early cancer genesis or pre cervical cancer, (Parsonnet, 1999). Specific genital strains of HRHPV infect different anatomical regions of the genital tract such as cervix or the vulva. High-risk strains of HPV such as genotypes 16 and 18 which are oncogenic and involved in the induction of cervical cancer are well studied, however although HPV is essential to the transformation of cervical epithelial cells, it is not sufficient, and a variety of other cofactors also influence cervical cancer development (Burd, 2003).

While virtually all women with cervical cancer are infected with HPV, about 85% of them actually develop cervical cancer disease from persistence of HR HPV (Parsonnet, 1999). The rest (15%) are believed to develop cervical cancer as a result of other infectious agents besides HRHPV (Samoff *et al.*, 2005). Current or previous exposure to sexually transmitted disease predisposes one to increased risks of acquiring cervical cancer. If an individual has, or had previously contracted a sexually transmitted disease, they are at an increased risk of cervical cancer. The American Cancer Society reports specifically points to HIV and Chlamydia as risk factors for cervical cancer (STD Trends in the United States, 2015). Chronic inflammation of virally invaded cells occurring over long periods in persistent

infections with viral agents results to indirect viral oncogenicity (Shelhaas *et al.*, 2008; Barcelos *et al.*, 2011).These infections are thought to interact with high risk HPV infections, in aiding to the progression of precancerous states to critical cervical cancer states. Organisms such as *chlamydia* and *Gonorrhoea* have also been associated with cervical cancer (Kiviat *et al.*, 1990; Viikki, 2000; Antilla *et al.*, 2001). These are obligate and intracellular microbes, hence entirely parasitic on the host cell for metabolism. The view of whether they have any role in protecting an individual from the known high risk HPV strains through an immune response defense invoked by their presence remains unclear and open for deliberation. Their detection will improve diagnostic accuracy and limit unnecessary colposcopy in patients with borderline or mildly abnormal cytologic test results.

2.7 Other pre cervical cancer determinants

The onset, development and progression of pre cervical cancer to invasive disease has been attributed to factors all which either independently or in association with each other, seem to greatly contribute to its continued proliferation.

2.7.1 Reproductive health-related factors

Multiple sexual partner practice has been identified as a risk factor for developing cervical cancer (Cancer Research UK, 2015). Early age onset of sexual activity is also linked as a risk factor for the development of cervical cancer. The link between sexual activity and or multiplicity of sexual partner practice with cervical cancer is linked to the transmission of HPV along with other STI. More over exposure to multiple ionizing radiation (X ray therapy) treatments in tissues and organs in close proximity to the RT is thought to initiate DNA damage resulting to the oncogenesis of cells of the RT (Camphausen, 2008). Cervical cancer development in women, whose family history has been plagued with a history of cancer of any etiology, increases their

chance of developing the disease. This is attributed to the inheritance of mutated genes which are incorporated into nascent stem cells post fertilization. Slow progression of oncogenesis during embryogenesis and post birth into different types of cancers including cervical cancer may then result (Cancer Research UK, 2015; Hodgson, 2007).

The lack of RT ill health awareness in asymptomatic HPV infection owing to non-manifestation of clinical sign may cause accelerated pre cervical cancer genesis (Ngugi *et al.*, 2012). HPV infection screen is not routinely conducted and neither integrated in MCH service in Kenya. It is a costly test, but in developed countries it is amongst the choice methods of early detection (Porika *et al*., 2010; NACB, 2015).

2.7.2 Lifestyle factors

Tobacco use e.g. smoking doubles a woman's risk of developing squamous cell cervical cancer. Cancer causing chemicals (benzyrene) from cigarette smoke have been detected in the cervical mucus of women who smoke (Appleby *et al.*, 2006; Appleby *et al.*, 2007; Cancer Research UK, 2015). Poor diet and being overweight and obese has been linked to an increase in the risk of cervical cancer onset. This is attributed to the general lack of not eating enough fiber content (fruits and vegetables). Essential anti-oxidants present in fruit and vegetables combat off the presence of free radicals produced from the breakdown of empty calories in the diet (Natural News Network, 2014). Food additives such as Sodium Nitrite are added in processed food like (meats found in sausages, hotdogs and salami) to give them a fresh meaty look rich in the red colour. This carcinogen is responsible for DNA damage that predisposes the cell to become oncogenic through altered mitotic divisions (Natural News Network, 2014).

2.7.3 Hormonal use

Long-term use of oral hormonal contraceptives has been predicted to increase a woman's risk of developing cervical cancer. Oral contraceptives use for more than five years, increases the chance of the development of cervical cancer up to four times higher than those women who do not use oral contraceptives (Cancer Research UK, 2015). The use of hormonal contraceptives before 20 years of age also increases the risk to cervical cancer (Cancer Research UK, 2015) as opposed to contraceptives involving non-hormonal methods. Diethylstilbestrol (DES) hormone use to prevent miscarriage and maintain pregnancy has been linked to a number of reproductive problems, such as vaginal clear cell adenocarcinoma and cervical cancer (Verloop, *et al.*, 2010). Women exposed to this hormone, developed cell adenocarcinoma (Veridiano *et al.*, 1981), a rare cancer that affects the vagina and cervix. They were also more likely to develop other precancerous and cancerous changes of the cervix, according to the American Cancer Society reports (Verloop, *et al.*, 2010). The contraceptive has since been disused by the early 1980's but it remains unclear if children born from affected mother developed the disease.

2.8 Pre cervical cancer disease management challenges in Kenya

In Kenya cervical cancer tops in female cancer mortality rate (Appendix VI). Statistics in Kenya have continued to rise despite increased awareness of the disease and aggressive screening and diagnostic interventions. This is attributed to speculatively a host of factors of which leading is socio-economic challenges that continue to plague the bulk of the gender that is affected in meeting the high cost of reproductive health treatments, when ill health presents (Rosser *et al.*, 2015). The high levels of poverty mainly due to inequality and limited access to assets as well as ownership of the same, owe to cultural belief practices which berate women in most

Kenyan communities (DPP, 2008). A consequence of this is attributed to the fact that a society such as ours is mainly patriarchal and as such women are subjected to fewer economic empowering opportunities that can give them economic freedom such as income generating projects not to mention the lack of occasions where they can participate in decision making in matters of community policing (DPP, 2008).

Other challenges according to observations presented in conference proceedings, have been demonstrated to result from the delay in the intervention of abnormal signs and cytologic finding in VIA/VILI and Pap smear screens which may progress to an increased cervical cancer disease severity (Muitta *et al*., 2015). In devolved public health services, challenges arising from constant industrial strike disputes resulting to loss of reproductive health services put the sick woman manifesting pre cancer signs at increased risk of developing advanced cervical cancer symptoms. Unnecessary re-booking of patients who have availed themselves for treatment but remain unattended to due to cancelled appointments is deplorable. Similar challenges arising from ill equipped facilities with limited and inconsistent fund allocation to run the hospital may contribute to the prolonged delay in pre cancer treatment conferment (Muitta *et al.*, 2015). Cervical cancer screening detects pre-invasive neoplasia, thereby making treatment possible before the disease becomes invasive. Uncontrolled and mismanaged pre cervical cancer signs results to high morbidity and mortality rate from invasive disease. Our economy is highly reliant on the Kenyan woman on several fronts of which the most recognizable is in the agricultural labour sector. Women health must then not be taken casually nor neglected altogether, because not only does this fail in upholding the intact family unit, but that of the community as a whole.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The Nakuru RVPGH recently upgraded to Nakuru County referral hospital in the devolved government served, as the suitable catchment area for female targets seeking reproductive health services (Gazette notice, 2013; Task force on North Rift hospital upgrade, 2016). It is a referral point for rural and urban health centres in the south rift including surrounding suburbs and its rural environs. As per the updated demarcations established by the Interim Election Boundaries Commission (IEBC), the county is divided 10 regions (Sub counties) which are Nakuru Town East, Nakuru Town West, Molo, Njoro, Subukia, Bahati, Naivasha, North Kuresoi, South Kuresoi and Rongai. (Appendix V, County map). The female population of the county as per the last Census and housing report of 2009 was recorded at 239, 230 females (KNBS, 2010). Nakuru County was chosen for this study owing to the high cervical cancer prevalence in pilot cancer registry reports yet to be made public in cohort on-going studies (Rajab *et al.*, 2014-2017).

3.2 Study design

This was a descriptive cross-sectional hospital based study on patients at various stages of pre-cervical cancer manifesting both pre-cancer and invasive cancer signs visiting the Nakuru County referral hospital.

3.2.1 Study variables

Dependent variables: These were characteristics obtained from pre cervical cancer status (grade) based on outcomes from Pap smear screens. They were classified as

Control, low grade squamous intraepithelial lesions (LSIL), high grade intraepithelial lesions (HSIL), and Atypia of glandular cells or adenocarcinoma in situ (AGC/AIS).

Independent variables: These were characteristics aimed at analyzing information of cytomorphologic, microbial STI exposure, lifestyle and nutritional practices as well as reproductive health characteristics and practices. Support information from socio-demographic characteristics served as baseline demographic features.

3.3 Study population

All females attending the reproductive health unit within the maternal child health department including those admitted in the gynaecological ward for various cervical cancer interventions were identified and enrolled during clinic visit days.

Inclusion criteria

1) Positively screened VIA/VILI females above the age of 18 years visiting the reproductive health clinic including those seeking maternal health and family planning services were considered.

2) Those admitted in the gynaecology ward (with confirmed pre cervical cancer and CIS diagnosis) awaiting management as well as patients with various gynaecological complications referred from comprehensive care center.

Exclusion criteria

1) Females below the age of 18 years particularly where sexual debutation had not begun.

2) All pregnant female patients including those who were not willing to participate in the study were excluded.

3.4 Sampling design

Purposive sampling technique was applied, where women subjects who had hitherto been scrutinized for cervical health through VIA/VILI visual cervical examination and tested positive were enrolled.

The following guidelines were taken into consideration in the sampling design.

1. That all participants were sourced from the MCH as well as those already admitted in the gynaecology ward) awaiting management for various gynaecological complications.

2. That all participants had undertaken VIA/VILI cervical visual screen.

3. That all participants had screened positive for visual cervical VIA/VILI test.

4. That all participants who tested VIA/VILI positive were subjected to Pap smear test.

5. All participants whose Pap smear results bore normal/no lesion manifestation were enrolled as the comparison (control) group.

6. That all participants whose Pap smear results bore positive results were categorized as the test cases (study groups grades) based on atypical cytomorphological feature manifestation of LSIL, HSIL and AGC/AIS.

7. Participant (subject) cluster employed a four group cohort as per the Pap smear report grading that considered the following Bethesda system (TBS) criteria (Nayar *et al.*, 2015). The study design data collection flow is as shown in Figure 3.1.

a) Study Group 1 (Control group) Normal or benign report -no evidence of cellular epithelial lesion/no pre cervical cancer.

b) Study Group 2 (LSIL) Pre cancer of low grade.

c) Study Group 3 (HSIL) Pre cancer of high grade.

d) Study Group 4 (AGC/AIS) Atypia of glandular cells (AGC/AIS).

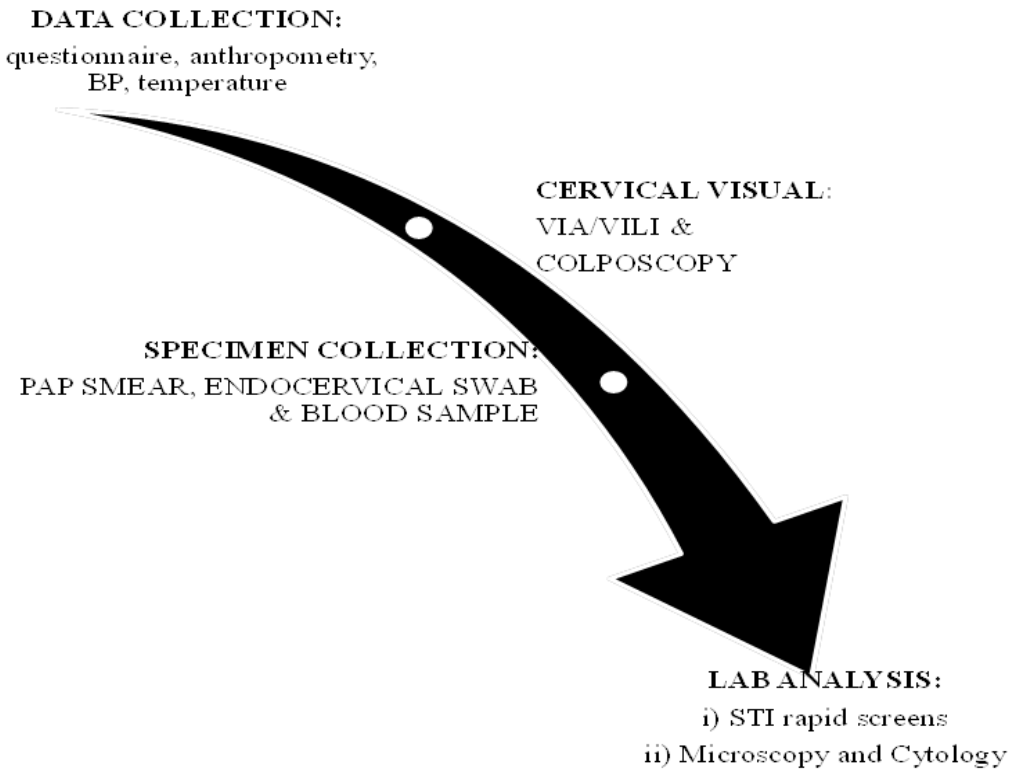

Figure 3.1: Algorithm of data collection flow

3.5 Sample size

The total population of female visits in the reproductive health clinic in any given month of the year usually falls between 150-200 women patients. These figures were captured from the client visit records registry book during the piloting period before subject recruitment was done. The study established the same number of women

attendance from the piloting survey hence served as the rationale for setting the desired sample size for the study. Kenya Census figure standings captured during the last Census survey indicates that the total female population in Nakuru was 798,743 (KNBS, 2010). Total female population in Kenya as per the same census was 19,417,639 (KNBS, 2010). The rationale did not consider current data on annual cervical cancer figure standings (2554) in Kenya as per Globocan and cancer registry pilot preliminary records (WHO/ICO, 2010; Ferlay *et al.*, 2012 and Rajab *et al.*, 2014-17). This study focused on pre-cervical cancer status where no specific data on prevalence of pre-cervical cancer predictors exists for Nakuru County. Sample size was hence determined from the upper limit of women monthly reproductive health clinic attendance of 200 women.

Therefore, the formula below was used to determine the desired sample size (Kothari, 2004).

nf = n/(1+n/N).

Where **nf** was the desired sample size; **n** is the estimate sample deduced from a seeming 71% out of 200 women clinic attendance level (142). **N** is total Nakuru women population (798743).

Therefore, 142/ 1+ (142/798743) = 52.5

Since four study groups were considered, then 52 X 4 = 208 **(nf)**. Nakuru County was the area of interest for an estimated sample size of 208 study participants. The subject enrolment period ran from January 2014 to November 2014 (Appendix I) during which 208 subjects were successfully enrolled. Some 66 study subjects data was invalidated and disregarded during data clean-up from a variety of challenges. These included non-valid questionnaire response entries and entries made from unsuitable specimens for analysis caused from deterioration leading to diagnostic pitfall. A

variety of specimen unsuitability causes included cytomorphologic atypia mimicry from non-specific artifact material in smears as well as cellular insufficiency experienced during microscopy. Moreover the lack of access to specimen storage facilities culminating from frequent hospital strikes resulting to hospital closure and reproductive health service loss occurred in the months of December 2013, April 2014 and 02 Jul 2014 (KNA, 2013; Standard Digital Media, 2013; News24 Kenya, 2014; Daily Nation 2014).

3.6 Research instruments

Structured questionnaires designed by the researcher were administered in a bid to obtain relevant information based on subject responses to lifestyle, nutritional and reproductive health history. Anthropometric parameter measurement was employed to gauge lifestyle predictors pertaining to body mass bulk gain and loss assessment. Select microbial STI occurrences and exposure was obtained from blood and endocervical specimens withdrawn from the subjects through rapid test kits. The use of cervical Pap smear screen for cytomorphological cellular characteristics using TBS 2001 (Appendix VIII) was employed for the identification of cytomorphologic characteristics of cervical epithelial cells.

3.6.1 Validity and reliability of research instruments

The instruments that were applied to capture the study data are protocols that have been used in other similar studies with success (DeVuyst, 2005; Abd El All *et al.*, 2007). Minor modifications were made in order to ensure that the most relevant information could be captured from the targeted group. The questionnaire probes were designed with utmost scrutiny through comparisons with methodology of studies such as those of Were *et al.*, 2010; Memiah *et al.*, 2012 and Ngugi *et al.*, 2012. The

cervical screens which enabled pre cervical cancer status establishment were obtained from approved systems accepted globally such as the Bethesda protocol (Nayar *et al.*, 2015). Smears were examined by qualified cyto technicians and confirmed by independent equally qualified cytologists. STI detection from rapid test assay protocols were subjected to internal quality control. These rapid screens met standard quality specification approval from the Chinese government.

3.7 Pilot study

An initial survey of Nakuru County referral hospital was conducted to determine its suitability in conducting the study. It was considered as it serves as a referral center to all the other health centers' within Nakuru County. In addition, it has minimal specialized treatment facilities and equipment from which female members of the community are more likely to access reproductive health services. The pilot survey also sought to establish the number of women who seek reproductive health services and their specific needs, so that projection on adequacy of sample size acquisition would enable meaningful interpretation of data from enrolled study subjects within the stipulated data collection period as captured in the work plan and budgetary needs.

3.8 Data collection techniques

1. Questionnaire survey was conducted in order to capture relevant information pertaining to their personal practices and characteristics including reproductive health status. Baseline attributes of body temperature and blood pressure readings were also captured.

2. Anthropometric measure of specific anatomical sites of the body, mainly the upper arm circumference, bust girth, waist circumference, weight and height scopes was conducted.

3. Endocervical specimens of scrapes and swabs were collected. Scrapes were for Pap smearing and cyto-morphological profiling following microscopic examination. This enabled subject clustering into four study group (LSIL,HSIL,AGC/AIS and control) as per the application of the Bethesda grading system (Nayar *et al.*, 2015). Additionally swabs were extracted for HPV, Gonorrhoea and Chlamydia antigen detection and blood specimens that were withdrawn from phlebotomy for HIV and Syphilis antibody detection.

3.8.1 Questionnaire administration

Identified subjects were counseled after consent approval on the importance of reproductive health awareness as well as on the study aims and undertakings (Appendix III). Questionnaire administration was conducted through a semi interview process as a number of subjects were illiterate and could not interpret the questions on the list. All information pertaining to their lifestyle, dietary habits, clinical reproductive health history, occupational activities including socio-demographic attributes were captured as per responses given by subjects. Questionnaire administration was conducted by the principle researcher with the aid of nursing officers attached to the clinic (Appendix IV).

3.8.2 Anthropometric and clinical measurements

The approach applied in determining lifestyle practices was based on a host of predictors such as anthropometric characteristics, types of diets consumed including tobacco and alcohol use, physical exercise regularity upon questionnaire responses obtained from open ended probes. These gave continuous data variables, of weight, height, mid upper arm circumference (MUAC), chest and waist measurements. Vital

clinical parameters such as body temperature and blood pressure (systolic blood pressure and diastolic blood pressure -SBP/DBP) were also recorded. Height, Weight arm, bust and waist girth (circumference) scopes were obtained from the women subjects using a standard tape measure. The S.I. unit applied for the physical measurements was centimeters (cm). Weight capacity was obtained using a standard calibrated weight scale and the S.I. unit applied was kilograms (Kg). Other parameters Blood pressure (BP) and body temperature readings were captured using calibrated blood pressure kit and digital thermometer. In the former, BP measure of systolic and diastolic limits were obtained from blood vessels of the upper arm captured in units of beats per minute (bpm) while value of temperature was recorded from readings obtained by aiming the sensitive thermometer probe on the fore head over the temporal artery of the subject in order to capture heat emanating from the head region in degrees centigrade (^{0}C). The information was recorded in a subjects' individual duly filled questionnaire form. BMI indices calculated from combined anthropometric measurements of height and weight were recorded for each individual study subject (S.I. unit kg/m^2). These parameters were established using standard WHO guideline as applied in the management of non-communicable disease (WHO, 2015).

3.8.3 Specimen collection

3.8.3.1 Endo-cervical specimens: Scrape and Swab collection

Through nursing officers' assistance, patients were counseled and verbal consent obtained as a precaution prior to guiding them on the examination couch in the dorsolithotomy position. They were required to be undressed from the lower torso. A sterile, single-use (or re-usable) bivalve speculum of appropriate size dependent on the average reproductive anatomical size of the subject was inserted gently into the

vagina using plain warm physiological saline or tap water lubrication (gel application was avoided). By manipulation of the speculum screws, the vaginal canal opening was expanded to expose the cervix that was identified by the OS region. The position of the speculum should allow for complete visualization of the Ossicle (OS) and ecto-cervix. Standard smear collection method was employed as routinely applied in the reproductive health clinic (Harrison *et al.*, 1993). Pap smear kit with cervical abrasion tools (Ayers spatula, cervical brush and cervical broom) was used to scrape cells of the ecto-cervix (upper and lower lips of the cervix) including the endocervical region of the OS for cellular morphology examination. Three smear preps were made from the three scrapping tools (two were backup smears). Smears made from cervical broom were used for cytomorphological profiling. Cotton Swab kits in transport media were also used to scrape the ecto-cervix for antigenic microbial STI detection. Four swab specimens were obtained, one in transport media (backup swab) and three in plain swab containers. They were used for HPV detection Gonorrhoea and Chlamydia detection respectively. Cotton swab specimens were preserved in transport media and frozen in ultra-low temperatures of between a range of -70° and -80°C.

3.8.3.2 Conventional Pap smear preparation technique

Conventional cytology protocol for Pap smear preparations was employed, where smear scrapping were applied using one directional sweep of abrasion tool onto frosted clean grease-free microscopic glass slides and immediately spray fixed before being taken to cytology laboratory for processes of staining and mounting. For those participants recruited from the ward, some diagnosed with CIS or AIS had their cervixes and uteri either partially excised off or the TAH surgical intervention done for the management of biopsy graded pre cancer. It was not possible to extract Pap smear specimens from them. Cytology reports from their personal hospital files

reports were re-analyzed for the most updated report for the purpose of confirming cervical cancer status. Archived Pap historical smears were retrieved and examined for cellular morphological manifestations. According to the national cervical cancer management all preliminary cancer screens that indicate suspicious test results (from VIA/VILI and Pap smear screens), secondary cervical screen using biopsy test reports must be conducted (DRHS/MOPH&S pocket handbook, 2010).

3.8.3.3 Blood specimen collection

Phlebotomy obtained blood specimens were collected from subjects in order to obtain sera for microbial STI antibody detection. Briefly subjects were verbally counseled and consent obtained for the procedure which involved fixing a tourniquet on the fore arm and tightening it to increase pressure that facilitates the pop up of veins. Vein identification and size was applied in determining the appropriate needle size. A suitable vein from the superficial ante-cubital region of the fore arm was identified from which a needle affixed to a syringe was inserted into. Using gentle pressure, the syringe piston was pulled outwards which aided in the extraction of blood from the vein through the needle into the barrel of the syringe. Blood was placed in a clot retraction container then centrifuged at 2000 RPM to separate serum which was aliquoted in eppendorf tubes and frozen at ranges of between -70° and -80°C.

All successfully recruited and enrolled study participants were on standard treatment and management program offered within the facility in line with the national cervical cancer management policy (DRHS/MOPH&S Pocket handbook, 2010).

3.8.4 STI microbial screening

Methods described in this section were standard laboratory protocols and procedures subjected to internal quality control for reliability and repeatability. Each protocol

applied had internal quality control assurance enforced. Antigen (STI marker) and or antibody expressed from microbial STI organisms were detected from endocervical swabs and blood specimen. A choice of select pathogen microbes were screened from blood and swab specimens. The kits used were Liming Bio®antigen rapid test for HPV16/18 and Chlamydia antigen detection, DRG RAPID® and Blue Cross® antigen rapid tests for *Neisseria gonorrhea* antigen detection Kehua Bio Engineering® antibody rapid test for HIV (1+2) colloidal gold antibody detection and Healgen® antibody rapid strip test for Syphilis antibody detection (Appendix ix). The detection of STI antigenic surface markers was conducted from endocervical and high vaginal swab specimens while sera samples separated from blood specimens were used for the detection of antibodies specific to Syphilis and HIV infection exposure.

Antigenic extraction for HPV, *Gonorrhoea* and *Chlamydia* STI detection was done through a series of two washes using diluent A and B to dislodge organisms from the swab tip. Two drops of extracted contents suspended in diluent fluid is then place on the reaction well and allowed to migrate for one minute. Coloured bands were examined post reaction on the cassette membrane surface. A control band incorporated onto the test confirms that the test is valid and that sufficient specimen volume and correct procedural technique has been followed (Appendix IX).

Antibody detection for *Treponema pallidum* and HIV1/2 in serum specimens was done in rapid tests by placing two drops of serum on the test area. Fluid was allowed to migrate from which coloured bands were read after one minute. A control band incorporated onto the test confirms that the test is valid and that sufficient specimen volume and correct procedural technique has been followed (Appendix IX).

The detection of these organisms indicated active, ongoing or previous STI infection exposures. The criterion applied in selecting microbial STI was based on the Kenya

demographic report detailing common STI infections within Nakuru residential community (Nakuru DSP, 2005–2010). These tests were conducted as per the particular kit's manufacturer guidelines in which principle mechanisms behind the test are also specified (Appendix ix).

3.8.5 Smear stain processing and microscopic examination

A series of manipulations and staining procedures according to the George Papanikolaou protocols (Appendix VIII) were employed. Briefly, the smears were fixed, dehydrated, re-hydrated and stained using appropriate stains (Hughes, 2000). Stained smears were further mounted with cover slips before the microscopic examination, as this affords permanent preservation.

Atypical cyto-cellular morphological manifestations present in both pre cancer and invasive cancer employed the 2014 revised Bethesda System (TBS) grade score classification (Nayar *et al.*, 2015). Revisions made to the system were also taken into consideration in order to classify cells using the most updated guideline following the TBS re-evaluation and revision in the 2001 workshop convention organized by the National Cancer Institute, USA, cosponsored by 44 professional societies represents in more than 20 countries (Nayar *et al.*, 2015). The reporting categories under the 2014 Bethesda System are summarized in a table (Appendix VIII). Non-neoplastic cellular categories in smears served as the control. These manifested benign characteristics such as cytolysis, irradiation, atrophic or progesterone effects. Neoplastic cellular cyto-morphology profiling that served as the test groups were classified into low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion(HSIL) and atypia of glandular cells (AGC) coupled with adenocarcinoma in situ (AIS).

Smear microscopy examination was only conducted from the cervical broom smears. Cellular grading was done using the battlement microscopic technique. This ensured that all cells within the smear can be focused within a microscopic field and critically described. Cellular characteristics applied in the grading cells were; nuclear volume relative to the cytoplasmic volume, multi-nucleation, nuclear membrane irregularity, prominence of chromatin granule inclusion, presence of prominent nucleolus and mitotic figure, including nuclear staining reactions that were hyperchromatic or hypochromatic pigmentation (Appendix VIII). Keratin deposition disproportionality was also considered as a feature for cellular segregation. The age of participants as well as the use of hormonal contraceptives influences for birth control in a bid to discriminate cellular morphology was also considered during microscopy.

This guideline was applied in both low power magnification (X 10 objective) for the degree or extent of cellular atypia within each examined field and high power magnification (X 40 objective) for critical discrimination of cell type and associated abnormality. The eye piece lens strength was X 20. Low power magnification gave a total magnification of X 200 (X 20 x X 10) while high power magnification gave a total magnification of X 800 (X 20 x X 40).

A simple criterion for cytological discrimination of epithelial cell varieties and other diagnostic smear finding for the study purpose was designed as per the varied cellular characteristics observed. This was for study result tabulation purpose only (Table 4.2). Cells were categorized as; Squamous (with squamous variants of atypia of undetermined significance and atypia of high grade (ASCUS and ASC-H), Koilocytes, Glandular (with glandular variants of atypia of undetermined significance and high grade glandular atypia (AGCUS and AGC-H). Both differentiated and undifferentiated squamous and glandular cells that manifested changes associated

with severe high grade atypia were examined. Inclusions were cellular components that were not epithelium derived but were none the less present during smear examination. They included bacteria, fungus, parasites and leucocytes (polymorphonuclear and histiocytes cells). All Pap smear microscopic examinations were conducted by an independent and experienced cyto technician and confirmed by a clinical cytologist. Smear microscopic feature images were captured using the Venus 2.0®programmemounted on computer CPU (specific for Venus camera imagery) launched on a Leitz compound microscope. Various photomicrograph images were captured in different pre cancer grades and saved in JPEG format. Each image captured was appropriately labeled as per its pre cancer grade. Experienced laboratory technicians were at hand to assist in the photo microscopic techniques.

The microscopic examination of cervical smear preparations (Pap smears) for any kind of cellular nucleic, cytoplasmic abnormality and or inclusions was used in the determination of the pre cancer grade which was used to assess its link with various pre cancer predictors as envisioned in the study's conceptual framework.

3.9 Data analysis

Cleaned computed and coded relevant data was assessed to sharpen measurements and to eliminate non-specific information. Data analysis was done using several analytical model platforms to test for significance in associations and relationships between dependent and independent variables. Summarized results were tabled to display study group proportions and *P* values. Interpretable information from which inferences were made from, were disclosed to make meaningful conclusions with the aim of predicting and linking risk factors to increased cancer grade severity leading to the onset and development of pre and invasive cervical cancer disease.

Successfully enrolled women subjects were assigned unique codes to distinguish them

from each other within the entire period when recruitment was ongoing (Appendix I). Subject names were not used for confidentiality purpose and instead a specified study number or code was used in place. Data obtained from characteristics of each individual subject was captured in a consolidated raw registration form and entries done into Excel spreadsheet package workbook (Microsoft office version 2010) by assigning characteristics as either categorical or continuous variables. Data entry was also duplicated by importing Excel captured data onto SPSS statistical application software programme (IBM SPSS Inc. application software version 21.0.1, 2010).

Data clean-up was done to identify and eliminate outliers and entries that were incomplete or invalid. Statistical analysis of the data was achieved using a triage system that considered the influence of variable attributes as per their distribution, proportion and extent of relationship or association with each other. The SPSS software was employed for both descriptive and inferential statistical analysis. Additional analysis was supplemented using Graph pad Prism (version 7, 2015) powered by Graph pad software Inc. Medians and interquartile range (IQR) were obtained for continuous data while absolute values and percentages were used for expressing magnitudes in categorical data. Differences in women proportions between the four groups or blocks of the study participants (control group) and the test groups (cases-which consist of categories of women with varying degrees of pre cancer severity (LSIL, HSIL and AGC/AIS) were compared. Univariate analysis to establish associations between pre cervical cancer status and microbial, lifestyle and reproductive health practice characteristics were conducted using the Pearson's Chi-square test for categorical variables and Kruskal-Wallis test for continuous variables. The probability (*P)* value cut off was set at $P \leq 0.05$ to gauge hypothesis. Multivariate logistic (linear) regression model where applicable, were used in determining specific

associations between study groups from the pre cancer grades of LSIL, HSIL and AGC that determined the pre cancer status. Only variables with $P<0.01$in univariate analyses were included in the regression analyses. Confounding effect of age, education level, birth control method, marital status and cervical disease awareness was controlled for in the regression models. In the model, correlation between the dependent variable (pre cancer grades) and independent variable (predictors) was evaluated through the degree of association differences (β coefficient), odds ratio (OR) values that indicated the magnitude of predictor risk association with the specific pre cancer grade including the probability (P) value.

3.10 Ethical considerations

This study involving human subjects was approved by Scientific and Ethical Review Committee of Kenyatta University. Specific elements pertaining to participant confidentiality and protection were:

1. Consent process that involved a debrief explaining personal queries to be asked on reproductive and sexual health history including performance of invasive procedures (speculum insertion in the vaginal canal and phlebotomy procedure).

2. Use of a special assigned study number/code for each participant instead of using their names.

3. Consent forms that were signed by participant if she agreed to participate.

4. Maintenance of strict data protection of all information collected from participants.

5. All approved procedures conformed to the international standards (Appendix X).

CHAPTER FOUR

RESULTS

4.1 Baseline characteristics of the study participants

Examination of the prevalence of pre cervical cancer grades among enrolled participants from Nakuru County referral hospital illustrated that most smears were of HSIL pre cancer grade (41.5%), in comparison to LSIL, 24.6% and atypia of combined glandular cells of AGC/AIS at 5.6% (Figure 4.1).

Analysis of baseline characteristics (Table 4.1) showed that age differed significantly across the study groups (P<0.0001). In between group comparisons showed that women presenting with high grade lesions of both glandular and squamous type (AGC/AIS and HSIL) were older (AGC/AIS, median, 65.0; range, 50.0-70.0and HSIL, median, 42.0; range, 27.0-63.0 compared to LSIL and controls (LSIL, median, 38.0; range, 20.0-57.0 and control, median, 34.0; range, 21.0-55.0 years. Education level finding indicated statistical difference among the study participants ($\chi 2$=10.226; df, 3; P=0.017) with HSIL and AGC/AIS (73% and 62.5% respectively), having more women without education or with primary school education levels compared to LSIL (48.6%) and controls (52.5%). Although marital status did not show statistical differences ($\chi 2$=2.940; df, 3; P=0.401), most of the study participants were married (HSIL, 59%; LSIL, 60.0% and controls, 75.0%). There were no statistical differences in the types of occupations undertaken across the study groups ($\chi 2$=2.408; df, 6; P=0.879). However, the occupations varied among the study participants, with a majority of the women engaging in informal sector occupations such as farmhands, quarry miners, pastoralists, peasant farmers, or in small businesses (AGC/AIS, 75%;HSIL, 56%; LSIL, 43%; and controls, 53%). Blood pressure, measured as

systolic (AGC/AIS, median, 125; range, 97-160; HSIL, median, 120; range, 99-159 ; LSIL, median, 119.0; range, 80.0-149.0; controls, median, 130.5; range, 118.0-143.0; *P*=0.237) and diastolic (AGC/AIS, median, 74; range, 55-100;HSIL, median, 72.5, range, 55-93; LSIL, median, 80.0; range, 60.0-97.0; controls, median, 86.0; range, 81.0-91.0; *P*=0.237) pressures (beats per minute), were within WHO normal reference range (WHO, 2015).Conversely, auxiliary body temperature measured in degrees centigrade (^{o}C) was significantly different across the study groups (*P*=0.001) and was higher in the LSIL pre cervical cancer group (median, 37.3; range, 37.0-37.8) compared to controls (median, 37.4; range, 37.0-37.4, but not in the HSIL pre cancer group (median, 37.1; range, 36.7-37.8). Blood pressure and body temperature readings were in accordance with the WHO reference guidelines (WHO, 2015).

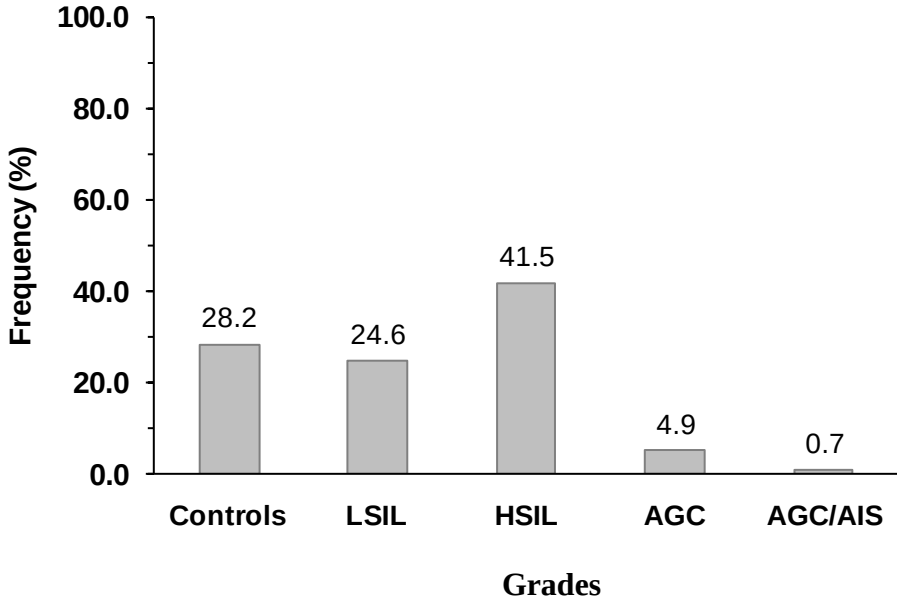

Figure 4.1: Pre cervical cancer classification of the study participants

Enrolled participants, n= 142. Controls, n=40 (28.2%); LSIL, n=35 (24.6%); HSIL, n=59 (41.5%); AGC, n=7(4.9%); and AGC/AIS, n=1 (0.7%)

Table 4.1: Baseline characteristics of the study participants

Characteristic	Controls, n=40	LSIL, n=35	HSIL, n=59	AGC/AIS, n=8	χ^2, df	*P*
Age (years)	34 (21-55)	38 (20-57)	42 (27-63)	65 (50-70)	-	**<0.0001**
Education						
≤Primary	21 (52.5)	17 (48.6)	43 (72.9)	5 (62.5)	10.226, 3	**0.017**
≥Secondary	19 (47.5)	18 (51.4)	16 (27.1)	3 (37.5)		
Marital status						
Married	30 (75)	21 (60)	35 (59.3)	3 (37.5)	2.940, 3	0.401
Single	10 (25)	14 (40)	24 (40.7)	5 (62.5)		
Occupation						
Informal sector	21 (52.5)	15 (42.9)	33 (55.9)	6 (75)		
Small businesses	15 (37.5)	15 (42.9)	19 (32.2)	2 (25)	2.408, 6	0.879
Formal employment	4 (10)	5 (14.3)	7 (11.9)	0 (0)		
BP, bpm						
SBP	130.5 (118-143)	119 (80-149)	120 (99-159)	125 (97-160)	-	0.237
DBP	86 (81-91)	80 (60-97)	72.5 (55-93)	74 (55-100)	-	0.353
Body Δ, ^oC	37.4 (37.0-37.4)	37.3 (37.0-37.8)	37.1 (36.6-37.1)	37.1 (36.7-37.8)	-	**<0.0001**

Data presented are number and frequency (%) of subjects or as median (range) for age, blood pressure (BP) and auxiliary body temperature. Study participants were categorized according to the Bethesda classification system into LSIL, HSIL and AGC/AIS pre cervical cancer grades (Nayar *et al.*, 2015). Controls were women with no smear epithelial lesions. Statistical comparisons were performed using the chi-square test for proportions and Kruskal-Wallis test for age; blood pressure and auxiliary body temperature. χ^2, chi-square statistic; df, degrees of freedom; bpm, beats per minute; SBP, systolic Blood Pressure; DBP, diastolic Blood Pressure; Δ, auxiliary body temperature; ^{o}C degrees centigrade.

4.2 Cytomorphologic features of the study participants

Cytomorphologic characteristics in smears were classified (Table 4.2) using the application of the Bethesda grading system (Nayar *et al.*, 2015). The frequency of koilocytes was higher in the LSIL study group, 77% in comparison to HSIL 52% and AGC/AIS study group, 25% (Figure 4.3). At least 12.5% of participants in the control study group smears displayed koilocytic cells (Figure 4.3). ASCUS presented with high proportions in both LSIL and HSIL study groups, 85.7% and 61% respectively while ASC-H were higher in HSIL Study group (39%) as compared to LSIL (14.3%). In contrast, the frequency of abnormal epithelial glandular cells in the AGC/AIS study group was higher in women presenting with AGCUS (87.5%) compared to those of AGC-H (12.5%). Epithelial cellular feature variants were significant (P<0.0001), koilocytes; χ^2 = 93.952, df, 24; squamous cells; χ^2= 193.573, df, 18 and glandular cells; χ^2 = 139.0, df, 3.

Analysis of smear inclusions revealed higher bacteria, fungi and inflammatory cells presence in AGC/AIC study group (<62.5%) relative to the control, LSIL and HSIL study groups collectively (<58%). One parasite (*Trichomonas vaginalis*, 2.5%) was detected only in the control group (Figure 4.2). Corresponding with the presence of microbes, inflammatory cells (polymorphonuclear neutrophils, histiocytes and plasma cells) were present in all the study groups (AGC/AIS, 100%; HSIL, 58%; LSIL, 46%; and controls, 55%; χ^2=73.863; df, 63; P=0.165).

Table 4.2: Cytomorphologic features in cervical smears of the study participants

Characteristic	Controls, n=40	LSIL, n=35	HSIL, n=59	AGC/AIS, n=8	χ^2, df	*P*
Type of cervical epithelia						
Koilocytes	5 (12.5)	27 (77)	31 (52)	2 (25)	93.952, 24	**<0.0001**
Squamous cells						
ASCUS	0 (0)	30 (85.7)	36 (61)	N/A	193.573, 18	**<0.0001**
ASC-H	0 (0)	5 (14.3)	23 (39)	N/A		
Glandular cells (endocervical and endometrial source)					139.000, 3	**<0.0001**
AGCUS	0 (0)	N/A	N/A	7 (87.5)		
AGC-H	0 (0)	N/A	N/A	1 (12.5)		
Inclusions						
Bacteria (cocci and bacilli)	4 (10)	6 (17.1)	20 (34)	5 (62.5)	73.863, 63	0.165
Fungi (hyphae and spores)	5 (12.5)	3 (8.6)	17 (29)	5 (62.5)		
Parasites (trophozoites)	1 (2.5)	0 (0)	0 (0)	0 (0)		
Inflammatory cells (polymorphonuclear histiocytes and plasma cells)	22 (55)	16 (46)	34 (58)	8 (100)		

Data presented are number and frequency (%) of subjects. Study participants were categorized according to the Bethesda classification system into LSIL, HSIL and AGC/AIS pre cervical cancer grades (Nayar *et al.*, 2015). Controls were women with no smear epithelial lesions. Statistical comparisons were performed using the chi-square test for proportions. χ^2, chi-square statistic; df, degrees of freedom, N/A (Not applicable for this cell type).

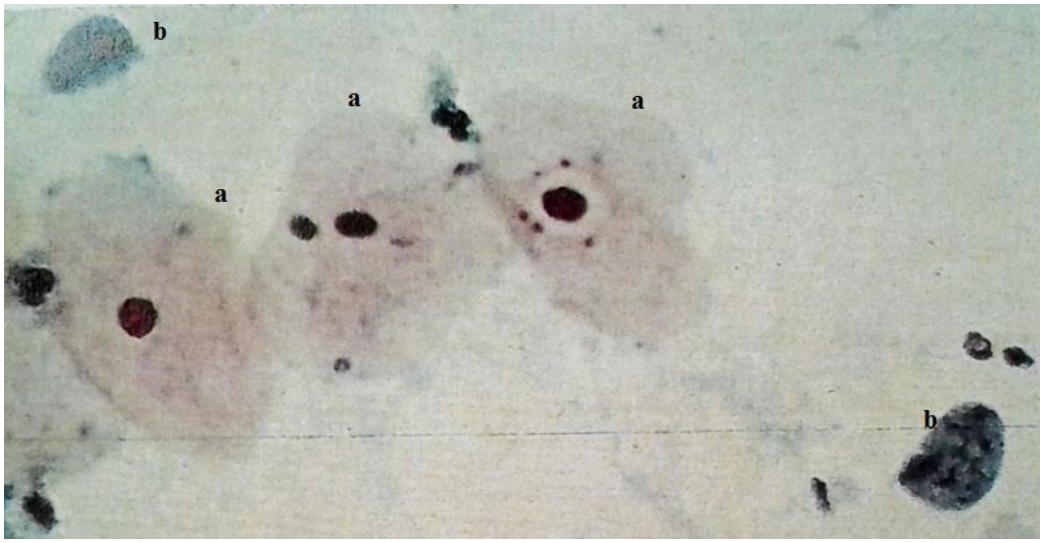

Figure 4.2: Cells derived from control study group smears

X800 Pap stain technique (Haematoxylin, EA 50 and OG 6) cytological presentation of faintly eosinophilic normal intermediate epithelial cells (a). Forms of ovoid trichomonad shape without characteristic flagella (b).

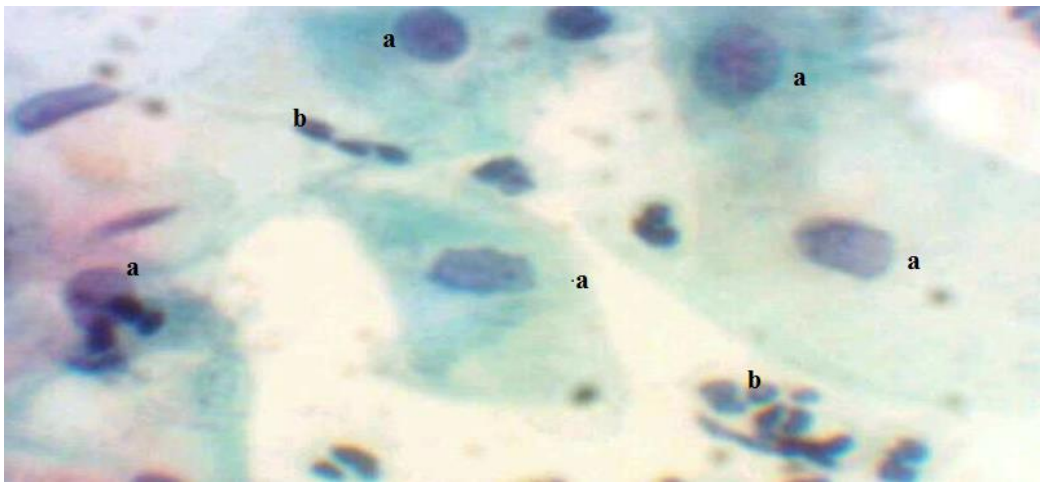

Figure 4.3: Cells derived from LSIL pre cancer study group smears

X 800 Pap stain technique (Haematoxylin, EA 50 and OG 6) cytological presentation of LSIL showing cyanophilic intermediate squames with varying dyskaryotic features of koilocytes (irregular nuclear membrane, enlarged nucleo-cytoplasmic volume ratio, hyperchromasia but no chromatin granulation (a) Evidence of candida connidia forms present with some connidia obscuring an intermediate epithelial cell to the left of field (b).

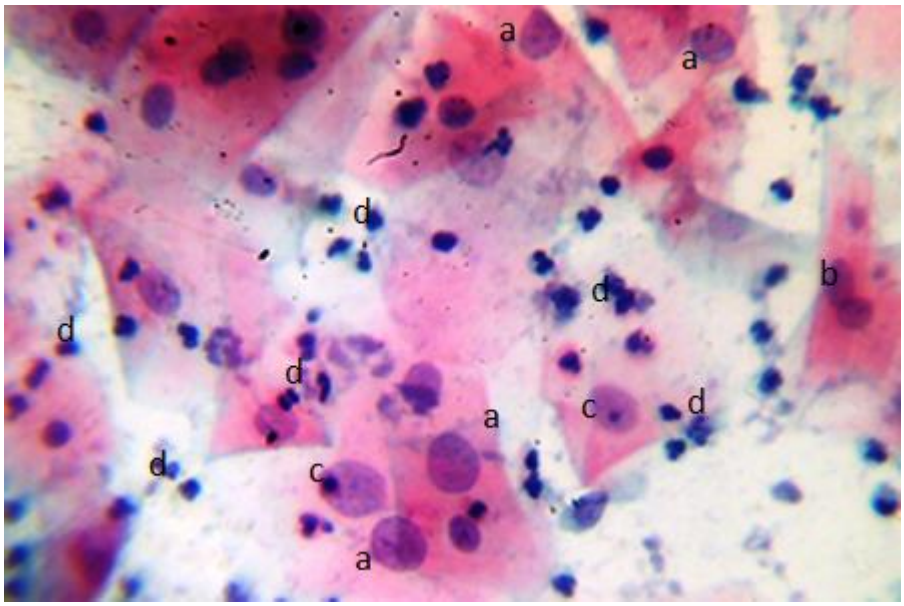

Figure 4.4: Cells derived from HSIL pre cancer study group smears

X 800 Pap stain technique (Haematoxylin, EA 50 and OG 6) cytological presentation of (HSIL) abnormal squamous cells. Eosinophillic superficial cell with grossly increased nucleo-cytoplasm (N/C) volume ratio (lower bottom 'a') and having dense hyperchromatic granules (upper top 'a'). multi-nucleate cell (b). Eosinophillic squames with prominent nucleolus (c) and polymorphs in smear (d).

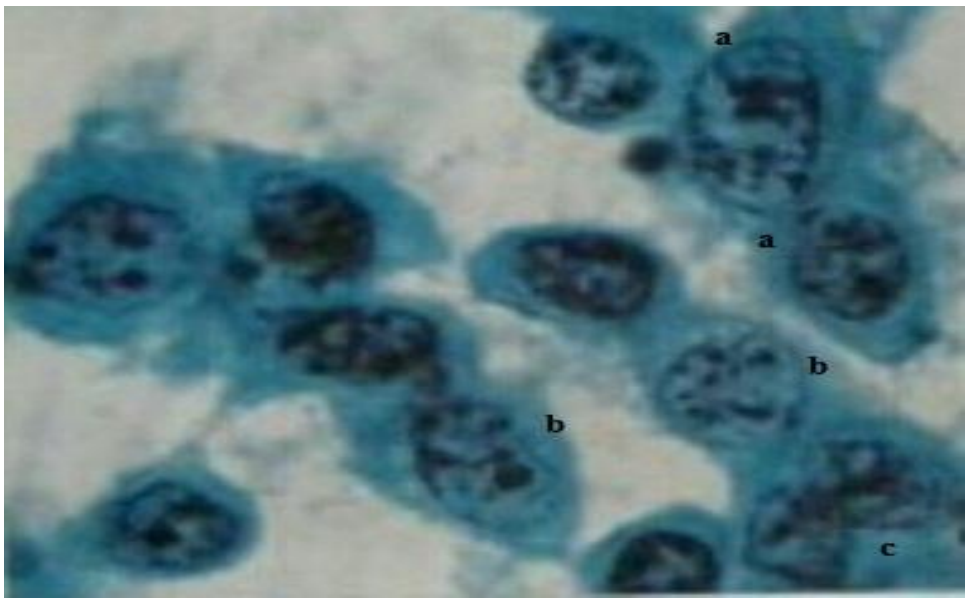

Figure 4.5: Cells derived from AGC/AIS study group smears

X 800 Pap stain technique (Haematoxylin, EA 50 and OG 6) cytological presentation of malignant cells of adenocarcinomatous type. The cytoplasm in this group of cells shows diffuse background and nuclei shows variation in sizes, shapes with hyperchromasia (a) as well as hypocromasia (b). Marginal condensation of chromatin pattern is evident which is grossly abnormal in distribution (a and b). A binucleated endocervical cell (c).

4.3 Select sexually transmitted infections among the study participants

Analysis of selected sexually transmitted immunosuppressive and/or oncogenic organisms (Table 4.3) indicated that the prevalence of HIV1/2 was higher in women presenting with HSIL grade (25.5%) relative to LSIL and control, 11.4% and 12.5% respectively(χ^2= 5.263; df, 3; *P*=0.154). Consistent with previous studies showing higher rates of HPV16/18 in cervical cancer patients, (1996; Burd, 2003; De Vuyst *et al.*, 2008; Memiah *et al.*, 2012 and Ai *et al*., 2015) at least three-quarters of the women presenting with pre cancer grades were positive for HPV16/18 serotypes (HSIL, 79.7%; LSIL, 82.9%; AGC/AIS, 100%) compared to controls (10.0%; χ^2= 62.681df, 3; *P*<0.0001). Subsequent regression analysis to determine whether STI microbial infections of HIV1/2 and HPV16/18 independently predicted pre cancer grades (Table 4.4) illustrated that only HPV16/18 was associated with increased risk of having pre cancer grade of AGC/AIS (β, 21.390; OR, 1.947; 95% CI, 1.940-1.947; *P*<0.0001); HSIL (β, 3.592; OR, 36.323; 95% CI, 9.456-139.525; *P*<0.0001) and LSIL (β, 3.913; OR, 50.055; 95% CI, 11.993-208.913; *P*<0.0001,Table 4.4).

Sero-positivity for syphilis was also detected in participants in all study groups (HSIL, 28.8%; LSIL,17.1%; AGC/AIS, 20% and controls,35.0%; χ^2= 3.214; df, 3; *P*=0.360). Surface marker antigen positivity for *N. gonorrhea* was only detected in HSIL (5.1%) study group while antigen positivity for *C. trachomatis* was present in both HSIL (6.8%); LSIL (2.9%) study groups.

Table 4.3: Select STI screened among study participants

Organism	Controls, n=40	LSIL, n=35	HSIL, n=59	AGC/AIS, n=8	χ^2, *df*	*P*
HIV1/2	5 (12.5)	4 (11.4)	15 (25.5)	0 (0)	5.263,3	0.154
HPV16/18	4 (10)	29 (82.9)	47 (79.7)	8 (100)	62.681,3	**<0.0001**
Treponema pallidum	14 (35)	6 (17.1)	17 (28.8)	3 (20)	3.214,3	0.360
Neisseriae gonorrhoea	0 (0)	0 (0)	3 (5.1)	0 (0)	4.158,3	0.245
Chlamydia trachomatis	0 (0)	1 (2.9)	4 (6.8)	0 (0)	3.458,3	0.326

Data presented are number and frequency (%) of subjects. Study participants were categorized according to the Bethesda classification system into LSIL, HSIL and AGC/AIS pre cervical cancer grades (Nayar *et al.*, 2015). Controls were women with no smear epithelial lesions. Statistical comparisons were performed using the chi-square test for proportions.χ^2, chi-square statistic; df, degrees of freedom.

Table 4.4: Association of HIV1/2 and HPV16/18 with pre cervical cancer grades

Pre cancer grade	β	OR	95% CI	*P*
		HIV1/2		
LSIL	-0.031	0.969	0.229-4.109	0.966
HSIL	0.993	2.699	0.780-9.342	0.117
AGC/AIS	1.880	6.556	0.241-178.435	0.265
		HPV16/18		
LSIL	3.913	50.055	11.993-208.913	**<0.0001**
HSIL	3.592	36.323	9.456-139.525	**<0.0001**
AGC/AIS	21.390	1.947	1.940-1.947	**<0.0001**

Multivariate logistic regress models were performed to identify microbial indicators of pre cancer grade outcome categorized according to the Bethesda classification system (Nayar *et al.*, 2015). In the analyses, grade outcomes of LSIL, HSIL and AGC/AIS were entered as the dependent variable, screening positive for HIV1/2 and HPV 16/18 as the predictor group while screening negative for the detection of both these organisms being entered as the reference. The confounding effect of age, birth control method choice, cervical cancer disease awareness, marital status and education level were controlled for in these regression models. Data are presented as odds ratios (OR) and 95% confidence interval (CI). β coefficient indicates the degree of association differences for model.

4.4 Lifestyle and Nutritional practices of the study participants

Lifestyle and nutritional profiling (Table 4.5) indicated that body weight was significantly different across the study groups (*P*=0.036) such that it was higher in AGC/AIS (median, 73.0; range, 52-86 kg) in comparison to controls (median, 71.0; range, 45-89 kg) and LSIL (median, 70.0; range, 45-70 kg). Height was similar amongst all the study group participants including control; median, 1.6 m (χ2, 1.158; df, 3; *P*=0.763). BMI (kg/m^2) was non-significantly (*P*=0.57) lower in women presenting with HSIL grade (median, 24; range, 16-33kg/m^2) relative to AGC/AIS (median, 27.5; range, 21-33kg/m^2); and controls (median, 26; range, 19-34kg/m^2). Corresponding with BMI, MUAC (cm) was also non-significantly lower (*P*=0.11) in HSIL (median, 30; range, 21.0-42.0 cm) in comparison to control (median, 33.0; range, 23-45cm).

Analogous to low body weight, circumferences of bust (*P*=0.004) and waist (*P*=0.002) were significantly lower in HSIL and AGC/AIS study groups compared to LSIL and controls (Table 4.5). The bust girth was lower in HSIL (median, 98.0; range, 74-126 cm) and AGC/AIS (median, 96.0; range, 76-101 cm) compared to LSIL (median, 100.0; range, 70-122 cm) and controls (median, 103.0; range, 78-122 cm). Similarly, waist circumference (cm) was lower in HSIL (median, 83.0; range, 56-112 cm) and AGC/AIS (median, 74.0; range, 64-90 cm) compared to LSIL (median, 86.0; range, 54-114 cm) and controls (median, 90.0; range, 51-109cm).

Most of the participants, ~80% were practicing physical activity in all the study groups. Analysis of this attribute indicates that there was a borderline association with pre cancer grades (*P*=0.05). Results demonstrating dietary practices illustrated that consumption of healthy diet was higher among HSIL (66%) group compared to LSIL

(31.4%) and controls (33%; χ^2, 13.175; df, 3; *P*=0.004). Unhealthy diet results illustrated that in LSIL (~69%), it was consumed in much higher proportions than in HSIL (~34%) and AGC/AIS (37%). Less than ~12% participants reported using both alcohol and tobacco products in all the study groups including control.

Multivariate regression analyses of lifestyle and nutritional practices in the attribute of anthropometric parameter associations with pre cancer grades revealed that lower median circumference values,≤ 99 of bust and ≤86 of waist were associated with more likelihood of manifesting HSIL grade (β,1.767; OR,5.851; 95% CI, 2.008-17.050; *P*=0.001 and β, 1.681; OR, 5.368; 95% CI, 1.870-15.409; *P*=0.002); and LSIL grade (β, 0.974; OR, 2.650; 95% CI,0.900-7.803; *P*=0.077 and β,1.052; OR,2.863; 95% CI, 0.994-8.244; *P*=0.051) respectively. In addition, analyses of nutritional practices assessing the types of consumed diets demonstrated that consuming unhealthy diet was associated with higher likelihood of developing LSIL as suggested by the trends illustrated in Table 4.6 that shows significant correlation of this practice with LSIL grade (β,-1.433; OR, 4.190; 95% CI,1.363-12.881; *P*=0.012).

Table 4.5: Lifestyle and nutritional characteristics of the study participants

Characteristic	Controls, n=40	LSIL, n=35	HSIL, n=59	AGC/AIS, n=8	χ^2, df	*P*
Weight, kg	71 (45-89)	70 (45-70)	70 (44-96)	73 (52-86)	-	**0.036**
Height, m	1.6 (1.46-1.83)	1.6 (1.46-1.77)	1.6 (1.49-1.78)	1.6 (1.53-1.63)	-	0.763
BMI, kg/m^2	26 (19-34.0)	25 (20-35)	24 (16-33)	27.5 (21-33)	-	0.57
MUAC, cm	33 (23-45)	31 (20-44)	30 (21-42)	32 (23-34)	-	0.11
Bust, cm	103 (78-122)	100 (70-122)	98 (74-126)	96 (76-101)	-	**0.004**
Waist, cm	90 (51-109)	86 (54-114)	83 (56-112)	74 (64-90)	-	**0.002**
Physical activity						
Yes	22 (80)	33 (94.3)	56 (94.9)	8 (100)	7.679,3	0.050
No	8 (20)	2 (5.7)	3 (5.1)	0 (0)		
Diet						
Healthy	13 (33)	11 (31.4)	39 (66)	5 (62.5)	13.175,3	**0.004**
Unhealthy	27 (70)	24 (68.6)	20 (34)	3 (37.5)		
Alcohol use						
Yes	3 (7.5)	3 (8.6)	6 (10.2)	1 (12.5)	0.902,3	0.825
No	37 (92.5)	32 (91.4)	53 (89.8)	7 (87.5)		
Tobacco use						
Yes	1 (2.5)	2(5.7)	6 (10.2)	1 (12.5)	3.44,3	0.328
No	39 (97.5)	33 (94.3)	53 (89.8)	7 (87.5)		

Data presented are number and frequency (%) of subjects for categorical variables and or as median (range) for continuous variables. Study participants were categorized according to the Bethesda classification system into LSIL, HSIL and AGC/AIS pre cervical cancer grades (Nayar *et al.*, 2015). Controls were women with no smear epithelial lesions. Statistical comparisons were performed using the Kruskal-Wallis test for continuous variables (weight, height, BMI, MUAC, bust and waist) and chi-square test for categorical variables. K-W, Kruskal-Wallis; χ^2, chi-square statistic; df, degrees of freedom; BMI, Body mass index; MUAC, mean upper arm circumference (WHO, 2015).

Table 4.6: Association of lifestyle and nutritional characteristics with pre cervical cancer grades

Pre cancer grade	β	OR	95% CI	*P*
Body weight **(**median≤68kg)				
LSIL	-0.087	0.916	0.339-2.480	0.863
HSIL	0.808	2.243	0.849-5.929	0.103
AGC/AIS	-1.839	0.159	0.004-5.831	0.317
Bust girth **(**median≤99cm)				
LSIL	0.974	2.650	0.900-7.803	0.077
HSIL	1.767	5.851	2.008-17.050	**0.001**
AGC/AIS	2.858	17.422	1.099-276.119	**0.043**
Waist circumference **(**median≤86cm)				
LSIL	1.052	2.863	0.994-8.244	0.051
HSIL	1.681	5.368	1.870-15.409	**0.002**
AGC/AIS	1.816	6.150	0.472-80.215	0.166
Unhealthy diet				
LSIL	-1.433	4.190	1.363-12.881	**0.012**
HSIL	-0.90	1.094	0.391-3.062	0.864
AGC/AIS	3.108	0.045	0.000-4.065	0.177

Multivariate logistic regress models were performed to identify lifestyle and nutritional indicators of pre cancer grade outcome categorized according to the Bethesda classification system (Nayar *et al.*, 2015). In the analyses, pre cancer grade outcomes of LSIL, HSIL and AGC/AIS were entered as the dependent variable, values ≤68kg median of weight, ≤99cm median of bust and ≤86cm median of waist as the predictor group while those above the stated medians as the reference. Unhealthy diet was also entered as predictor while healthy diet as the reference. Confounding effect of age, birth control method choice, cervical cancer disease awareness, marital status and education level were controlled for in these regression models. Data are presented as odds ratios (OR) and 95% confidence interval (CI). β coefficient indicates the degree of association differences for model.

4.5 Reproductive health history and practices of the study participants

The reproductive health history and practices of the study participants is summarized in Table 4.7. Evaluation of reproductive health history showed that the frequency of women reporting concurrent lower abdominal pain, vaginal bleeding and discharge was high in all groups including control (>75%; χ^2=11.926; df, 3; *P*<0.008).However, women reporting having both lower abdominal pain and bleeding (>85%) was higher in all the pre cancer groups with an exception to controls (50.0%; χ^2=27.553; df, 3; *P*<0.0001). In contrast, the rates of women reporting lower abdominal pain and discharge was similar across the study groups (~69%) with an exception to control, which was lower (62%; χ^2=0.938; df, 3; *P*=0.816).

Most of the women were practicing birth control use with hormonal methods dominating in all the study groups (HSIL, 56%, LSIL, 51% and control, 45%) compared to non-hormonal methods (HSIL, 24%,LSIL, 29% and control 40%. AGC/AIS study group had the highest rates of non-birth control users (100%;χ^2=21.993; df, 6; *P*=0.001, respectively. Parity variable yielded statistical difference across study groups (χ^2=1.834, df, 3; *P*=0.001), with AGC/AIS and HSIL study group indicating the highest rates of participants with at least two or more live births (88% and 62% respectively)compared to LSIL and control (~45%) that had less than two children. Moreover, the frequency of women reporting having multiple sexual partners (i.e., more than one) was highest in HSIL study group, 50.8 % in comparison to LSIL, 37% and controls 25%; χ^2=6.776; df, 3; *P*=0.079. There was significant statistical difference obtained on the perception of cervical cancer cause across all the study groups (χ^2=25.843; df, 9; *P*=0.002) with varied responses

obtained. At least 11% attributed its cause to infections while ~50% responded that they did not know the cause.

Additional multivariate regression analyses indicated that only presenting with clinical history of lower abdominal pain and vaginal bleeding was associated with higher odds of having AGC/AIS, HSIL or LSIL pre cancer (β, 25.347; OR, 1.019; 95% CI, 1.000-1.019; $P<0.0001$; β, 2.183; OR, 8.873; 95% CI, 2.576-30.561; $P=0.001$ and β, 1.758; OR, 5.800; 95% CI, 1.795-18.739; $P=0.003$) respectively.

Table 4.7: Reproductive health history and practices of the study participants

Characteristic	Controls, n=40	LSIL, n=35	HSIL, n=59	AGC/AIS, n=8	χ^2, *df*	*P*
History of lower abdominal pain, vaginal bleeding and discharge						
YES	30 (75)	33 (94.3)	56 (94.9)	8 (100)	11.926,3	**0.008**
NO	10 (25)	2 (5.7)	3 (5.1)	0 (0)		
History of lower abdominal pain and vaginal bleeding						
YES	20 (50)	30 (85.7)	54 (91.5)	8 (100)	27.553,3	**<0.0001**
NO	20 (50)	5 (14.3)	5 (8.5)	0 (0)		
History of lower abdominal pain and vaginal discharge						
YES	25 (62.5)	25 (71.4)	41 (69.5)	6 (75)	0.938,3	0.816
NO	15 (37.5)	10 (28.6)	18 (30.5)	2 (25)		
Birth control use						
Hormonal	18 (45)	18 (51.4)	33 (56)	0 (0)	21.993,6	**0.001**
Non-hormonal (Devices and Barriers)	16 (40)	10 (28.6)	14 (23.7)	0 (0)		
None	6 (15)	7 (20)	12 (20.3)	8 (100)		
Parity						
≥2	22 (55)	19 (54.3)	37 (62.7)	7 (87.5)	1.834,3	**0.001**
<2	18 (45)	16 (45.7)	22 (37.3)	1 (12.5)		
Number of sexual partners						
>1	10 (25)	13 (37.1)	30 (50.8)	2 (25)	6.776,3	0.079
≤1	30 (75)	22 (62.9)	29 (49.2)	6 (75)		
Perception of cervical cancer cause						
Don't know	20 (50)	24 (68.6)	32 (54.0)	2 (25)	25.843,9	**0.002**
Infections	7 (17.5)	4 (11.4)	7 (11.9)	0 (0)		
Poor hygiene	4 (10)	7 (20)	18 (30.5)	6 (75)		
Witchcraft	9 (22.5)	0 (0)	2 (3.4)	0 (0)		

Data presented are number and frequency (%) of subjects. Study participants were categorized according to the Bethesda classification system into LSIL, HSIL and AGC/AIS pre cervical cancer grades (Nayar *et al.*, 2015). Controls were women with no smear epithelial lesions. Statistical comparisons were performed using the chi-square test for proportions. χ^2, chi-square statistic; df, degrees of freedom.

Table 4.8: Association of reproductive health history and practices with pre cervical cancer grades

Pre cancer grade	β	OR	95% CI	*P*
History of lower abdominal pain and vaginal bleeding				
LSIL	1.758	5.800	1.795-18.739	**0.003**
HSIL	2.183	8.873	2.576-30.561	**0.001**
AGC/AIS	25.347	1.019	1.000-1.019	**<0.0001**
Parity ≥2				
LSIL	-0.216	0.806	0.270-2.408	0.699
HSIL	-0.654	0.520	0.178.-1.515	0.231
AGC/AIS	0.465	1.592	0.034-74.450	0.813
Multiple sex partners >1				
LSIL	0.125	1.134	0.256-5.016	0.755
HSIL	1.350	3.859	0.905-16.450	0.068
AGC/AIS	-0.602	0.548	0.013-23.896	0.216

Multivariate logistic regress models were performed to identify reproductive health indicators of pre cervical cancer grade outcome categorized according to the Bethesda classification system (Nayar *et al.*, 2015). In the analyses, pre cancer grade outcomes of LSIL, HSIL and AGC/AIS were entered as the dependent variable, predictors of having clinical history of lower abdominal pain and vaginal bleeding with reference group as the non-clinical manifestation of pain or bleeding history; having more than two children with reference as having less than two children and having more than one sexual partner with reference as having less than one sexual partner. Confounding effect of age, birth control method choice, cervical cancer disease awareness, marital status and education level were controlled for in these regression models. Data are presented as odds ratios (OR) and 95% confidence interval (CI). β coefficient indicates the degree of association differences for model.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Introduction

Cervical cancer disease is the third most common cancer amongst women worldwide but second to breast cancer in developing countries such as Kenya where presently 86% of the new cervical cancer cases and account for 88% women deaths (Ferlay *et al.*, 2012, and Torre *et al*., 2015). Regionally countries south of the Sahara particularly in East and Central Africa are most affected with an age rate of 34.5 per 100,000 women each year (WHO/ICO, 2010). Distinct determinants (risk factors) on pre cervical cancer screen grade outcomes among study participants are hereby described. By critically evaluating arguments for and against their role in risks to the development as well as progression of pre cancer, justification based on both descriptive and inferred statistical analysis was established in support of their association with pre cervical cancer grades as determined among women study participants in Nakuru County referral hospital.

Cytomorphological results illustrating a reducing trend in the frequency of koilocytes (~77%, ~52% and ~25% in LSIL, HSIL and AGC/AIS) accompanied by an increasing trend in the frequency of abnormal epithelial cells of high grade cellular lesion types (ASC-H), ~14% in LSIL and ~39% in HSIL, suggests that higher pre-cancer grade is characterized by abnormal epithelial cells with increased nuclear-to-cytoplasm volume ratio, increased hyperchromasia, irregularity of nuclear membrane and increased chromatin prominence. This pattern is consistent with features as described in TBS grading system guideline (Nayar *et al.*, 2015). These results are similar to previous studies among women at Kenyatta National Hospital showing higher rates of abnormal

epithelia of ASC-H in HSIL pre cancer grade relative to Koilocytic atypia in LSIL pre cancer grade (Karuri *et al.*, 2014) and similarly in another cross-sectional study conducted in a resource-limited settings at Nazareth Hospital ART clinic in Kiambu, central Kenya (Memiah *et al.*, 2012). The results also mirror previous findings in American and British women presenting with pre cervical cancer indicating higher rates of koilocytes in LSIL compared to HSIL grades (Solomon and Kurman, 2002; Solomon and Nayar *et al.*, 2004; Solomon and Breen, 2007; Denton *et al.*, 2008; Solomon, 2015). Interestingly, a small proportion of women in the control study group displayed koilocytic cells in their smears (12.5%). This suggests that women is this group are exposed to high risk viral agents that cause cytopathic effects and may potentially develop pre cervical cancer signs should persistence of infection ensue. A study conducted in American women shows similarity in the sense that normal healthy women without cervical epithelial lesions may be candidates of pre cervical cancer development (Burd, 2003).

Only eight study participants (5.6%) with a median age of 65 years, and range 50-70 years had atypia of glandular cells (pre cancer group AGC/AIS) in which only one was characterized as high grade lesion (AGC-H) in her Pap smear. These cells constitute cellular atypia found in the rarer variant of cervical cancer (Adenocarcinomatous type). This low rate is consistent with reports from a collaborative survey of 23 different cervical cancer studies globally (Appleby *et al.*, 2006), indicating that low occurrence of glandular atypia in women is due to the inversion of the transformation zone in cervixes of post-menopausal women making the endocervical layer where endocervical cells can be sampled inaccessible. In advanced age the TZ moves deeper into the endocervical canal away from reach where cellular sampling for pre cervical

cancer screening via scrap, colposcopy or cervicography can be done. This therefore contributed to a less effective detection of this pre cancer grade type by way of the Pap smear pre cervical cancer screening method as used in this study.

Altogether, the findings of higher prevalence of abnormal epithelial cells having high grade lesions (HSIL) is attributable to increasing HPV-driven cellular transformation of koilocytic cells to cells of severer levels of abnormality manifesting increased nuclear-to-cytoplasm volume ratio, increased hyperchromasia, and increased course chromatin. These assertions are consistent with previous studies showing that persistence of HRHPV such as serotypes 16 and 18 infection promotes the transformation of koilocytic cells to a degree of higher cellular abnormality (Burd, 2003; Eurocytology, 2016), especially in the presence of impaired body defense mechanisms due to cervical cancer-triggered chronic immunosuppression (Massad, *et al*., 2001). Therefore, identification of koilocytes in cervical smears is an important indicator of pre-cancer diagnosis of LSIL. Of equal significance, the identification of high rates of high grade abnormal epithelial cells is an indicator of pre-cancer progression and increasing cancer severity from pre cancer grade of LSIL grade to that of HSIL grade.

Evaluation of cervical smear inclusions revealed higher rates of bacterial and fungal organisms in atypia of glandular cells-AGC/AIS (over 60%) while only approximately ~34% prevalence in atypia of squamous cells (LSIL and HSIL study groups). This is a routine finding in women during Pap smear microscopic screening. For instance, the prevalence of vaginal infections (i.e., *Gardnerella vaginalis*, *Trichomonas vaginalis* and *Candida albicans*) in adult women attending cytology clinic in Nigeria, Lagos was

14.5% (Konje *et al*., 1991), and up to 27% in prospective cytopathology laboratory study records of women with biopsy-proven CIS and invasive carcinoma of the uterine cervix in the United States (Mitra, *et al.*, 2016). The AGC/AIS study group yielded results showing higher rates of these infections, which are cited in studies as common occurences especially in post-menopausal women whose advanced age is also noted in this study group (Table 4.1).This is supported by findings documented in previous research on atrophic smear evaluation of post-menopausal in Kenyan and European women (Were *et al.*, 2010; Eurocytology, 2016). It is likely that most of these organisms thrived as a result of diminishing cyclic endocervical pH changes. In support of this explanation, previous study conducted in Gynecology and Perinatology Clinic, Maribor Teaching Hospital, Slovenia-Eastern Europe allude that bacterial and yeast organisms in different grades of CIN are frequent as a result of hormonal peaks that affect cervical pH thereby interfering with their symbiotic relationships with residential commensals in the RT (Takac, 1998).

Additional smear inclusion analyses illustrated that at least ~58 % of women in atypia of squamous cells study groups (LSIL and HSIL) and absolute (100%) for those in atypia of glandular cells (AGC/AIS), manifested leucocyte inflammatory cells which suggests that there was increased inflammatory response in the reproductive tract. This finding prudently demonstrates that the presence of inflammatory cells is a para-neoplastic syndrome indicator in early LSIL and HSIL pre cancer manifestation (Ashour *et al.*, 1997 and Rahkola *et al*., 2009). It is likely that this inflammatory response is a first-line non-specific immune response resulting from the presence of microbes in the RT or cervical epithelium tissue injury in pre-cancer. These results are consistent with a study conducted by Gordon (1993) in the US showing that increased

detection of inflammatory cells in cervical smears is associated with the presence of pre cancer. Additionally, low rates of inflammatory cells in LSIL smears (46%) compared to ~55% in controls may be attributable to depressed immunity prompted by pre cervical cancer-triggered chronic immunosuppression (Massad *et* al., 2001).

While univariate analyses of HIV1/2 infection exposure did not yield statistical difference amongst the variant pre cervical cancer study groups (Table 4.3), high prevalence of HR HPV16/18 in LSIL, HSIL and AGC/AIS(> 79%) with an exception to the control study group (10%) was obtained. Further additional multivariate regression modeling analyses showing association of HRHPV16/18 with pre-cervical cancer grades (Table 4.4) confirms the fact that the high risk HPV strains 16/18 are endemic in this population. These analyses are similar with those in cross-sectional hospital based study conducted at Nairobi, Kenya in Kenyatta National hospital showing associations between high risk HPV with HSIL in over 70% of high grade lesions and all squamous cell carcinomas detected in this study (Karuri *et al.*, 2014). Marked prevalence of HRHPV 16/18 among cervical cancer subjects in Nakuru may be related to the fact that HPV infections are not only asymptomatic but also that high risk variants which are oncogenic and persistent may have resulted from protracted immunosuppression resulting to their persistence within the RT of HSIL and AGC/AIS pre cervical cancer subjects. It is also believed that HPV transmission may not have only been through sexual means but additionally transmission may have occurred through skin-skin contact in genital areas not protected by condom coverage such as the scrotum, vulva and labia. These assertions are supported by evidence from a Kenyan study (Were *et al.*, 2010) as well as from American surveys (Burd, 2003; Blake *et al.*, 2015) that indicate precipitated transmission of HRHPV.

Anthropometric attributes of lifestyle practices revealed that low median values for weight, and circumferences of bust and waist in HSIL and AGC/AIS study groups, and supplementary regression analysis indicating associations of low median values for bust and waist circumferences with HSIL pre cervical cancer grades (Table 4.5 and 4.6) suggests development of wasting syndrome within the upper trunk and lower abdominal sections of the body. These findings are similar to a previous study conducted in Texas M.D. Anderson Cancer Center, Houston in the United States showing associations between rapid loss of fat and lean mass in pre cervical neoplasms (Ashour *et al.*, 1997).

The fact that bust and waist girth associated with pre cancer grades of LSIL and HSIL, implies that there is progressive body mass wasting with increasing pre cancer grade characterized by loss of upper and middle abdominal adiposity. This is supported by previous studies illustrating that wasting and weight loss are cachectic paraneoplastic manifestations observed in many pathological conditions including cervical cancer (Fearon et al., 2006; Argiles *et al.*, 2011; Mantovani *et al.*, 2013). The syndrome is attributed to high burn out of nutrients in muscles and fat body reservoirs in early and progressive cancer disease to provide energy needed during increased metabolic demand from accelerated proliferation of growing populations of abnormally differentiating cells forming lesions, masses and tumours. An American scientist, (Koppenol *et al.*, 2011) has demonstrated in his study that cancerous cells are predatory as they deprive nutrients to normal cells in the body as a result of their aggressive increased metabolic rates necessary for their uncontrolled proliferation thereby selfishly depleting the body's resources for themselves resulting to wasting and body bulk loss.

Dietary assessment illustrating lower subject frequencies of healthy diet consumption in LSIL study group relative to control as well as HSIL and AGC/AIS at < 34% (Table 4.5), and additional regression analyses showing increased risk of pre cancer manifestation among LSIL unhealthy diet subject consumers (β, -1.443; OR, 4.190; 95% CI, 1.363-12.881; *P*=0.012, Table 4.6), suggests likelihood of pre cervical cancer development. This may be influenced by the possibility of lowered practice of healthy diet consumption as observed in the LSIL study group. These results are consistent with previous studies among normal healthy women paralleled with women manifesting LSIL and HSIL pre cervical cancer signs in Lok Nayak Hospital, New Delhi India. Consuming healthy organic foods in participants enrolled in Indian women imparted a protective effect that may have prevented the development and progression of low grade cancer to grades of higher severity and potentially afforded recovery from high grade cancer as well (Labani *et al.*, 2009). Similarly, a study conducted in Louisiana State University Medical Center-Shreveport in the United States demonstrated experimental evidence illustrating consumption of cruciferous vegetables such as cabbage and kale rich in indole-3-carbinol was more effective than placebo in the prevention of low grade cervical epithelial cell abnormality among women with pre cervical cancer (Bell *et al.*, 2000). The same pattern was established among American women populations (Bouvard *et al.*, 2015).

The demonstration of higher incidence of LSIL participants (~69%) relative to HSIL and AGC/AIS (~34%) consuming unhealthy type of diet is presumed to be as a result of inconsistent and non-committal adherence to advisory and edification in regards to nutritional counseling given during clinic follow-up visits. It is believed that LSIL subjects were not as adherent and attentive to health diet benefit advice given to them

as compared to HSIL and AGC/AIS subjects, who were noted to consume considerably less unhealthy diet possibly due to their acute awareness of their critical and severer cervical health status. It is also suggested that based on the low the economic activities most of them were engaged in (Table 4.1) healthy diets varieties of fruit and vegetable may not have been consumed as much among LSIL subjects due to their unaffordability and high cost. Findings among women in the rural community of both Kenya and Cape Town, South Africa indicates dietary fatalistic beliefs, attitudes and practices in pre cervical cancer disease consistent with dietary practice observations perceived in Nakuru women (Gatune *et al.*, 2005; Mosavel *et al.*, 2009).

Evaluation of reproductive health history and practices indicating higher rates of simultaneous manifestation of lower abdominal pain (pelvic pain), vaginal bleeding and vaginal discharge among all pre cancer study groups (LSIL, HSIL, AGC/AIS, Table 4.7) and multivariate regression model analysis illustrating that presenting reproductive signs of lower abdominal pain and vaginal bleeding is associated with higher odds for HSIL and AGC/AIS grades, (Table 4.8), proposes that presenting a clinical history of lower abdominal pain and vaginal bleeding is diagnostic for increasing and progressing severity of pre cervical cancer disease. These results mirror previous studies conducted in Uganda and in the United States of America. Patients with advanced and late stage cervical cancer disease attending reproductive health care at a private-not-for-profit (PNFP) and a public Regional Referral Hospital, both in Gulu, northern Uganda, were assessed and findings indicated that all presented with lower abdominal pain and vaginal bleeding (Mwaka *et al.*, 2015). Similarly, in Texas M.D. Anderson Cancer Center, Houston in the United States, increased incidence of lower abdominal pain discomfort and vaginal bleeding was observed among advanced pre cervical cancer patients with grades exceeding CIS attending

treatment (Krucik, 2016). The appearance of a combination of abnormal vaginal bleeding and pelvic pain are usually the initial signs of metastasis in cervical ill health as previously documented in study reports by Oriel *et al.*, 1999 and Boardman *et al.*, 2012 indicating that protracted vaginal bleeding and pelvic pain are important clinical RT complications of HSIL. During routine reproductive health clinic visits, abnormal RT findings as disclosed in this study, support suspicion for undertaking cervical cancer screens in women with such signs.

Birth control involving the utility of both hormonal and non-hormonal methods was significantly associated with pre cervical cancer grades ($\chi 2$=21.993; df, 6; P=0.001) suggesting risk of usage with pre cervical cancer development. This is similarly reflected in studies implicating hormonal contraception use in European women and elsewhere (Moreno *et al.*, 2002; Skegg, 2002; Appleby *et al.*, 2007; Luhn *et al.*, 2013). Synthetic hormonal contraceptives made up of progesterone and oestrogen elicit similar effects to naturally secreted ovarian hormones which are involved in several functions in the RT including the growth, differentiation, proliferation and maturation of epithelial cells (Atia, 2010). The usage of synthetic contraceptive hormones among study participants may be implicated with abnormality in maturation and desquamation of epithelial cells localized within the epithelium layer either through their suppression or overexpression. Epithelial development and maturation disruption was markedly manifested by their abnormality in morphology observed as atypia of undetermined significance in examined cervical smears which were scored and graded as ASCUS (Table 4.2).Similarly to birth control use, parity variable demonstrated significant association with pre cervical cancer grades ($\chi 2$=1.83; df, 3; P=0.001). Studies conducted in America and Europe give strong indication of similarity with pre cancer manifestation (Brisson *et al.*,1994; Muñoz *et al.*, 2002; Jensen *et al.*, 2013;

Luhn *et al.*, 2013).The probable explanation of parity result resemblance in this study with the other afore mentioned studies are that with high parity, there is increased chance of ovarian hormonal upheavals. These hormones regulate cell growth processes within the epithelium layer of the cervix. Hormonal upheavals may affect metaplastic processes during columnar cellular conversion to squamous cells within the TZ. Cells undergoing metaplasia are most venerable to HR HPV due to numerous mitotic events which may concurrently propagate HR HPV DNA transcription in infected cells (Burd, 2003) Virally infected cells manifest Koilocytic atypia which may further transform to severer forms in persistent HR HPV infections recognized as pre cervical cancer signs of LSIL or HSIL within the cervical epithelium (Burd, 2003).

The prevalence of at least >25% subjects with multiplicity of sexual partners practice(i.e. exceeding one partner) in all three test study groups including control, suggests that having multiple sexual partners is a risk factor for developing cervical cancer (Table 4.8). These findings parallel previous studies in the north rift of Kenya (MTRH) among histology confirmed cervical cancer study participants accessing MCH clinic for well-baby services and family planning services, showing that about 54% had multiple sex partners (Were *et al.*, 2010). Similarly, in a study conducted in Kenyatta National referral hospital (Nairobi), demonstrated that having invasive cervical cancer was associated with the practice of multiplicity of sexual partners among participants (OR= 1.9, 95% CI: 1.6-6.2) in the hospital-based study (Williams *et al.*, 1994). A study conducted amongst both cancer free and cervical cancer women victims in the rural population of Gulu district in the neighbouring country of Uganda, indicated that 88% of study participants had knowledge on multiplicity of sexual partners as a risk factor to cervical cancer (Mwaka *et al.*, 2015).The higher risk of multiple-sexual partners is mainly linked to the increased transmission of high risk

HPV strains. In deed this assertion is supported by previous studies showing higher prevalence of HRHPV16/18 strains in American women with pre and advanced cervical cancer (Burkett *et al.*, 1992; Burd, 2003; Luhn *et al.*, 2013).

The lack of knowledge and perception of cervical cancer causes among study participants was significantly associated with pre cancer grades($\chi 2$=25.843; df, 9; *P*=0.002, Table 4.7).In one study conducted in Thika, Kenya, similarity in the lack of cervical cancer disease awareness and causes was quite high (Ngugi *et al.*, 2012).A likely reason could be high illiteracy and ignorance among study subjects which may be supported from the findings on baseline characteristics of the study participants that demonstrate the fact that education level was quite low and also portrayed significant difference with the pre cancer grades with most participants having attained only primary level education ($\chi 2$=10.226; df, 3; *P*=0.017,Table 4.1).

Study limitations encompassed the non-employment of modern LBC techniques over the utility of conventional cytology technique. This contributed to diagnostic pitfalls and clarity challenges during smear microscopy examination particularly in the distinction of epithelial atypia from normal undifferentiated cells, inflammatory cells and non-diagnostic artifacts and debris. Insufficient financial research resources restricted the application of sensitive molecular (DNA) based techniques in distinguishing HPV variants 16/18 from each other. Likewise the cultivation of bacterial STI was not done due to limited storage facilities (freezers). Rapid test kits were locally unavailable hence were out-sourced (imported) and employed for the qualitative detection of STI infections. Highly fragmented questionnaire responses were encountered resulting to non-valid response entries possibly from exaggeration

or falsehood stimulated by reluctance, introversion, and perceived loss of dignity and stigma. Significant information on whether HPV immunization had been administered, types of consumed micro-nutrient and dietary supplements, including the specific source of vaginal bleeding within the RT whether due to abnormal or dysfunctional uterine bleeding was not established and neither was parity frequency distinction of live births from still births. Non-inclusion of Caucasian and Indian Nakuru women residents including women covered in private health insurance schemes was inevitable as this study was confined in a public county hospital. Two major industrial hospital strikes that occurred within the period of data collection affected data collection operations (storage logistics) hence some human specimens were unsuitable for laboratory analysis either as a result of poor sample storage and handling thereby affecting overall operational sample size.

Considered delimitation through the recommendation of a comparative study to address the gaps that served as restrictions is applicable. Additionally longitudinal research study would serve as an effective mechanism to recall women with abnormal cytologic abnormalities as a follow up which will also address the challenge of default rate associated with invalidity of questionnaire responses.

5.2 Conclusion

1. Findings indicating high frequency of Koilocytic cells in LSIL grade smears contrasting with low rates of ASC-H cells in LSILgrade smears and low rates of koilocytes inHSIL grade smears, illustrates that Koilocytic cells indeed transformed to higher grade atypia propagated by persistence of high risk oncogenic HPV16/18 infection in women who had productive HPV exposure.

2. Compelling results of high prevalence of HRHPV16/18 was remarkably associated with the three cervical cancer test cases of LSIL, HSIL and AGC/AIS. This confirms that productive HR HPV16/18 infection is a definite predictor of cervical cancer genesis whose persistence is linked to the development of low grade pre cancer of LSIL as well as the advancement and progression of low grade cervical cancer to cervical cancer of higher severity characterized by HSIL and AGC/AIS.

3. Lifestyle atribute of anthropomentry analysis indicated thatsignificantly associaed low bust and waist median circumferences suggested wasting syndrome in the upper trunk and lower abdominal section in HSIL pre cervical cancer grade while consuming healthy diet was protective against the development of HSIL pre cancer type.

4. Discoveries of clinical manifestations of lower abdominal pain coupled to vaginal bleeding,usage of hormonal contraception and high parity were important predictors of LSIL, HSIL and AGC/AIS hence careful monitoring and patient follow-up through mandatory quarterly cervical screening for cervical health assessment to avert progression to invasive cancer can be applied as a preventive tool in positive prognosis of cervical cancer management outcomes.

5.3 Recommendations

The prevention of pre cervical cancer incidence and its progression to advanced invasive cancer are achievable as hereby suggested. These recommendations are directed to all consumers of reproductive health information including health seeking individuals of Nakuru County. Affiliated organizations (in research, reproductive health science academia and personnel involved in health care policy making) will find the account documented here useful.

1. Detected cytomorphologic features in smears can be applied as predictive laboratory diagnostic tools to foretell dynamic events within the cervical wall hence limit or inhibit pre cervical cancer genesis. These features include the identification of koilocytes and other grades associated with pre cervical cancer. Additionally distinguishing various assortments of epithelial atypia which point out to transformed koilocytic cells is actual motivation to suspect advancing low grade cancer to an escalating degree of atypia severity. This can be enhanced by introduction of LBC technique to increase atypia harvest for smears in the Nakuru referral hospital cytopathology laboratory. The detection of increased leucocytic aggregation in smears coupled to detection of cellular forms such as spores, cocci forms and cytopathic effects in epithelial cells they point-out suspicions for conducting cervical cancer screens and investigations. The integration of advanced cytologic technique and initiation of appropriate pre cervical cancer treatment and therapy as per atypia detection is therefore hereby proposed.

2. Positively screened outcomes of HR16/18 HPV can be a pragmatic diagnostic tool for identifying pre cervical cancer genesis and its prevention in women. This is substantiated from subsequent data analysis in this study, which established its predicted association with different grades of pre cervical cancer. This signified that it is a true precursor of cervical cancer disease. Laboratory screening of HRHPV infections in Nakuru County which is currently not undertaken should promptly be introduced and possibly integrated to that of other STI such as HIV in the prevention of cervical cancer genesis as well as inhibition of persistence which drives the transformation of infected cervical cells to higher degrees of abnormality. Relevant treatment is recommended for identified STI’s.

3. Favorable application of protocols on the prevention and aversion of cachectic syndrome (body wasting syndrome) such as anthropometry vigilance to gauge observed signs of low weights, low median circumferences of bust and waist is recommended. Additionally the solicitation and incorporation of healthy diet consumption deemed protective against the development of pre cervical cancer signs can be exploited as realistic tools to initiate early pre cervical cancer prevention. Types of consumed dietary products by patients during clinic visits can be sought by health care providers hence they would be in a position to provide nutritional counseling based on responses given by women on the positive outcome

4. Presenting at the reproductive health clinic with acute or chronic ill health of the RT with signs of protracted bleeding and lower abdominal pain are valid suspicions for conducting a cervical cancer screen, including visual cervical colposcopic examination. Conductance of a purposive, exhaustive and intensive clinical history review background to establish type of contraception used, parity and practices of multiplicity of sexual partners by the health care provider can aid in the establishment of both symptomatic and asymptomatic signs as well. Through edifying, sensitization and counseling, valuable pre cervical cancer management and support can alleviate further progression of perceived pre cervical cancer signs on the lower RT. Additionally clinic visits should be made by both couples/sexual partners to discourage discordant sexual practices that lead to sexually transmissible RT complications. Relevant further investigation (colposcopy and cervicography) and treatment is supported on the identification of RT ill health.

5.3.1 Recommendations for future research

The following suggestions are appropriate for future exploration in pre cervical cancer disease research with the aim of refining what was previously known, what has been discovered and what remains to be revealed in Nakuru County and how further this information can be applied in the alleviation of cervical cancer disease burden. Prospectively, multi-disciplinary and multi institutional partnering amongst the scientific community, students, care givers, local leaders and the Nakuru local community would be beneficial from the varied shared ideas in the field of reproductive health for improvement in pre cervical cancer disease management.

1. Cytomorphological profiling would have best been studied under longitudinal research studies which are supported in the cancer prevention handbook (IARC, 2015). Similarly, cervical cancer risk may differ according to the characteristics of the population studied when interpreting results from randomized controlled trials. The distinction and segregation of squamous and glandular atypia in pre cervical cancer smears based on their unique cytomorphological characteristics in Nakuru is recommended. The utility of cytological confirmatory test such as maturation index count and morphometric nuclear-cytoplasmic volume ratio test including hormonal cytochemistry is advocated to limit mimicry and ambiguities of cyto-cellular characteristics. The application of LBC cyto techniques will exclude and eliminate non-diagnostic inclusions hence limit dilemma and microscopic examination pitfalls due to challenges of cellular atypia discrimination during smear inspection (Wildra *et al.*, 1994; Lee *et al.*, 1997; Maeda *et al.*, 1997; Wesola, *et al.*, 2014 and Gupta, *et al.*, 2016).

2. The enrollment of HR HPV vaccinated women subjects in future cervical cancer studies would give clear distinctions of variant types of HRHPV genotypes in future studies. The consideration of a DNA based all-inclusive STI spectrum screen to establish high risk genotypes of viral, fungal, protozoan and bacterial organisms having predilection of pre cancer genesis would increase sensitivity and specificity, moreover research funding facilitation from stake holders will ensure standard findings and results from highly sensitive and specific screening methods are delivered.

3. Highly fragmented questionnaire responses can be limited in the establishment of prediction to pre cervical cancer caused by the risk factors of lifestyle, dietary and reproductive health history and practice attributes, through the use of close ended enquiries. In addition for consumed dietary products analysis, the use and duration of traditional and commercial supplementation use should be included in the questionnaire list to establish their protagonistic effect as should queries on source of vaginal bleeding and duration and types of birth control methods used particularly on their specific dates of commencement and cessation to establish association with pre cervical cancer grades.

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APPENDICES

Appendix I: Study work plan

Table I Work plan captured in Gantt chart representation

MONTH	PROPOSAL DEVELOPMENT	PILOT STUDY	PROPOSAL DEFENSE	ETHICAL APPROVAL	DATA COLLECTION	DATA ANALYSIS	REPORT WRITTING	FINAL DEFENSE	PUBLICATION
JAN-AUG 2012									
SEPT-DEC 2012									
JAN-JUL 2013									
AUG-DEC 2013									
JAN-DEC 2014									
JAN 2015-APR 2016									
JAN 2016-JUN 2017									
JUL-NOV 2017									

Appendix II: Study budget

Table II Study Budget and financial allocation

ITEM	SPECIFICS	COST PER UNIT	TOTAL COST PER 6 MTHS
STATIONERY	PENS (PER BOX)	K. SH. 20	K. SH. 480
	PENCILS (PER BOX)	K. SH. 20	K. SH. 480
	PHOTOCOPY PAPER (PER RIM)	K. SH. 450	K. SH. 3,000
	STAPLES/PINS (PER BOX)	K. SH. 50	K. SH. 1,000
FACILITATION FEE AT DATA COLLECTION SITE	FACILITATION FEE (PAID ONCE)	-	K. SH. 50,000
	INCENTIVES FOR TARGET GROUPS	OPTIONAL	OPTIONAL
COMPUTER SERVICES	TYPE SETTING (PER PAGE)	K. SH. 35	K. SH. 20,000
	PHOTOCOPYING (PER PAGE)	K. SH. 5	K. SH. 10,000
	INTERNET (PER MINUTE)	K. SH. 10	K. SH. 40,000
	FAX & SCANNING (PER PAGE)	K.SH. 40	K. SH. 10,000
LABORATORY EXPENSES	LAB. REAGENTS (PER MONTH)	K. SH. 50,000	K. SH. 300,000

	CONTROL KITS (PER 6 MONTHS)	K. SH. 25,000	K. SH. 150,000
	CALIBRATION AND SERVICE OF LAB. EQUIPMENT (PER MONTH)	K. SH. 25,000	K. SH. 150,000
	GLASSWARE (PER CARTON)	K. SH. 50,000	K. SH. 50,000
STIPEND FOR ASSISTANTS	NURSES/RESEARCH ASSISTANTS (PER 6 months)	K. SH. 20,000	K. SH. 20,000
	DATA ENTRY CLERK (PER 6 months)	K. SH. 10,000	K. SH. 10,000
SUPERVISOR VISITS	CONSULTATION FEE (PER VISIT)	K. SH. 2,000	K. SH. 12,000
DATA ANALYSIS SERVICES	FEE FOR ANALYSIS (PER MONTH)	K. SH. 10,000	K. SH. 60,000
PEER REVIEW MEETINGS	CONSULTATION FEES (PER VISIT)	K. SH. 5,000	K. SH. 30,000
	PUBLISHING FEE (PAID ONCE)	-	K. SH. 60,000
COMMUNICATION EXPENSES	MOBILE CALLS & TEXTS (PER MONTH)	K. SH. 10,000	K. SH. 60,000
	COURIER SERVICES (AS PER MONTH)	K. SH. 1,000	K. SH. 6,000
ETHICAL COMMITTEE FEE	REGISTRATION OF RESEARCH CONCEPT (PAID ONCE)	-	K. SH. 30,000
TOTAL EXPENDITURE			K.SH. 1,072,960

Appendix III: Informed consent form

My name is Esther Muitta I am a PhD student from Kenyatta University. I am conducting a study on the Microbial Occupational and Lifestyle determinants of Cervical Cancer in Nakuru County. The information will be used by the Ministry of Medical Services and Ministry of Public Health and Sanitation to improve access and the quality for screening of cervical cancer in this hospital as well as other regions of Kenya.

Procedures to be followed

Participation in this study will require that I ask you some questions, take some body measurements and also examine you in order to screen you for cervical cancer. Some specimens will be taken from you for further tests. I will record the information from you in a questionnaire.

You have the right to refuse participation in this study. You will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from this clinic or any other organization now or in the future.

Discomforts and risks

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. You may also stop the interview atany time. The interview may take approximately an hour.

The procedures that require to be done in your screening for your cervical health include the pap smear withdrawal from your cervix as well as a blood sample from your arm. These procedures are relatively harmless, but you may experience some uncomfortable dull sensation during the exercise. The pap smear test will involve the opening of your birth canal using speculum in order to access the cervical wall. This procedure may cause you some discomfort of a mild degree but once finalized you will return to normal. The withdrawal of blood from the ante-cubital region of your arm is an invasive procedure involving the use of a needle and syringe. This procedure may cause you discomfort of a mild degree as well, but once complete you will feel perfectly normal. You may request to have the procedures stopped at any time, as this does not compromise on your freedom of choice. A specialized physician and nursing officer on reproductive health matters within the clinic will be readily available for any consultation should you have any queries or require further advice prior to the withdrawal of the specimens.

Benefits

If you participate in this study you will help us learn how to provide effective screening services that can improve the health of women and reduce the risk of cervical cancer. You will also benefit from being screened for cervical cancer and if found to have a problem, you will be referred to a specialist for advice on treatment and the management of your condition. Further support for social and welfare address will be referred to the social worker assigned the clinic.

Confidentiality

The interviews and examinations will be conducted in a private setting within the clinic. Your name will not be recorded on the questionnaire. The questionnaires will

be kept in a locked cabinet for safe keeping at Kenyatta University. Everything will be kept private.

Contact Information

If you have any questions you may contact Dr. Tom Were on 0720326127 or Dr. Anthony Kebira on 0715032643 or the Kenyatta University Ethical Review Committee Secretariat on kuerc@ku.ac.ke

Participant's Statement

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care I will receive from the clinic today or that I will get from any other clinic at any other time.

Name of Participant…… Signature/Thumb print……..…

Date…….....

Investigator's statement

I, the undersigned, have explained to the volunteer in a language she understands the procedures to be followed in the study and the risks and benefits involved.

Name of Interviewer…….

Signature...…….

Date…….....

Appendix IV: Study questionnaire

Instructions: Please give all information required. Please tick as appropriate in the spaces provided and fill in the correct responses where necessary.

Date of interview …….
Name of interviewer ……
Questionnaire number …….

(A) BIOMETRICS

1. STUDY CODE NO:……
DATE:……..
MOBILE NUMBER:……..
2. AGE:
   - 15-44
   - 45-54
   - 55-64
   - > 65
3. PARITY:
   - 0
   - 1
   - 2-4
   - ABOVE 4
   - OTHER (SPECIFY)
4. MARITAL STATUS:
   - SINGLE
   - MARRIED
   - WIDOWED
   - OTHER (SPECIFY)

(B) SOCIO-ECONOMIC CHARACTERISTICS

5. RELIGION:
   - CHRISTIAN
   - MUSLIM
   - TRADITIONAL
   - OTHER (SPECIFY)
6. SOURCE OF INCOME:
   - HOUSE WIFE
   - BUSINESS
   - FORMAL EMPLOYED (GO TO 8)
   - OTHER (SPECIFY)
7. WHAT IS YOUR FORMAL OCCUPATION?
   - ADMINISTRATIVE OFFICE
   - HOSPITAL WORKER
   - INDUSTRIAL FACTORY
   - CHEMICAL PLANT
   - OTHER
8. WHAT IS THE HIGHEST LEVEL OF EDUCATION HAVE YOU ATTAINED?
   - PRIMARY
   - SECONDARY
   - VOCATIONAL/COLLEGE

- UNIVERSITY
- OTHER (SPECIFY)

(C) LIFESTYLE CHOICES:

9. DO YOU SMOKE?
   - YES
   - NO
10. DO YOU CONSUME INTOXICATING DRINKS (ALCOHOL)?
   - YES
   - NO
11. DO YOU EXERCISE?
   - YES
   - NO
12. DO YOU CONSUME ANIMAL (MEAT) PRODUCTS?
   - YES (GO TO 13)
   - NO
13. HOW MANY TIMES DO YOU CONSUME MEAT?
   - EVERYDAY
   - WEEKLY
   - FORTNIGHTLY
   - MONTHLY
   - OTHER (SPECIFY)
14. DO YOU INCOPORATE FRUIT AND VEGETABLE IN YOUR DIET:
   - YES (GO TO 15)
   - NO
15. HOW MANY TIMES DO YOU CONSUME FRUIT AND VEGETABLE?
   - EVERYDAY
   - WEEKLY
   - FORTNIGHTLY
   - MONTHLY
   - OTHER

(C) REPRODUCTIVE HEALTH STATUS:

16. HAVE YOU EVER BEEN SCREENED FOR ANY DISEASE OR GONE FOR A HEALTH TEST IN THE PAST ONE YEAR:
   - YES (GO TO 17)
   - NO
17. INDICATE UNDER WHICH CONDITIONS YOU SOUGHT SOME MEDICAL INTERVENTION:
   - MEDICAL ISSUE
   - SURGICAL ISSUE
   - REPRODUCTIVE ISSUE (GO TO 18)
   - OTHER (SPECIFY)
18. DO YOU CURRENTLY SUFFER FROM ANY URINARY TRACK PAINS OR LOWER ABDOMINAL DISCOMFORTS?
   - YES
   - NO
   - OTHER (SPECIFY)
19. HAVE YOU EVER VISITED A VCT CENTRE?
   - YES (GO TO 20)

- NO
- OTHER (PSECIFY)

20. HOW MANY TIMES DO YOU VISIT THE VCT CENTER?
- EVERY 6 MONTHS
- EVERY YEAR
- EVERY 2 YEARS
- OTHER (SPECIFY)

21. HAVE YOU EVER BEEN DIAGNOSED WITH A SEXUALLY TRANSMITTEDINFECTION (STI) IN THE LAST 6 MONTHS:
- YES (GO TO 22)
- NO

22. WHAT INFECTION WAS IT?
- SYPHILLIS
- GONORRHOEA
- CHLAMYDIA
- HPV
- CANDIDA SPECIES
- HIV
- OTHER (SPECIFY)

23. IN THE LAST 6 MONTHS HAVE YOU HAD MORE THAN ONE SEXUAL PARTNER?
- YES (GO TO 24)
- NO

24. HOW MANY SEXUAL PARTNERS:
- >2
- >4
- >6
- OTHER (SPECIFY)

(D) GENETIC PREDESPOSITION

25. IS THERE ANYONE IN YOUR IMMEDIATE FAMILY SUFFERING FROM CANCER?
- YES (GO TO 26)
- NO

26. WHAT IS THEIR RELATION TO YOU?
- PARENT
- SPOUSE/PARTNER
- SISTER/BROTHER
- AUNTY/UNCLE/COUSIN
- GRANDPARENT
- DISTANT RELATIVE
- OTHER (SPECIFY)

27. WHAT TYPE OF CANCER IS IT?
- CERVICAL CANCER
- BREAST CANCER
- OVARIAN CANCER
- THROAT CANCER
- COLON/STOMACH CANCER

(E) ADDITIONAL INFORMATION

28. HAVE YOU EVER HEARD ABOUT CERVICAL CANCER?
- YES
- NO

29. HAVE YOU EVER HAD A PAP SMEAR TEST?
- YES
- NO

30. WOULD YOU CONSENT TO HAVE A PAP SMEAR TEST TAKEN FROM YOU IF ADVISED ON ITS BENEFITS?
- YES
- NO
- MAYBE

31. ARE YOU ON ANY TREATMENT FOR CERVICAL CANCER MANAGEMENT?
- CRYOTHERAPY
- LEEP
- TAH
- RADIOTHERAPY?CHEMOTHERAPY
- ANTIBIOTICS ANTIFUNGAL ANTIPARASITIC ANALGESIC

32. ANTHROPOMETRIC PARAMETERS:
- WEIGHT
- HEIGHT
- MUAC
- BUST
- WAIST
- BODY TEMP
- BP

COMMENTS FROM THE RESPODENTS:……..
……
……

Appendix V: Map showing Nakuru county patient referral extent

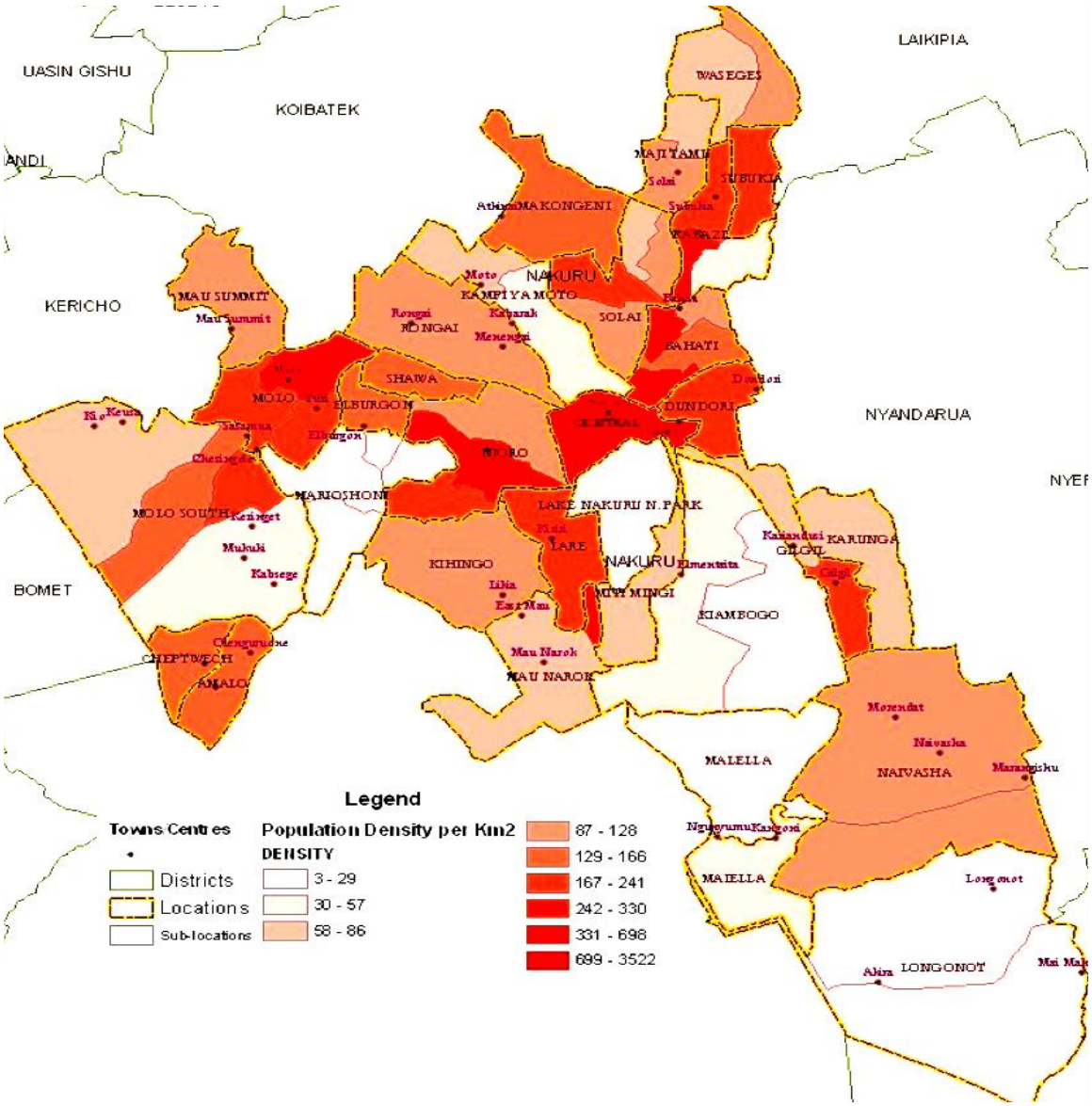


Figure V Map of Nakuru County. Source: National coordinating agency for population and development-Nakuru district strategic plan 2005-2010.

Appendix VI: Incidence and mortality rate of cervical cancer compared to other cancers

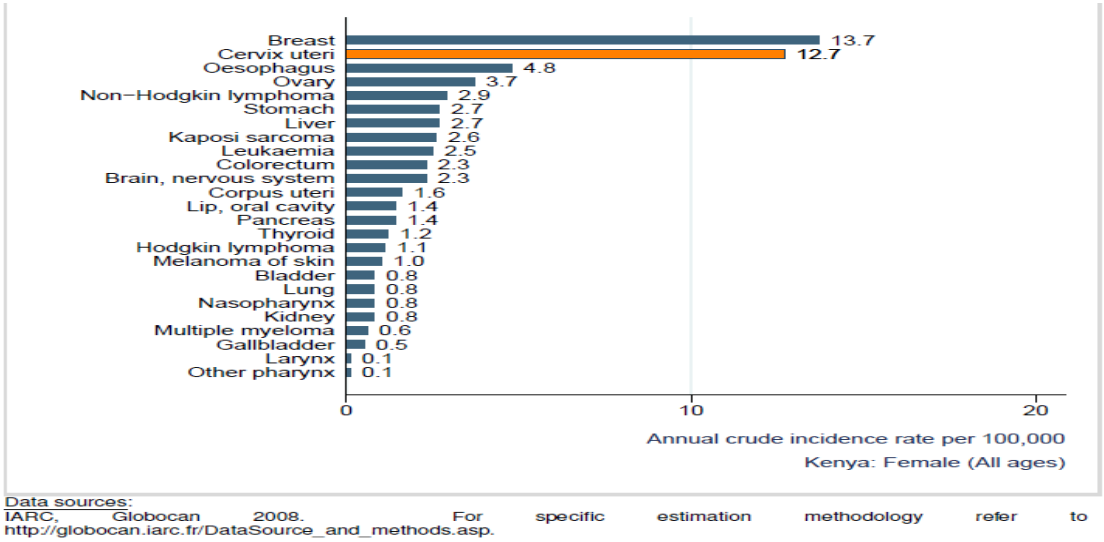


Figure VI.I Incidence of cervical cancer compared to other cancers in women of all ages in Kenya as indicated in GLOBOCAN statistics (Ferlay, 2012).

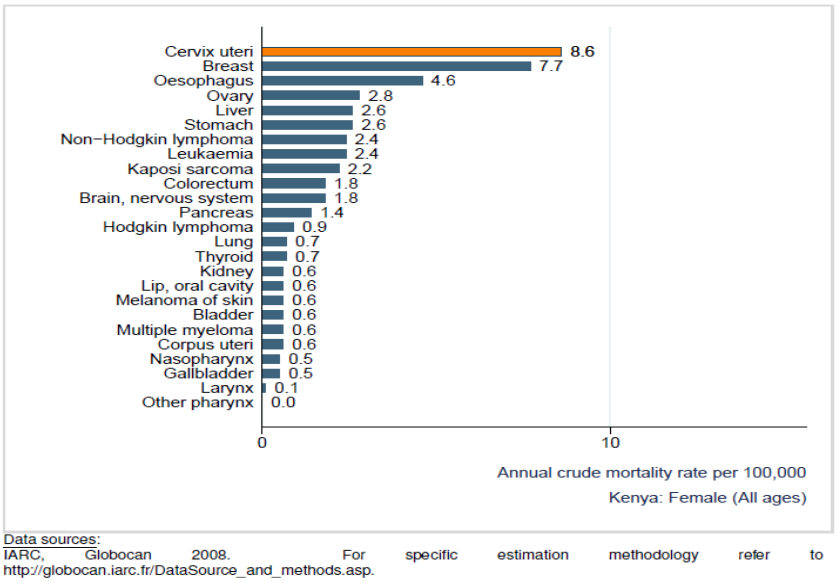


Figure VI.II Cervical cancer mortality compared to other cancers in women of all ages in Kenya. Incidentally, Cervical Cancer tops the list in being the cause of death of women suffering from all general cancer cases (Ferlay *et al*., 2012).

Appendix VII: Cervical cancer Age specific incidences and mortality rates

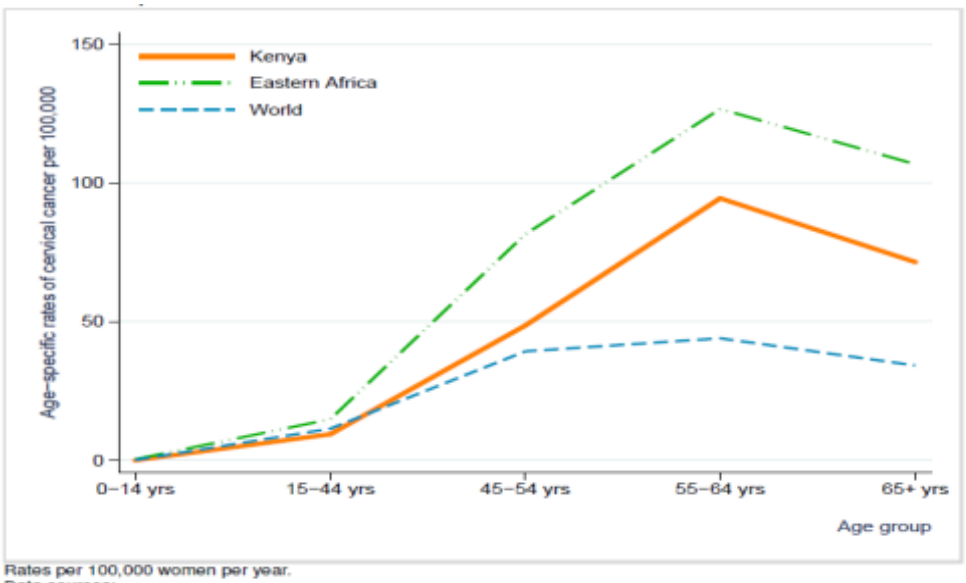


Figure VII.I Age-specific incidence rates of Cervical Cancer in Kenya compared to estimates in Eastern Africa and the World (Ferlay *et al.*, 2012)

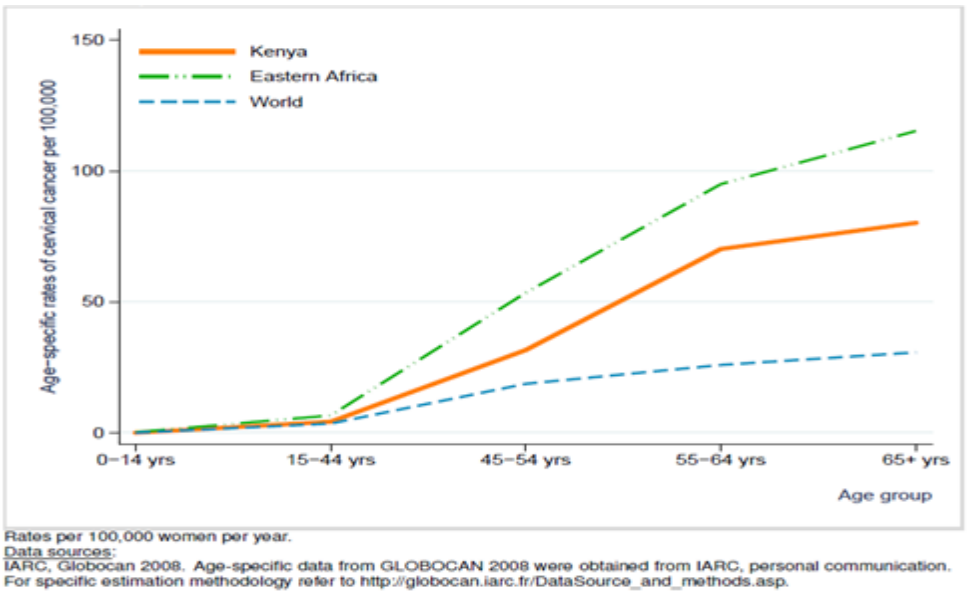


Figure VII.II Age-specific mortality rates of Cervical Cancer in Kenya compared to estimates in Eastern Africa and the World (Ferlay *et al.*, 2012)

Appendix VIII: TBS grading system and pap smear staining protocol

(a) Table VIII Cervical cytology grading summary guideline

Bethesda System Classification (2014)	**Description of Cell Sample**	**Follow-up Recommendation**
Within normal limits	Only normal cells in sample. Nuclear size and shape are within normal physiological limits for the level of maturation of the epithelium. Nuclear membranes are even. Cells are cohesive, architecture is maintained. Mitosis normally only occur at the basal layer so are rarely seen in a cervical smear (repair/regeneration). Healthy and cancer free.	Return in one year for a routine Pap smear
Atypical squamous cells of unknown significance (ASCUS)	Most cells in sample are normal, but some (few) cells have irregular colors, shapes, sizes. Rare hyperchromasia, nuclear membrane irregularity, increased nuclear/ cytoplasmic volume ratio and chromatin is fine and evenly distributed.	Treat any infection; may need colposcopy and/or repeat Pap smears
PRE-CANCER-LSIL overlapping with Cytopathic effects –Koilocytic smear	Mild abnormal number of koilocytic cells in HPF sample with irregular colors, shapes, sizes chromatin is course, clumped and unevenly distributed, Nuclei showing size and shape variation within the same cell population, Nuclear	Treat any infection; may need colposcopy and/or repeat Pap smears.

	membranes are irregular in outline and show indentation and/or thickening due to chromatin margination and clumping, Nuclei are usually hyperchromatic and staining varies from one group to the next, Multinucleation is common, reflecting rapid cell turnover, If present, nucleoli are large, irregular and vary from nucleus to nucleus; nucleoli are more prominent in cancer than dysplasia/dyskaryosis, Loss of cohesiveness (cells coming away from their groups). Nuclei tend to crowd each other in sheets/clusters, Mitotic figures may be seen.	
HIGH GRADE CANCER-HSIL	Increased large number of abnormal cells in HPF with above characteristics and orwhole sample in every examined HPF contains cells that are probably cancerous with the above characteristics.	Perform colposcopy and biopsy; treat if confirmed
AGC/AIS	Whole sample in every examined HPF contains endocervical (glandular cells) that are probably cancerous with the above characteristics.	Perform colposcopy and biopsy; treat if confirmed. Further intervention includes administration of short course oestrogen therapy to initiate proliferation due to mimicry with parabasal cells in atrophic smears (post menopause).

Adapted from Hirschowitz *et al*, 2012 (Histopathology cancer screen reporting NHS).

(b) Pap smear scrapings staining protocol

Epithelial cells are found in mucous membranes as well as sensory organs where they line the underlying tissues egbuccal cavity, cervical tissues, stomach lining etc. Pap smears are thick films or preparations made from the scrapings obtained from the lining of the cervical tissues. They are used for the detection and examination of the metastatic changes of either the nucleus or cytoplasm within epithelial cells that line the cervical tissues, useful for the diagnosis of cervical cancer. The cervix is the opening canal of the uterus and one of the leading cancer diseases are those that involve the cervix in females.

Specimen Collection:

In the collection and preparation of smears for cytological examination, the major objectives are:

1. Specimens should have a sufficient number of cells from the area in question and smears should contain well preserved cells uniformly distributed so that each cell can be individually examined.
2. The staining procedure should clearly define the details of all structures.
3. Cytological preparations are obtained from patient by approved methods and techniques. Scraping, obtained from both the ecto and endo cervix are spread directly on a clean slides.
4. The smear is immediately fixed with a cytological spray fixative or in an alcohol-ether dip.
5. Fixation or preservation is one of the most important steps in the procedure. Drying of the cells prior to fixation will usually result in artifacts such as nuclear distortion and vacuolization.
6. Fixed smears must be air dried before posting to laboratory for staining.

PROCEDURE

1. Dehydrate in absolute ethanol immediately by gentle application on the slide contents.
2. Dip slide(s) gently 5-10 times in 95% ethanol to commence re-hydration.
3. Dip slide(s) gently 5-10 times in 70% ethanol.
4. Dip slide(s) gently 5-10 times in distilled water.
5. Stain 5 minutes in Harris Heamatoxylin.
6. Place smears in distilled water. Rinse in successive changes of distilled water until the water remains colourless.
7. Dip slide(s) gently 5-10 times in 70% ethanol.
8. Dip slide(s) in a 1% solution of HCl in 70% ethanol until the smear shows salmon colour.
9. Rinse slide(s) well in 2 changes of 70% ethanol.
10. Dip slide(s) gently in a 3% solution of ammonium hydroxide in 70% ethanol until the smear takes on a blue colour.
11. Rinse the slide(s) in two changes of 70% ethanol.
12. Dip slide(s) 5-10 times in 95% ethanol.
13. Stain slide(s) in OG-6 for 2 minutes.
14. Rinse slide(s) in two changes of 95% ethanol.
15. Stain slide(s) in EA (eosin azure)-50 for 3-6 minutes.
16. Rinse slide(s) well in two changes of 100% methanol.
17. Rinse slide(s) in one part absolute methanol one part xylene.
18. Clear smear in xylene.
19. Mount in DPX mountant (not necessary) and allow to dry before microscopy.
20. Apply the above TBS scoring system to grade the smear.

Results:

Normal epithelial cells: Nuclei are stained blue while cytoplasm displays varying shades of clear or transparent shades, pink (eosinophilia), orange, yellow and blue-green (cyanophilia).

Limitations: Proper specimen collection and fixation of cells is essential for interpretation.

Fixed Pap smear ready for staining

Smear staining station in Nakuru cytology laboratory

Appendix IX: STI microbe rapid test insert

(Reproduced from provided kit inserts)

1. Diagnosis-related group (DRG) Gonorrhoea antigen rapid test kit

DRG® Gonorrhea Rapid (RAP-4867)
Revised 28 Jan. 2011 rm (Vers. 3.1) USA: RUO
DRG International Inc., USA
Please use only the valid version of the package insert provided with the kit.
This kit is intended for Research Use Only.
Not for use in diagnostic procedures.

INTENDED USE

The Gonorrheal Rapid Test is a rapid, direct binding test for the visual detection of gonorrhoea antigen, in the secretoryspecimen from urogenital system.
Test results are unambiguous and can be read in 10-20 minutes. The test kit is easy to operate and does not involvewashing or comparison to standards.

MATERIALS PROVIDED

o Gonorrhoea **test cassette** in foil pouch (20 per kit box)
o**Flocked swabs** (20 per kit box)
o**Test tubes** and **dropper tips** (20 per kit box)
o**Workstation**(1 per kit box)
o**Reagent 1** (1 per kit box)
o**Reagent 2** (1 per kit box)
o Package insert (1 per kit box)

MATERIALS NEEDED BUT NOT PROVIDED

o Timer

PRINCIPLE OF TEST

The Gonorrhoe Rapid Test is based on the principle of double sandwich immunoassay for the detection of gonorrhea antigen in the secretory specimen. Monoclonal and polyclonal antibodies are employed to identify gonorrhea specifically. Both sensitivity and specificity of the test are higher than those of the present methods, which often involve long hours of culturing the collected specimen. Test results are not affected by any medication that is being taken. Results are read visually without any instrumentation. This test is ideal for screening specimen samples containing at least 1 x105 bacteria per ml.
The assay is conducted by adding diluted swabbed discharge specimen to the test device and observing the formation of coloured lines. The specimen migrates via capillary action along the membrane to react with the coloured conjugate.
Positive specimens react with the specific coloured antibody conjugates and form a coloured line at the test line region of the membrane. Absence of this coloured line suggests a negative result. To serve as a procedural control, a coloured line will always appear at the control line region if the test has been performed properly.

REAGENTS

Coated Antibodies:

Control region: Goat anti-mouse (IgG) polyclonal antibody

Test region: Mouse monoclonal anti-gonococcus antibody A

Labeled Antibodies:

Colloidal gold conjugate of mouse monoclonal anti-gonococcus antibody B

WARNINGS & PRECAUTION

In research use only, For professional use only.

Do not use test kit beyond the expiry date.

The test device should not be reused.

Specimens may contain infectious agents and should be handled as though capable of transmitting disease.

Wear disposable gloves throughout the specimen collection and assay procedures.

SPECIMEN COLLECTION

1. Use a swab to collect specimen in the following suggested method:

a. ***Male sample specimens:***

Swab discharge from the opening of the urinary tract. If no discharge is present, insert the swab 2-3 cm into the urinary tract, gently move a few turns and retrieve the swab.

b. ***Female sample specimens:***

Swab discharge from the vaginal opening, then insert swab into vagina for half a minute and

2. Place the swab into the test tube and add 6 drops (300 µl) of reagent 1 onto the swab.

Place the flocked swab in the test tube and rotate the flocked swab between two fingers for 10-15 seconds.

Discard the swab according to federal state and local regulations.

Then add 2 drops (100 µl) of reagent 2 into the test tube and mix well. Fit the dropper tip on top of the extraction tube.

Specimen collected in the solution should be stored at 4 °C to 8 ºC and tested within 24 hours.

DIRECTIONS FOR USE

Allow the test and the specimen to equilibrate to room temperature (15 °C to 30 °C) prior to testing

1. To begin testing, open the sealed pouch by tearing along the notch. Remove the test cassette from the pouch and use it as soon as possible.

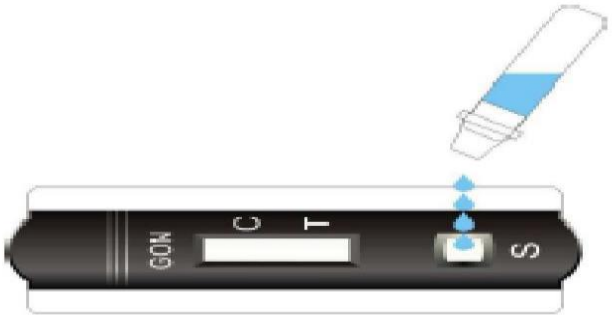
2. Dispense 4 drops (approx. 0.2 ml) into the sample well of the cassette (see figure).

3. Wait for the coloured lines to appear. Depending on the concentration of bacteria in the test specimen, positive

results may be observed in as little as few seconds.

To confirm negative results, the test must be read again in 20 minutes.

Do not read results after more than 20 minutes.



Note: A low bacterial concentration might result in a weak line appearing in the test region (T) after an extended periodoftime; therefore, do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

NEGATIVE: Only one colouredline appears on the control region. No apparent line on the test region.

POSITIVE: Distinct colour lines appear on the control and test regions. The test result can be read as soon as the
distinctcoloured lines appear in the test region.

INVALID: No line appears in the control zone "C", the test should be voided since an improper test procedure may have been performed or deterioration of reagents may have occurred. This is due to the internal control built in which adistinct control region (C) line should always appear. Repeat the test using a new device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

STORAGE AND STABILITY

The test kit can be stored at temperatures between 2 °C to 30 °C in the sealed pouch to the date of expiration.

The test kit must be kept away from direct sunlight, moisture and heat.

The expiration dating was established under these storage conditions.

QUALITY CONTROL

A coloured line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen
volume and correct procedural technique.

A clear background is an internal negative background control. If the test is working properly, the background in theresult area should be white to light pink and not interfere with the ability to read the test result.

External controls may also be used to assure that the reagents are working properly and that the assay procedure isfollowed correctly.

It is recommended that a control be tested at regular intervals as good laboratory testing process. Users should follow theappropriate federal, state, and local guidelines concerning the running of external quality controls.

References / Literature

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A positive rapid Gonorrhoea test

2. Healgen Orient Gene Syphilis antibody rapid detection

Zhejiang Orient Gene Biotech Co., Ltd.
CECertificate(CE0197)
ISO 13485 Certificate
Syphilis rapid test kits;TP test device;medical diagnostic test kits
Port: Shanghai
Minimum Order Quantity: 10,000 Piece/Pieces
Supply Ability: 500,000 Piece/Pieces per Week
Storage 2^0-30^0 C
Packaging Details: 25 devices/Box or as request
Specifications
Syphilis One Step Rapid Test
CE Certificate
ISO 13485 Certificate

Introduction:

Syphilis is a disease caused by Spirochete bacterium called Treponema pallidum (TP).

If untreated the organism moves throughout the body in fluids and can cause damage to many visceral organs making syphilis a life threatening disease if not fully treated.

The serological response to syphilis involves production of antibodies to a wide range of antigens including non-specific antibodies and specific anti-Tp antibodies.

The first detectable response to infection is the production of specific anti-treponemalIgM which can be detected within 4 to 7 days after chancre appearance post the second week of infection.

Anti- treponemal IgG appears at about four weeks later. By the time Syphilis disease symptoms develop, most patients have both detectable IgG and IgM.

Principle:

The TP One Step Rapid Test is a lateral flow chromatographic immunoassay based on the principle ofdouble antigen– sandwich technique.

Procedure:

In this test procedure, recombinant Syphilis antigen is immobilized in the test line regi on of the strip.
Using provided disposable dropper aspirate serum and place two drops on the well of the strip.

Allow one minute for the migration of seum on the membrane surface so that it antibody present in the test will react with antigen bound on the membrane.
After specimen is added to the specimen test area of the device, it reacts with the Syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized syphilis antigens on the membrane of the device.

If the specimen contains Tp antibodies, a coloured line will appear in the test line indicating a positive result.

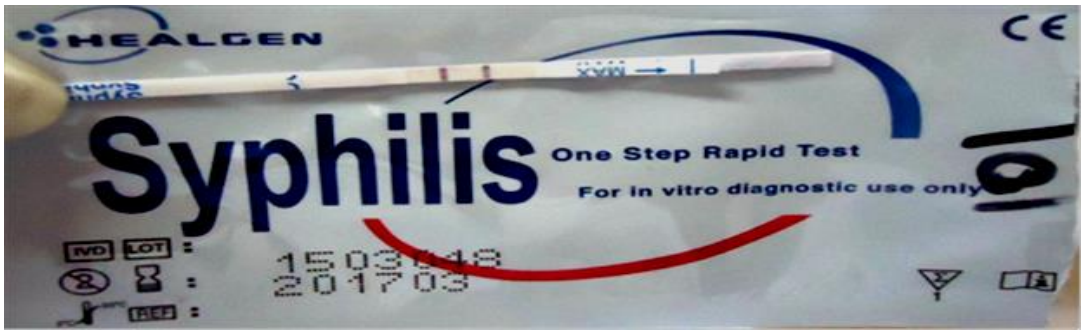
The double antigen test can detect both IgM and IgG in specimens. If the specimen do es not contain Tp antibodies, a coloured line will not appear in this region indicating a negative result.

To serve as a procedural control, a coloured line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

Materials Supplied:

Each test contains :
1. One cassette device
2. One pipette dropper
3. One desiccant
Each kit contains:
25 test devices
One instruction

A positive Syphilis rapid test



3. Kehua Bio Engineering (KHB) HIV1/2 antibody rapid cassette

DIAGNOSTIC KIT FOR HIV(1+2)ANTIBODY (COLLOIDAL GOLD)

Intended Use

The rapid test for HIV (1+2) from KHB is an in-vitro, visually read test for the qualitative determination of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. The test is intended to be used as an aid to detect antibodies to HIV (1+2) from infected individuals.

Summary and Explanation of the Test

AIDS (Acquired Immunodeficiency Syndrome) is an immunosuppressive disease characterized by depletion of the helper T-cell, which leaves the infected individual susceptible to opportunistic infections and some malignancies. The viruses that have been detected to cause AIDS are HIV-1, first isolated in 1983 and HIV-2, a distinctive but related type isolated in 1985. The presence of the AIDS virus elicits the production of the antibodies specific to either HIV-1 or HIV-2.

Principle of the Procedure

The rapid test of HIV (1+2) from KHB adopts the solid phase colloidal gold immunochromatographic technology for the qualitative detection of antibodies to HIV-1/HIV-2. The gold-gp160 conjugate and the gold-gp36 conjugate are coated to the conjugate pad in advance. The Test Line (HIV type 1+II antigens) and the Control Line (monoclonal antibody against gp160) are pre-coated on the surface of the NC membrane. When the sample that added to the sample pad migrates through the conjugate pad, it reconstitutes and mixes with the colloidal gold-antigen conjugates. The mixture continues to migrate through the NC membrane to the pre-coated antigens or antibody that present on the membrane. A reddish-purple Test Line will be visible in the strip if there are enough antibodies to HIV-1/HIV-2 in the sample. If antibodies to HIV-1/HIV-2 are absent, or are present at very low level, then no color will appear in the Test Line. The Control Line of reddish-purple is for quality control only and thus does not affect the test result.

Materials provided

- HIV (1+2) antibody test cassettes — 1x 50 cassettes
- Instruction sheet — 1 piece
- Sample diluent — 1 bottle (4 ml)

Warnings and Precautions

The precautions are included, but not limited to the following:

- Wear gloves during the entire testing process.
- Do not use a pipette by mouth. Do not eat or smoke while handling specimens.
- Clean and disinfect all spills of specimens or reagents using a suitable disinfectant.
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials as if they were infectious wastes.

The test is sensitive to humidity and the temperature:

- Run the test immediately after removing the test cassette from the foil pouch.
- Bring all reagents to room temperature (~20℃) before testing. The tests should be performed at the room temperature.
- Do not use the test cassette if the pouch is damaged or the seal is broken. Contact to the supplier when this is happened.
- The test is for *In Vitro* Diagnostic Use only.

Storage

The test cassettes and the sample diluent must be stored at 4~30℃ until expiration date.

Kit components are stable until expiration date when handled and stored as directed. Do not use kit components beyond the expiration date.

Sample Collection

Human serum, plasma, and whole blood sample shouldbe collected by venipuncture (or by fingerstick for whole blood) and the hemolysis of the sample should be avoided.

Sample Storage

- Serum and plasma samples should be stored at 2~8℃ if the test is to be run within one week after collection. Otherwise, the samples should be frozen (-20℃ or colder).
- Whole blood collected by venipuncture should be stored at 2~8℃ if the test is to be run within one week after collection. Do not freeze whole blood samples.
- Whole Blood Collection by Fingerstick should be tested immediately.

Test Procedure

1. Remove a test cassettes from a foil pouch, and place it on a flat surface
2. Use the sample of either serum/plasma or whole blood: Add 40 μ l of sample (precision pipette) to the sample area first (see figure 1), and then slowly instill 1 drop (~40 μ l) of sample diluent to the same area (see figure2). Direct contact of the diluent bottle with the sample area should be avoided.
3. The results can be seen within 2-3 minutes with strong positive samples. Please do not interpret the test result after 30 minutes. Do record the result on cassettes.

Results

- Positive Result (two bands)

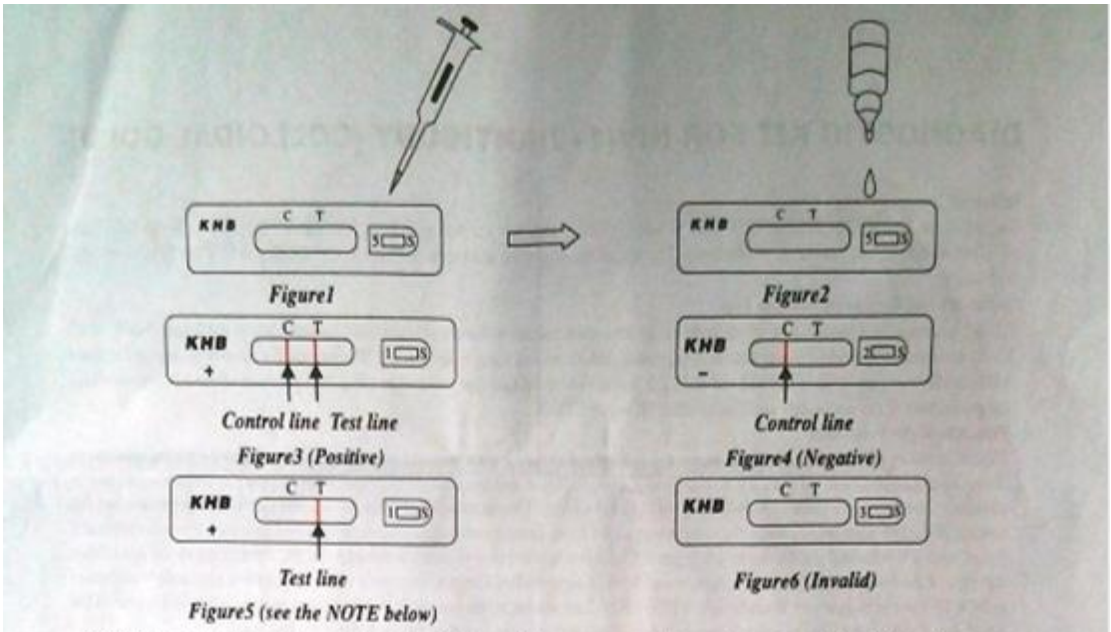
A reddish- purple band appears both on the control line (C-line) and the test line(T-line) of the cassette (see figure 3).

- Negative Result (one band)

A reddish- purple band appears only at the control line (C-line) of the cassette (see figure 4).

- Invalid Result (no band)

No reddish-purple band appears neither at the control line nor the test line of the cassette (see figure 6).



NOTE: The test result is valid even if the band of the Control line appears much lighter or darker than the band of Test line. The control band may not appear when very strong positive samples are tested as shown in Figure 5 (only one band appears in the result window at the test line). If so, it is strongly recommended to make a series of sample dilution with HIV negative serum/plasma, and repeat the test. If at least one of the dilutions shows positive result (Figure 3), it further confirms that the sample is strong positive. If the result remains the same (Figure 5) or turns to be invalid (Figure 6), it indicates that the test may have deteriorated. It is strongly recommended that the sample be retested or contact your KHB supplier.

Limitations of the Procedure

- The HIV-1/HIV-2 test is designed to detect antibodies to HIV-1 and HIV-2 in human serum, plasma, and whole blood. Other body fluids or pooled samples may not give accurate results.
- No test absolutely guarantee that a sample does not contain low levels of antibodies to HIV-1 and HIV-2 which may occur at a very early stage of infection. Therefore a negative result should not exclude the possibility of exposure to or infection by HIV-1 or HIV-2 viruses.
- Positive samples should be retested, and additional testing using other clinical methods is recommended.

Performance Characteristics

Specificity: Compared with HIV (1+2) antibodies (ELISA)

Sample	KEHUA HIV(1+2)antibodies Rapid test cassette (colloidal gold)		HIV(1+2) antibodies ELISA	
	+	-	+	-
Blood donor	0	3540	0	3540
HCV positive	0	100	0	100
Healthy	0	85	0	85

(Samples are collected from Shanghai blood center and Beijing 302 hospital)

Sensitivity: Compared with HIV (1+2) antibodies (ELISA)

Sample	KEHUA HIV(1+2)antibodies Rapid test cassette (colloidal gold)		HIV(1+2) antibodies ELISA	
	+	-	+	-
HIV patients	578	6	583	1

(Samples are collected from national HIV Lab of CDC in China and the HIV Lab of CDC in Shanghai)

Comparison to Abbott Determine HIV-1/2

Sample	KEHUA HIV(1+2)antibodies Rapid test cassette (colloidal gold)		Abbott Dtetrmine HIV-1/2 Rapid test card	
	+	-	+	-
Healthy	0	20	0	20
HIV patients	50	3	50	3

(Samples are collected from HIV Lab of CDC in Shanghai)

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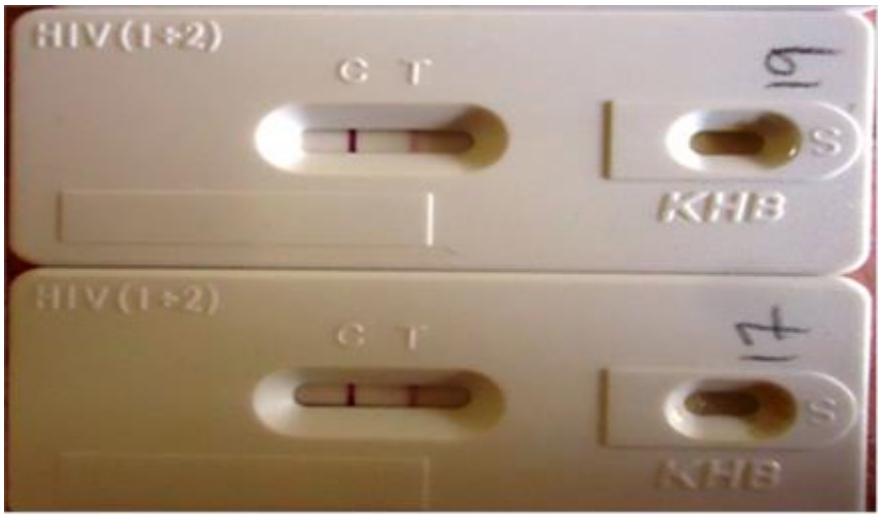
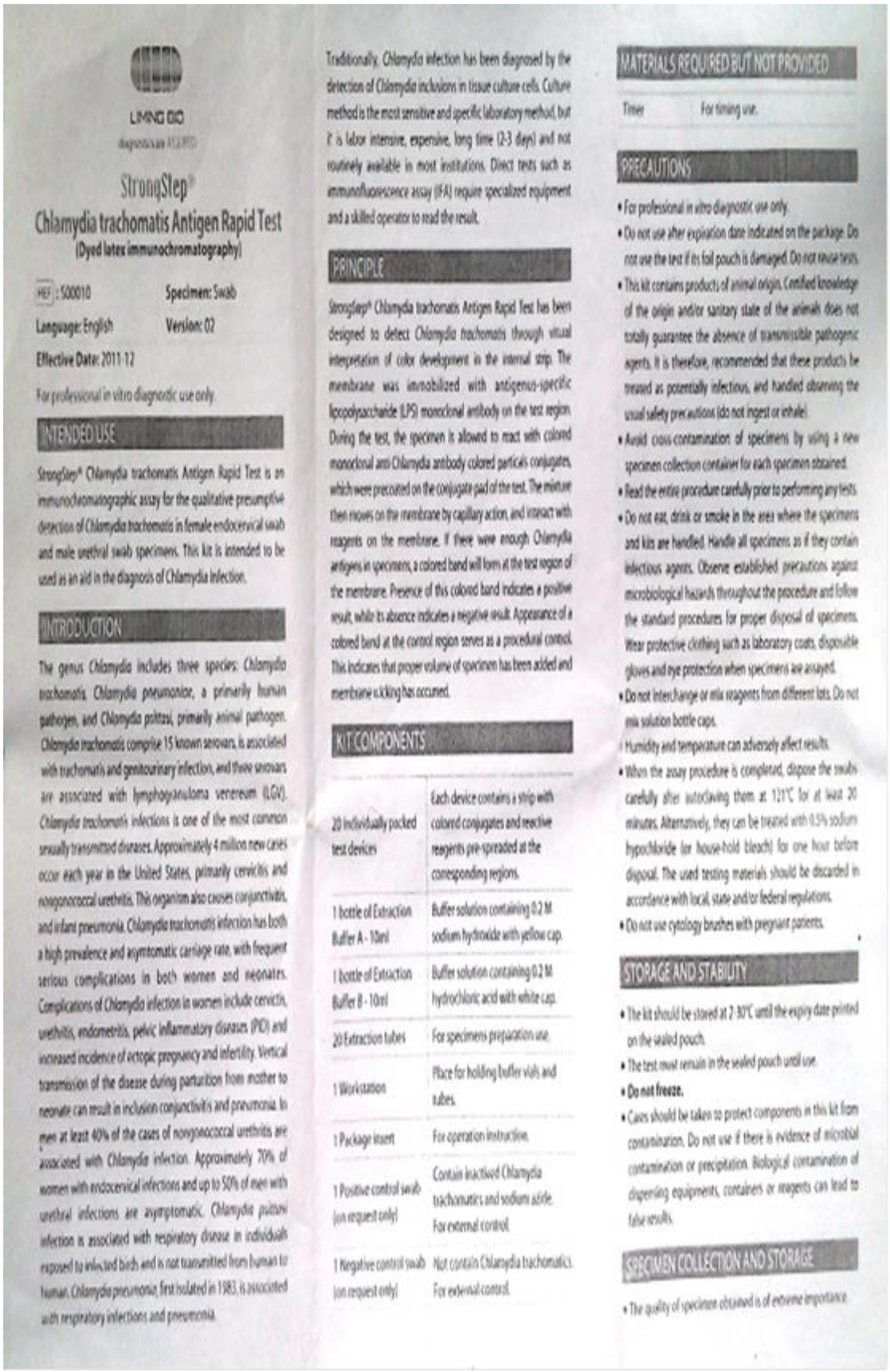
KHB SHANGHAI KEHUA BIO-ENGINEERING CO., LTD
TEL: +86-21-54500744, 64850088 (Sales Dept.);+86-21-64950625(Technical Support)
FAX: +86-21-64854051;WEB: http://www.skhb.com Rev: w02-07-10-38

Positive HIV1/2 rapid tests



4. Liming Bio Chlamydia trachomatis antigen rapid test



LIMING BIO

StrongStep®

Chlamydia trachomatis Antigen Rapid Test

(Dyed latex immunochromatography)

REF : S00010 Specimen: Swab

Language: English Version: 02

Effective Date: 2011-12

For professional in vitro diagnostic use only.

INTENDED USE

StrongStep® Chlamydia trachomatis Antigen Rapid Test is an immunochromatographic assay for the qualitative presumptive detection of Chlamydia trachomatis in female endocervical swab and male urethral swab specimens. This kit is intended to be used as an aid in the diagnosis of Chlamydia infection.

INTRODUCTION

The genus Chlamydia includes three species: Chlamydia trachomatis, Chlamydia pneumoniae, a primarily human pathogen, and Chlamydia psittaci, primarily animal pathogen. Chlamydia trachomatis comprise 15 known serovars, is associated with trachomatis and genitourinary infection, and three serovars are associated with lymphogranuloma venereum (LGV). Chlamydia trachomatis infections is one of the most common sexually transmitted diseases. Approximately 4 million new cases occur each year in the United States, primarily cervicitis and nongonococcal urethritis. This organism also causes conjunctivitis, and infant pneumonia. Chlamydia trachomatis infection has both a high prevalence and asymtomatic carriage rate, with frequent serious complications in both women and neonates. Complications of Chlamydia infection in women include cervicitis, urethritis, endometritis, pelvic inflammatory diseases (PID) and increased incidence of ectopic pregnancy and infertility. Vertical transmission of the disease during parturition from mother to neonate can result in inclusion conjunctivitis and pneumonia. In men at least 40% of the cases of nongonococcal urethritis are associated with Chlamydia infection. Approximately 70% of women with endocervical infections and up to 50% of men with urethral infections are asymptomatic. Chlamydia psittaci infection is associated with respiratory disease in individuals exposed to infected birds and is not transmitted from human to human. Chlamydia pneumoniae, first isolated in 1983, is associated with respiratory infections and pneumonia.

Traditionally, Chlamydia infection has been diagnosed by the detection of Chlamydia inclusions in tissue culture cells. Culture method is the most sensitive and specific laboratory method, but it is labor intensive, expensive, long time (2-3 days) and not routinely available in most institutions. Direct tests such as immunofluorescence assay (IFA) require specialized equipment and a skilled operator to read the result.

PRINCIPLE

StrongStep® Chlamydia trachomatis Antigen Rapid Test has been designed to detect Chlamydia trachomatis through visual interpretation of color development in the internal strip. The membrane was immobilized with antigenus-specific lipopolysaccharide (LPS) monoclonal antibody on the test region. During the test, the specimen is allowed to react with colored monoclonal anti-Chlamydia antibody colored particals conjugates, which were precoated on the conjugate pad of the test. The mixture then moves on the membrane by capillary action, and interact with reagents on the membrane. If there were enough Chlamydia antigens in specimens, a colored band will form at the test region of the membrane. Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

KIT COMPONENTS

20 individually packed test devices	Each device contains a strip with colored conjugates and reactive reagents pre-spreaded at the corresponding regions.
1 bottle of Extraction Buffer A - 10ml	Buffer solution containing 0.2 M sodium hydroxide with yellow cap.
1 bottle of Extraction Buffer B - 10ml	Buffer solution containing 0.2 M hydrochloric acid with white cap.
20 Extraction tubes	For specimens preparation use.
1 Workstation	Place for holding buffer vials and tubes.
1 Package insert	For operation instruction.
1 Positive control swab (on request only)	Contain inactived Chlamydia trachomatis and sodium azide. For external control.
1 Negative control swab (on request only)	Not contain Chlamydia trachomatis. For external control.

MATERIALS REQUIRED BUT NOT PROVIDED

Timer	For timing use.

PRECAUTIONS

- For professional in vitro diagnostic use only.
- Do not use after expiration date indicated on the package. Do not use the test if its foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to performing any tests.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Do not interchange or mix reagents from different lots. Do not mix solution bottle caps.
- Humidity and temperature can adversely affect results.
- When the assay procedure is completed, dispose the swabs carefully after autoclaving them at 121°C for at least 20 minutes. Alternatively, they can be treated with 0.5% sodium hypochloride (or house-hold bleach) for one hour before disposal. The used testing materials should be discarded in accordance with local, state and/or federal regulations.
- Do not use cytology brushes with pregnant patients.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- The quality of specimen obtained is of extreme importance

Detection of Chlamydia requires a rigorous and thorough collection technique which provides cellular material rather than just body fluids.

For endocervical specimens:

- Use only Dacron or Rayon tipped sterile swabs with plastic shafts. It is recommened to use the swab supplied by the kits manufacturer(The swab are not contained in this kit, for the ordering information, please contact the manufacturer or local distributor, the catalog numbers are 207000-female swab, 208000-male swab). Swabs from other suppliers have not been validated. Swabs with cotton tips or wooden shafts are not recommended.
- Before specimen collection, remove excess mucus from the endocervical area with a separate swab or cotton ball and discard. The swab should be inserted into the endocervical canal, past the squamocolumnar junction, until most of the tip is no longer visible. This will permit acquisition of columnar or cuboidal epithelial cells which are the main reservoir of Chlamydia organismn. Firmly rotate the swab for 15 - 20 seconds without contamination with exocervical or vaginal cells.
- Put the swab into the extraction tube, if the test may be run immediately.

For Urethral specimens:

- Standard wire-shafted fiber-tipped swabs should be used for urethral specimen collection. Instruct the patients not to urinate at least two hours prior to specimen collection.
- Insert the swab into the urea about 2-4 cm, rotate for 3-5 seconds and withdraw it, and place it into the extraction tube, if the swab may be tested immediately.
- Do not place the swab in any transport device containing medium since transport medium interferes with the assay and viability of the organisms is not required for the assay. If immediate testing is not possible, the patient samples should be placed in a dry transport tube for storage or transport. The swabs may be stored for 24 hours at room temperature (15-30°C) or 1 week at 4°C or no more than 6 month at -20°C. All specimens should be allowed to reach room temperature of 15-30°C before testing.
- Do not use 0.9% sodium chloride to treat swabs before collecting specimens.

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. Prepare Endocervical or Urethral swab specimens:

- Place a clean extraction tube in the designated area of the workstation.

Add 8 drops of extraction buffer A into the extraction tube.

- Immerse the patients swab into the extraction tube and extract 2 minutes at room temperature. During extraction, use a circular motion to roll the swab against the side of the extraction tube so that the liquid is expressed from the swab and can reabsorb.
- At the end of the extraction time, add 8 drops of extraction buffer B into the tube and extract for another 1 minute in the same way. Then squeeze the swab firmly against the tube to expel as much liquid as possible from the swab. Discard the swab following guidelines for handling infectious agents.
- The specimens extracted can retain at room temperature for 60 minutes without affecting the result of the Chlamydia test.

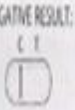
2. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within one hour.

3. Add 3 drops (approximately 100 µl) of extracted sample from the extraction tube to the sample well on the test cassette. **Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.**

As the test begins to work, you will see color move across the membrane.

4. Wait for the colored band(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

POSITIVE RESULT: 	Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).
NEGATIVE RESULT: 	Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).
INVALID RESULT: 	Control band fails to appear. Results from any test which has not produced a control band in the specified reading time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of the color in test region (T) may vary depending on the concentration of aimed substances present in the specimen. But the substances level can not be determined by this qualitative test.
2. Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered as an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.
- External procedural controls may provided on request only) in the kits to ensure that the test are functioning properly. Also, the Controls may be used to demonstrate proper performance by the test operator. To perform a positive or negative control test, complete the steps in the Test Procedure section treating the control swab in the same manner as a specimen swab.

LIMITATIONS OF THE TEST

1. *StrongStar*® Chlamydia trachomatis Antigen Rapid Test is for professional in vitro diagnostic use, and should be used for the qualitative detection of *Chlamydia trachomatis* only. There is no meaning attributed to line color intensity or width.
2. The *Chlamydia* Test does not specifically differentiate *C. trachomatis, C. pneumonia* or *C. psittaci*.
3. Detection of Chlamydia is dependent on the number of organisms present in the specimen. This may be affected by specimen collection methods and patient factors such as age, history of STD, presence of symptoms, etc. The minimum detection level of this test may vary according to serovar.
4. Women with vaginal discharge should be evaluated for risk factors of cervicitis and pelvic inflammatory disease caused by other organisms including Neisseria gonorrhoeae, Candida albicans, Trichomonas vaginalis or Bacterial vaginosis (These can also be diagnosed by Uniengbio's other products: S00020 Neisseria gonorrhoeae antigen rapid test; S00050 Neisseria gonorrhoeae/Chlamydia trachomatis antigen combo rapid test; S00030 Candida albicans antigen rapid test; S00040 Trichomonas vaginalis Antigen Rapid test; S00060 Candida albicans /Trichomonas vaginalis antigen combo rapid test; S00080 Bacterial vaginosis rapid test).
5. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings

have been evaluated.

6. Excessive blood (>50 µL in case of female swabs and >20 µL in case of male swabs) may cause false positive results. Endocervical samples from female patients should not be collected during menstrual period.

PERFORMANCE CHARACTERISTICS

Table: StrongStep® Chlamydia trachomatis Antigen Rapid Test vs. Another Branded Chlamydia Test Plus PCR*

Female Endocervical Specimens

Relative Sensitivity: 94.12% (86.85%-98.00%)*			Another Branded Chlamydia Test Plus PCR (Expanded gold standard)		
Relative Specificity: 99.13% (97.85%-99.77%)*			+	-	Total
Overall Agreement: 98.39% (96.98%-99.26%)*	StrongStep® Chlamydia Test	+	80	4	84
*95% Confidence Interval		-	5	469	474
			85	473	558

Male Urethral Specimens

Relative Sensitivity: 91.59% (88.62%-97.59%)*			Another Branded Chlamydia Test Plus PCR (Expanded gold standard)		
Relative Specificity: 99.8% (99.12%-99.99%)*			+	-	Total
Overall Agreement: 99.08% (97.54%-99.58%)*	StrongStep® Chlamydia Test	+	105	0	105
*95% Confidence Interval		-	6	417	423
			111	417	528

*: This clinical study is performed using a famous branded Chlamydia Antigen Rapid Test as the comparison test, of which the discrepant results were confirmed by Real-time PCR.

The antibody used in the Chlamydia test has been shown to detect all 15 Chlamydia serovars. In addition, Chlamydia psittaci and Chlamydia pneumonie strains have been tested with the test and gave a positive result. Cross reactivity with other organisms has been studied using suspensions of 10^7 CFU/ml. The following organisms were not detected using the test:

Acinetobacter calcoaceticus	*Proteus vulgaris*
Salmonella typhi	*Acinetobacter spp.*
Staphylococcus aureus	*Candida albicans*
Neisseria catarrhalis	*Neisseria gonorrhoeae*
Neisseria meningitidis	*Neisseria lactamica*
Escherichia coli	*Gardnerella vaginalis*
Streptococcus faecalis	*Streptococcus faecium*
Pseudomonas aeruginosa	*Trichomonas vaginalis*
Ureaplasma Ureolyticum	*Mycoplasma hominis*

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GLOSSARY OF SYMBOLS

REF	Catalog number
	Temperature limitation
	Consult instructions for use
LOT	Batch code
IVD	In vitro diagnostic medical device
	Use by
	Manufacturer
	Contains sufficient for <n> tests
	Do not reuse
EC REP	Authorized representative in the European Community
CE	CE marked according to IVD Medical Devices Directive 98/79/EC

Liming Bio-Products Co., Ltd.

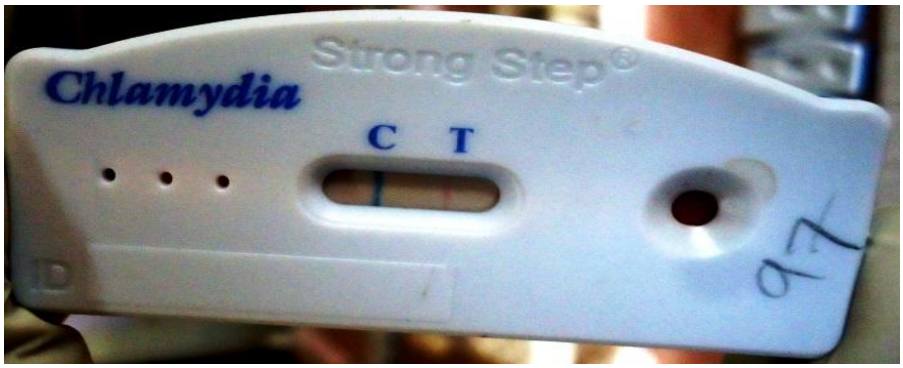
Add: No. 12, Huayuan Road,Nanjing, Jiangsu, 210042 P.R. China
Tel: (0086)25 8528 8566 **Fax:** (0086)25 8547 6307
E-mail: sales@limingbio.com
Website: www.limingbio.com
www.stddiagnostics.com
www.stddiagnostics.com

EC REP

WellKang Ltd. (www.CE-marking.net)

Add: 29 Harley St., London W1G 9QR,UK
Tel: +44(0)7939 4946
Fax: +44(0)1603 38674

A positive Chlamydia rapid cassette test



5. Liming Bio HPV16/18 antigen rapid test

LIMING BIO

HPV 16/18 Antigen Rapid Test Device

REF 500140	Specimen: Swab
Language: English	Version: 02
Effective Date: 2012-03	

For professional in vitro diagnostic use only.

INTENDED USE

The StrongStep® HPV 16/18 Antigen Rapid Test Device is a rapid visual immunoassay for the qualitative presumptive detection of *HPV 16/18 E6&E7* oncoproteins in female cervical swab specimens. This kit is intended to be used as an aid in the diagnosis of Cervical Pre-cancer and Cancer.

INTRODUCTION

In developing countries, cervical cancer is a leading cause of cancer related death of women, due to the lack of implementation of screening tests for cervical pre-cancer and cancer. A screening test for low resource settings should be simple, rapid, and cost effective. Ideally, such a test would be informative regarding HPV oncogenic activity

Expression of both HPV E6 and E7 oncoproteins is essential for cervical cell transformation to occur. Some research results demonstrated a correlation of E6 &E7 oncoprotein positivity with both severity of cervical histopathology and risk for progression. Hence, E6&E7 oncoprotein promises to be an appropriate biomarker of HPV-mediated oncogenic activity.

PRINCIPLE

The StrongStep® HPV 16/18 Antigen Rapid Test Device has been designed to detect *HPV 16/18 E6&E7* Oncoproteins through visual interpretation of color development in the internal strip. The membrane was immobilized with monoclonal anti-HPV 16/18 E6&E7 antibodies on the test region. During the test, the specimen is allowed to react with colored monoclonal anti-HPV 16/18 E6&E7 antibodies colored particals conjugates, which were precoated on the sample pad of the test. The mixture then moves on the membrane by capillary action, and interact with reagents on the membrane. If there were enough HPV 16/18 E6&E7 oncoproteins in specimens, a colored band will form at the test region of the membrane. Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

KIT COMPONENTS

20 Individually packed test devices	Each device contains a strip with colored conjugates and reactive reagents pre-spreaded at the corresponding regions.
1 bottle of Extraction Buffer A - 10ml	Buffer solution containing 0.2 M sodium hydroxide with yellow cap.
1 bottle of Extraction Buffer B - 10ml	Buffer solution containing 0.2 M hydrochloric acid with white cap.
20 Extraction tubes	For specimens preparation use.
1 Workstation	Place for holding buffer vials and tubes.
1 Package insert	For operation instruction.

MATERIALS REQUIRED BUT NOT PROVIDED

Timer	For timing use.

PRECAUTIONS

- For professional in vitro diagnostic use only.
- Do not use after expiration date indicated on the package. Do not use the test if its foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to performing any tests.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Do not interchange or mix reagents from different lots. Do not mix solution bottle caps.
- Humidity and temperature can adversely affect results.
- When the assay procedure is completed, dispose the swabs carefully after autoclaving them at 121°C for at least 20 minutes. Alternatively, they can be treated with 0.5% sodium hypochloride (or house-hold bleach) for one hour before disposal. The used testing materials should be discarded in accordance with local, state and/or federal regulations.
- Do not use cytology brushes with pregnant patients.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- The quality of specimen obtained is of extreme importance. As much as cervical epithelial cell should be collected by the swab.
  For cervical specimens:
- Use only Dacron or Rayon tipped sterile swabs with plastic shafts. It is recommend to use the swab supplied by the kits manufacturer(The swab are not contained in this kit, for the ordering information, please contact the manufacture or local distributor, the catalogo number is 207000). Swabs from other suppliers have not been validated. Swabs with cotton tips or wooden shafts are not recommended.
- Before specimen collection, remove excess mucus from the endocervical area with a separate swab or cotton ball and discard. Insert the swab into the cervix until only the bottommost fibers are exposed. Firmly rotate the swab for 15-20 seconds in one direction. Pull the swab out carefully!.
- Do not place the swab in any transport device containing medium since transport medium interferes with the assay and viability of the organisms is not required for the assay. Put the swab to the extraction tube. If the test may be run immediately. If immediate testing is not possible, the patient samples should be placed in a dry transport tube for storage or transport. The swabs may be stored for 24 hours at room temperature (15-30°C) or 1 week at 4°C or no more than 6 month at -20°C. All specimens should be allowed to reach a room temperature of 15-30°C before testing.

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. **Prepare swab specimens:**
   - Place a clean extraction tube in the workstation. Add 8 drops of Extraction Buffer A to the extraction tube.
   - Immerse the patient swab into the extraction tube and wait 2 minutes. While waiting, use a circular motion to roll the swab against the side of the extraction tube so that the liquid is expressed from the swab and can reabsorb.
   - At the end of the extraction time, add 8 drops of Extraction Buffer B to the tube and extract for another 1 minute in the same way. Then squeeze the swab firmly against the tube to expel as much liquid as possible from the swab. Discard the swab following guidelines for handling infectious agents.
   - The extracted specimen can remain at room temperature for 60 minutes without affecting the test result.
2. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within one hour.

3. Add 3 drops (approximately 100 μl) of extracted sample from the Extraction Tube to the sample well on the test cassette.
   Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.
   As the test begins to work, you will see color move across the membrane.
4. Wait for the colored band(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

POSITIVE RESULT: 	Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).
NEGATIVE RESULT: 	Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).
INVALID RESULT: 	Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of the color in test region (T) may vary depending on the concentration of aimed substances present in the specimen. But the substances level can not be determined by this qualitative test.
2. Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. The HPV 16/18 Antigen Rapid Test Device (Swab) is for professional in vitro diagnostic use, and should be used for the qualitative detection of HPV 16/18 E6&E7 Oncoproteins only. There is no meaning attributed to lines color intensity or width.
2. Detection of HPV 16/18 E6&E7 Oncoproteins is dependent on the number of proteins present in the specimen. This may be affected by specimen collection methods and patient factors such as age, history of STD, presence of symptoms, etc. The minimum detection level of this test may vary according to serovar.
3. The expression of E6&E7 oncoprotein is only indicate the risk of cervical cancer and pre-cancer occures, the positive results do not confirm the cancer or pre-cancer occures and the negative results can not exclude the happening of cervical cancer and pre-cancer.
4. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

Table: *StrongStep*® HPV 16/18 E6&E7 Oncoproteins Test vs. IHC

Relative Sensitivity: 72.3%			IHC		
Relative Specificity: 88.7%			+	-	Total
Overall Agreement: 84.9%	*StrongStep*® HPV Test	+	16	8	24
		-	6	63	69
			22	71	93

The antibody used in the HPV 16/18 Antigen test has been shown to detect HPV type 16 and 18. Cross reactivity with other organisms has been studied using suspensions of 10^7 org/ml. The following organisms were not detected using the test:

Acinetobacter calcoaceticus	*Proteus vulgaris*
Salmonella typhi	*Acinetobacter spp.*
Staphylococcus aureus	*Candida albicans*
Neisseria catarrhalis	*Neisseria gonorrhoea*
Neisseria meningitidis	*Neisseria lactamica*
Escherichia coli	*Gardnerella vaginalis*
Streptococcus faecalis	*Streptococcus faecium*
Pseudomonas aeruginosa	*Trichomonas vaginalis*
Ureaplasma Urealyticum	*Mycoplasma hominis*

LITERATURE REFERENCES

1. Giovane C., et al., J. Mol. Recog. 1999;12:141-152.
2. Joo-Ho Kim, et al., Bull. Korean Chem. Soc. 2009;30;2999-3005
3. Sexually Transmitted Diseases, 4th Edition. King K. Holmes. McGraw-Hill Professional, 2007.

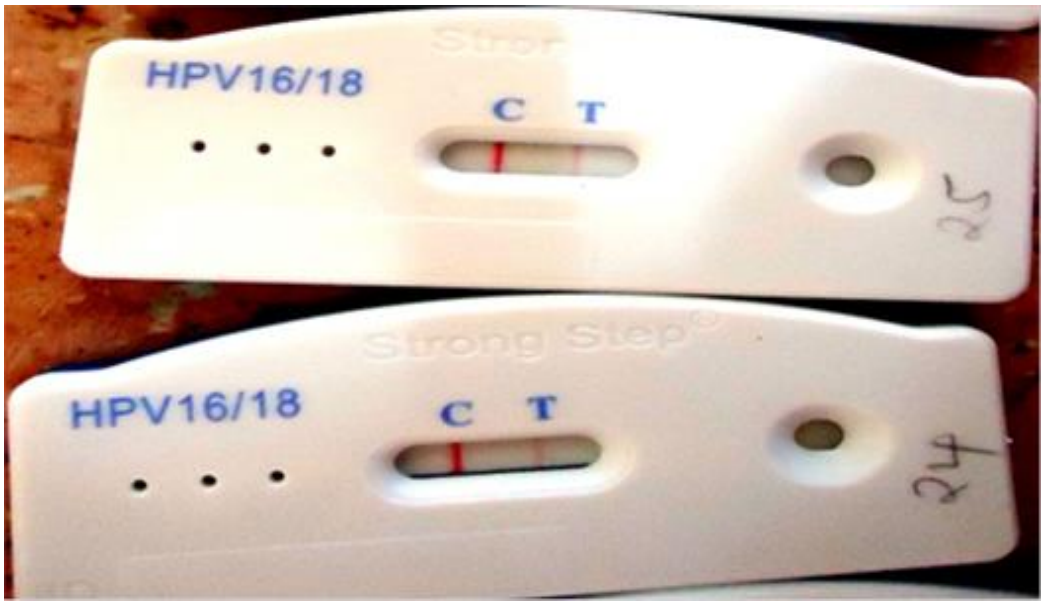
GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community
CE	CE marked according to IVD Medical Devices Directive 98/79/EC		

Liming Bio-Products Co., Ltd.
No. 12 Huayuan Road,Nanjing, Jiangsu, 210042
P.R. China.
Tel: (0086)25 85476723 Fax: (0086)25 85476387
E-mail: sales@limingbio.com
Website: www.limingbio.com
www.stddiagnostics.com
www.stdiagnostics.com

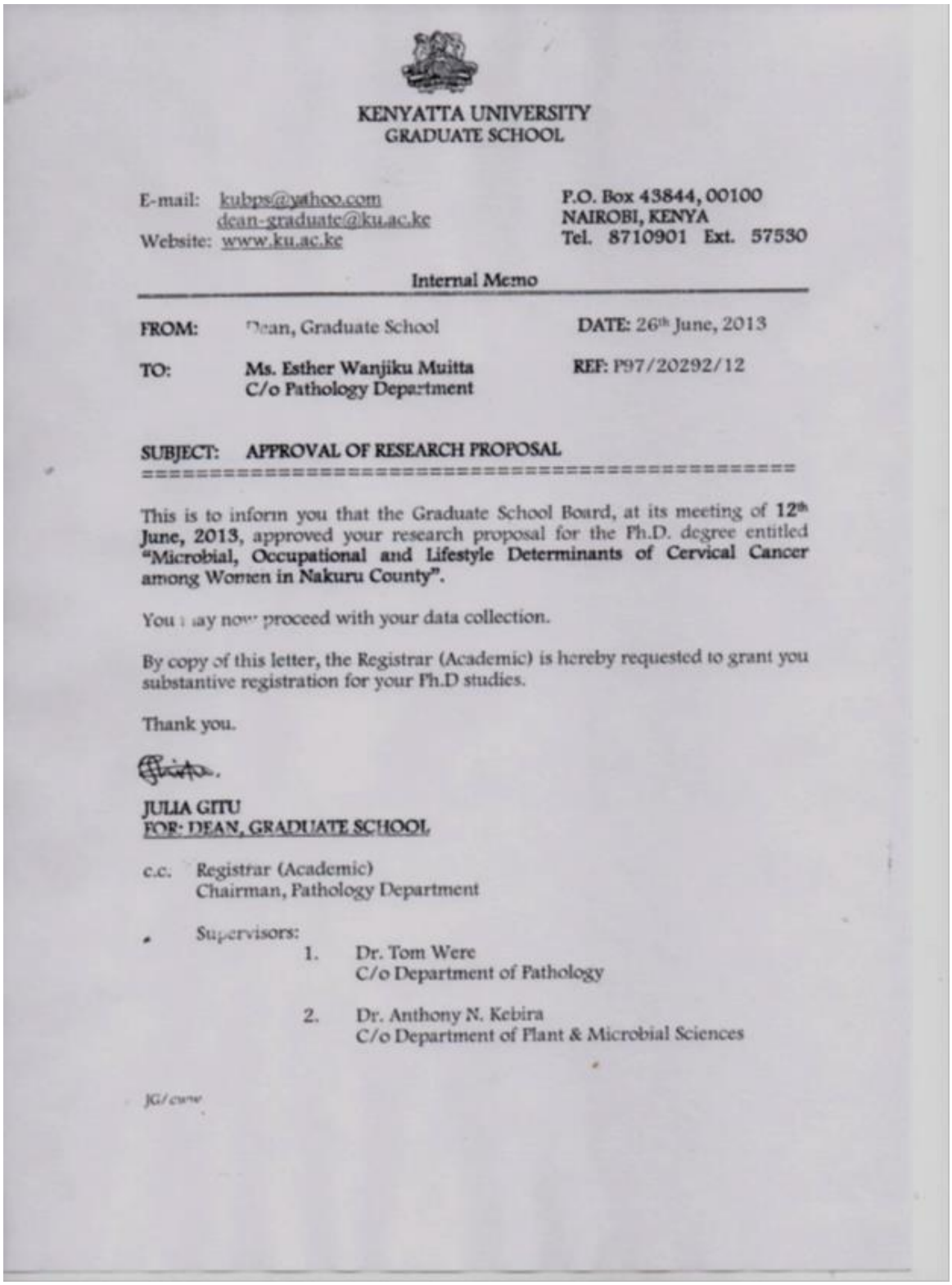
EC REP WellKang Ltd (www.CE-marking.eu) Tel: +44(20)79934346
29 Harley St., London W1G 9QR,UK Fax: +44(20)76811874

Positive HPV 16/18 rapid tests



Appendix X: Research study authorization permits and ethical clearance

KU research approval



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 26th June, 2013

TO: Ms. Esther Wanjiku Muitta
C/o Pathology Department

REF: P97/20292/12

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board, at its meeting of 12th June, 2013, approved your research proposal for the Ph.D. degree entitled "Microbial, Occupational and Lifestyle Determinants of Cervical Cancer among Women in Nakuru County".

You may now proceed with your data collection.

By copy of this letter, the Registrar (Academic) is hereby requested to grant you substantive registration for your Ph.D studies.

Thank you.

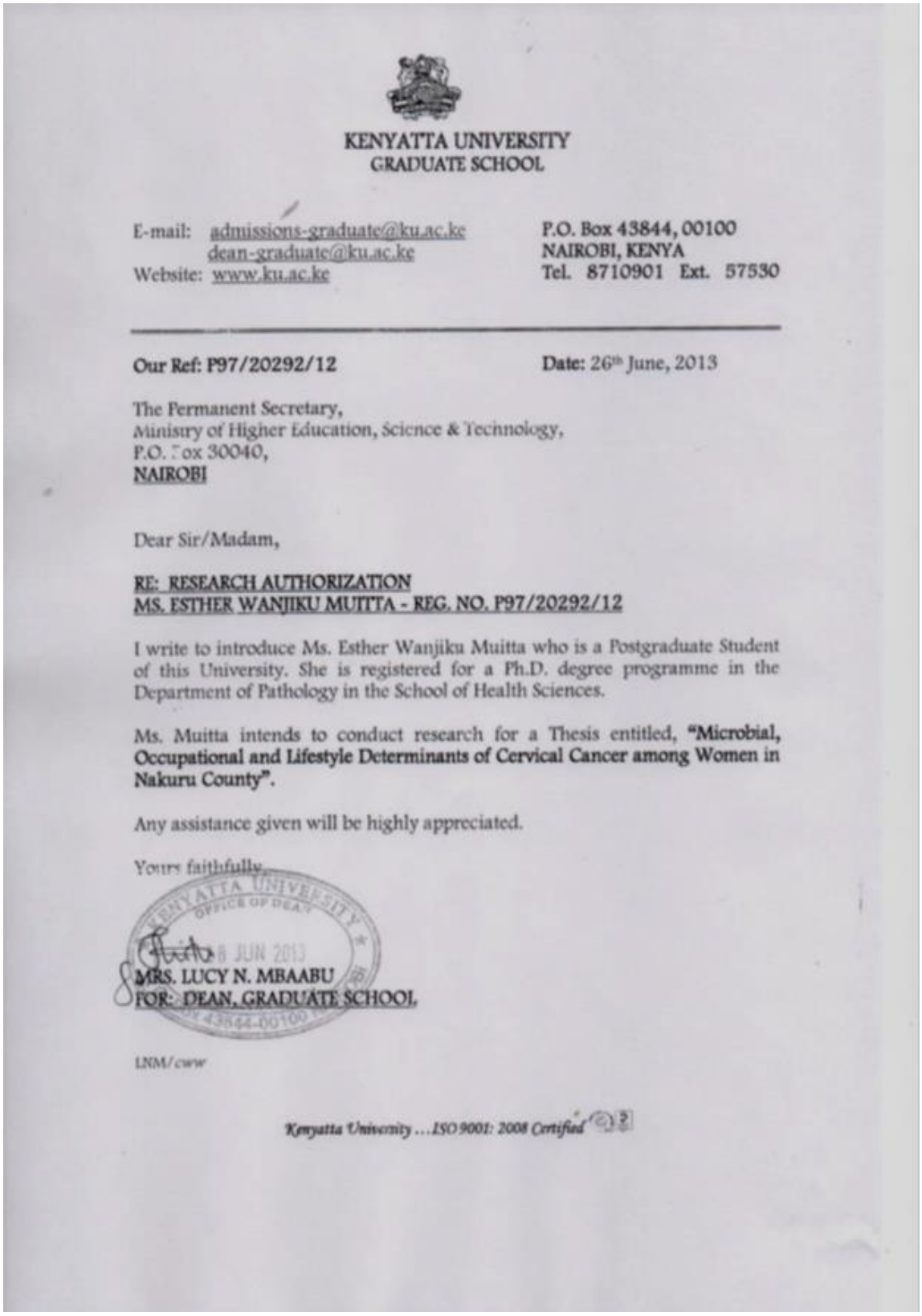
JULIA GITU
FOR: DEAN, GRADUATE SCHOOL

c.c. Registrar (Academic)
Chairman, Pathology Department

Supervisors:
1. Dr. Tom Were
C/o Department of Pathology
2. Dr. Anthony N. Kebira
C/o Department of Plant & Microbial Sciences

JG/cww

KU research authorization



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: admissions-graduate@ku.ac.ke
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: P97/20292/12 Date: 26th June, 2013

The Permanent Secretary,
Ministry of Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION
MS. ESTHER WANJIKU MUITTA - REG. NO. P97/20292/12

I write to introduce Ms. Esther Wanjiku Muitta who is a Postgraduate Student of this University. She is registered for a Ph.D. degree programme in the Department of Pathology in the School of Health Sciences.

Ms. Muitta intends to conduct research for a Thesis entitled, **"Microbial, Occupational and Lifestyle Determinants of Cervical Cancer among Women in Nakuru County".**

Any assistance given will be highly appreciated.

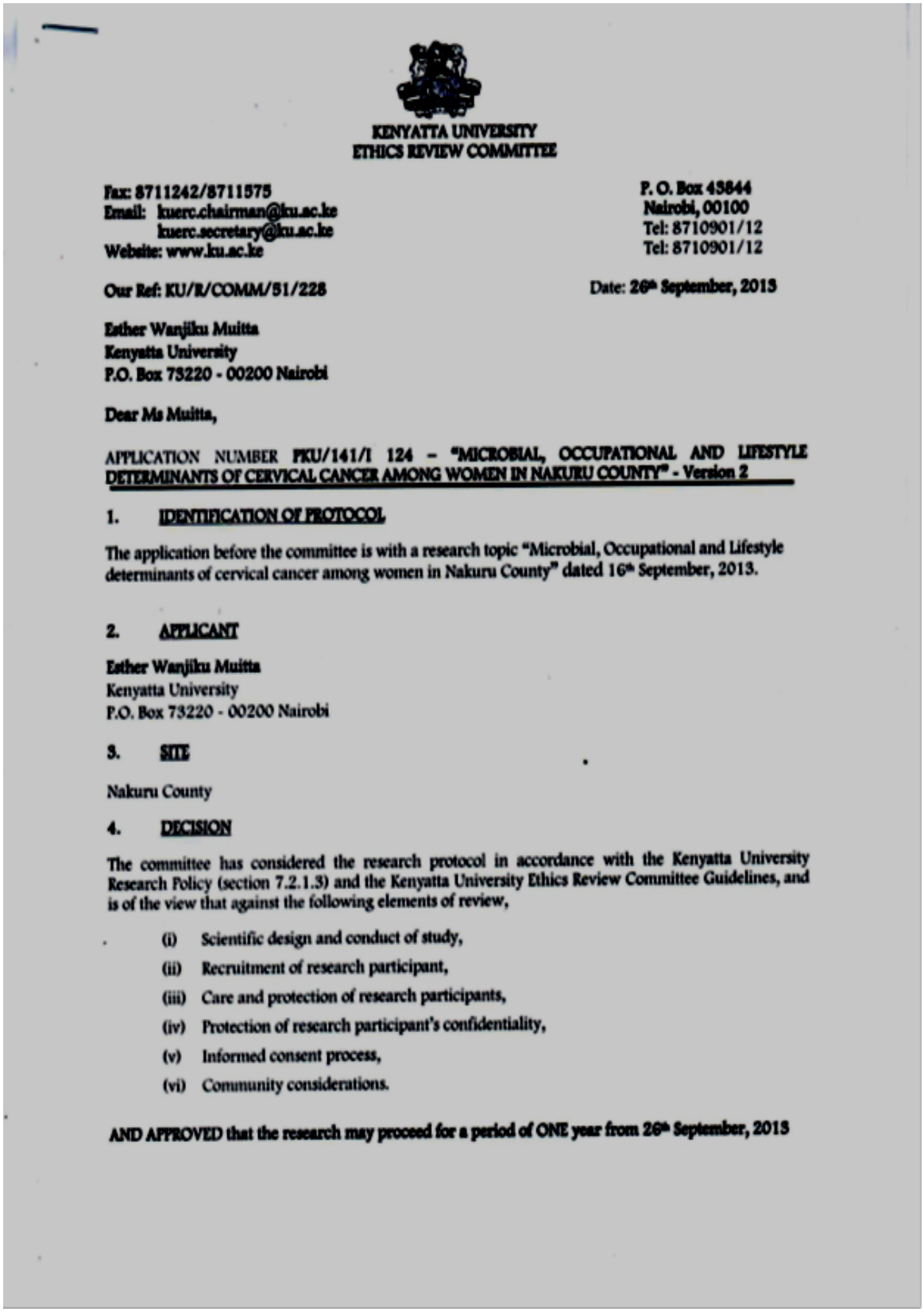
Yours faithfully,

KENYATTA UNIVERSITY OFFICE OF DEAN 26 JUN 2013 P.O. BOX 43844-00100

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

LNM/cww

KU Ethical clearance



KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844
Nairobi, 00100
Tel: 8710901/12
Tel: 8710901/12

Our Ref: KU/R/COMM/51/228

Date: 26th September, 2013

Esther Wanjiku Muitta
Kenyatta University
P.O. Box 73220 - 00200 Nairobi

Dear Ms Muitta,

APPLICATION NUMBER PKU/141/I 124 – "MICROBIAL, OCCUPATIONAL AND LIFESTYLE DETERMINANTS OF CERVICAL CANCER AMONG WOMEN IN NAKURU COUNTY" - Version 2

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic "Microbial, Occupational and Lifestyle determinants of cervical cancer among women in Nakuru County" dated 16th September, 2013.

2. APPLICANT

Esther Wanjiku Muitta
Kenyatta University
P.O. Box 73220 - 00200 Nairobi

3. SITE

Nakuru County

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines, and is of the view that against the following elements of review,

(i) Scientific design and conduct of study,
(ii) Recruitment of research participant,
(iii) Care and protection of research participants,
(iv) Protection of research participant's confidentiality,
(v) Informed consent process,
(vi) Community considerations.

AND APPROVED that the research may proceed for a period of ONE year from 26th September, 2013

5. **ADVICE/CONDITIONS**

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.

iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

KENYATTA UNIVERSITY
OFFICE OF THE CHAIRMAN
26 SEP 2013
ETHICS REVIEW COMMITTEE
P.O. BOX 43844 - 00100 NAIROBI

PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I ESTHER WANJIKU MUITTA accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of 26TH SEPTEMBER 2013.

cc. Vice-Chancellor
DVC: Research, Innovation and Community Outreach.
Director: Institute for Research Science and Technology

NACOSTI authorization

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471, 2241349, 310571, 2219420
Fax: +254-20-318245, 318249
Email: secretary@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No.

Date:

29th October, 2013

NACOSTI/RCD/12A/013/148

Esther Wanjiku Muitta
Kenyatta University
P.O.Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application dated *17th October, 2013* for authority to carry out research on *"Microbial, occupational and lifestyle determinants of cervical cancer among women in Nakuru County,"* I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for a period ending **31st December, 2016.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Coordinator of Health, Nakuru County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. M. K. RUGUTT, PhD, HSC.
DEPUTY COMMISSION SECRETARY
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Copy to:

The County Commissioner
The County Director of Education
The County Coordinator of Health
Nakuru County.

PAGE 2

THIS IS TO CERTIFY THAT:
Prof./Dr./Mr./Mrs./Miss/Institution
Esther Wanjiku Muitta
of (Address) Kenyatta University
P.O.Box 43844-00100, Nairobi.
has been permitted to conduct research in

Location
District
County

Nakuru

On the topic: Microbial, occupational and lifestyle Determinants of cervical cancer among women In Nakuru County.

for a period ending: 31st December, 2016.

PAGE 3

Research Permit No. NACOSTI/RCD/12A/013/148
Date of issue 29th October, 2013
Fee received KSHS. 2000

Applicant's Signature

For: Secretary
National Commission for Science Technology & Innovation

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

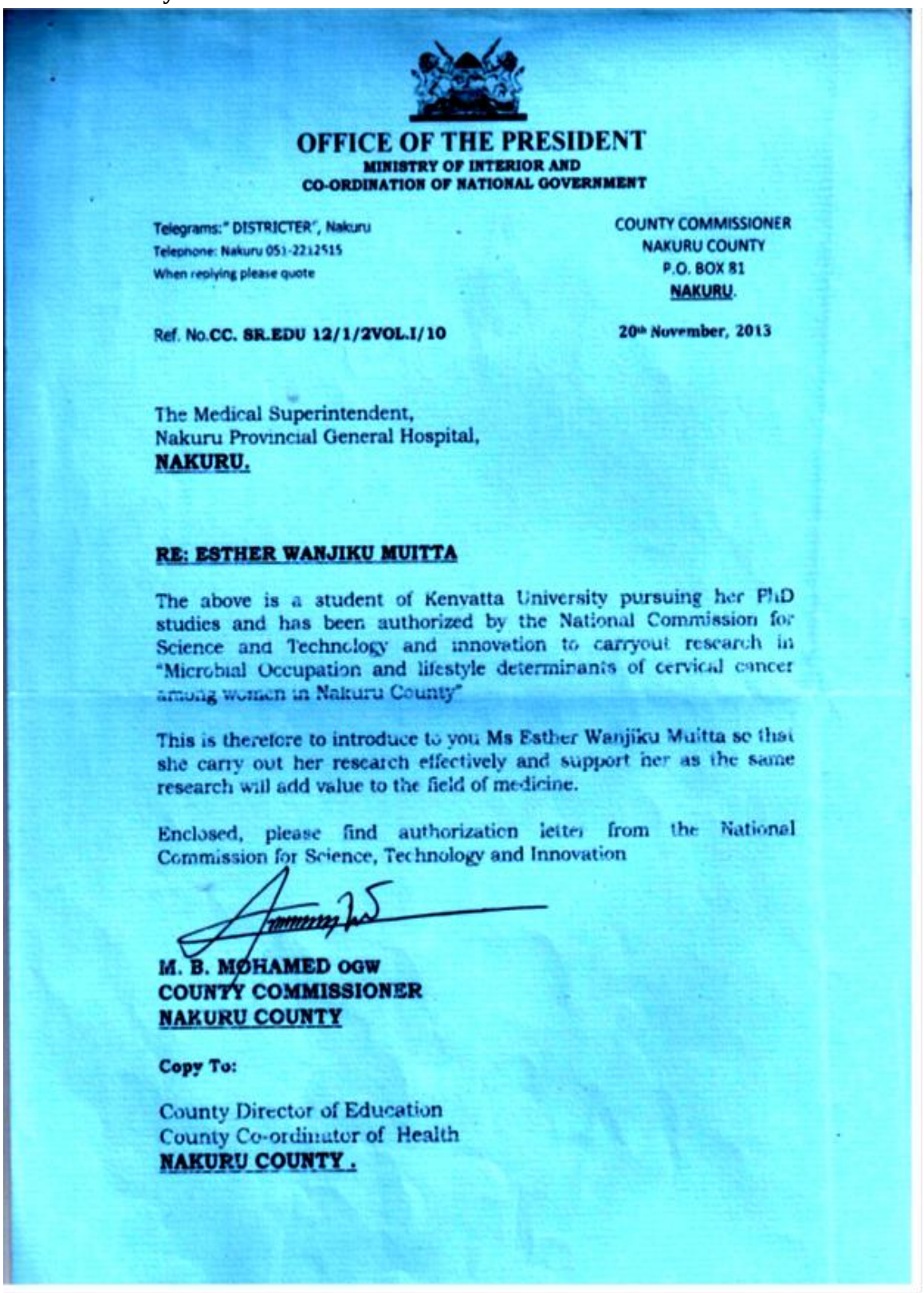
National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A 510

CONDITIONS: see back page

Nakuru County authorization



OFFICE OF THE PRESIDENT
MINISTRY OF INTERIOR AND
CO-ORDINATION OF NATIONAL GOVERNMENT

Telegrams:" DISTRICTER", Nakuru
Telephone: Nakuru 051-2212515
When replying please quote

COUNTY COMMISSIONER
NAKURU COUNTY
P.O. BOX 81
NAKURU.

Ref. No.CC. SR.EDU 12/1/2VOL.I/10

20th November, 2013

The Medical Superintendent,
Nakuru Provincial General Hospital,
NAKURU.

RE: ESTHER WANJIKU MUITTA

The above is a student of Kenyatta University pursuing her PhD studies and has been authorized by the National Commission for Science and Technology and innovation to carryout research in "Microbial Occupation and lifestyle determinants of cervical cancer among women in Nakuru County"

This is therefore to introduce to you Ms Esther Wanjiku Muitta so that she carry out her research effectively and support her as the same research will add value to the field of medicine.

Enclosed, please find authorization letter from the National Commission for Science, Technology and Innovation

M. B. MOHAMED OGW
COUNTY COMMISSIONER
NAKURU COUNTY

Copy To:

County Director of Education
County Co-ordinator of Health
NAKURU COUNTY .

MINISTRY OF HEALTH

Telegrams "PROVMED"Nakuru
Tele: Nakuru 2216710 Fax 22103
When replying please quote

COUNTY DIRECTOR OF HEALTH
NAKURU COUNTY
P.O. BOX 2060
NAKURU

REF.NO. A1VOL.11/546

18th November, 2013

The Medical Superintendent
Provincial General Hospital
P.O. BOX 71
NAKURU

RE: PERMISSION TO CONDUCT RESEARCH IN NAKURU COUNTY

Ms ESTHER WANJIRU MUITTA is a PHD student from Kenyatta University. Her, research is in the area of Cervical Cancer in women in Nakuru County.

Please allow her to collect the necessary data for the intended purpose..

DR. B.OSORE
COUNTY DIRECTOR OF HEALTH
NAKURU

MINISTRY OF MEDICAL SERVICES

Telegrams: "PROVMED", NAKURU
Telephone: Nakuru 051-2215580-90
When replying please quote
FAX 051 2216497

PROVINCIAL GENERAL HOSPITAL
RIFT VALLEY PROVINCE
P.O. Box 71
NAKURU.

RII/VOL.I/08

Date 28/11/2013

To: ESTHER W. MUITTA
KENYATTA UNIVERSITY
BOX 43844-00100 NAIROBI

Dear ESTHER MUITTA

RE: APPROVAL TO UNDERTAKE RESEARCH AT THE RIFT VALLEY PROVINCIAL GENERAL HOSPITAL

Reference is made to your letter dated 18/11/2013 seeking approval to conduct a research on "MICROBIAL OCCUPATIONAL AND LIFESTYLE DERMINANTS OF CERVICAL CANCER AMONG WOMEN IN NAKURU COUNTY"

Permission has been granted/~~Not granted~~ for the research. It is hoped that you will adhere to the ethics and standards that relate to research at our institution.

Thank you.

Yours sincerely,

MEDICAL SUPERINTENDENT

C3 DEC 2013

CHAIRPERSON
RESEARCH AND ETHICS COMMITTEE

CPD PROVIDERS LIST OF PARTICIPANTS

Name of CPD provider: Rift Valley Provincial General Hospital Nakuru

Date of activity: 14TH MAY 2015

Venue: BOARDROOM

Brief description of Activity: PRESENTATION: EVALUATION OF PRE CERVICAL CANCER SCREENING AND MANAGEMENT IN RIFT VALLEY PROV BY: MUITTA E.W

No	REG. No.	Title	SURNAME	FIRST NAME	MIDDLE NAME	EMAIL	TEL. NUMBER	SIGN
1	B579	DR	KABATH	JOHN	KIMUTAI	jkimkimotho@yahoo.com	0722221406	(CME CHAIR
2		DR.	MUHINDI	FAITH	N.	faithmuhindi@gmail.com	0725626930	
3		DR	KHATETE	RICHARD	GIBENGO	rkhate2@gmail.com	0713477825	
4		DR.	ARAKA	DANNY	MAGOMA	arakadanny@gmail.com	0722104523	
5		DR.	KOECH	STANLEY	KIPKEMBOI	anpoonde@gmail.com	0720356701	
6	AG176	DR.	IDRIS	NZAO	CHRISTOPHE	onzeo@yahoo.com	0721436946	
7		MRS	KINYANJUI	KARUIRI	NJOROGE	kinyanjui@gmail.com	0721941610	
8		MR	TOYON	DAVID	KIPKEMOI	david.toyon@yahoo.com	0729892335	
9		MR	Ngaaga	Philip		Ngaagadut@yahoo	0724101641	
10		MISS	Onyuka	LISPER	MYABOKI	onyukalisper@gmail.com	0722432437	
11		Dr.	Mutua	James	Ndenge	Jendenge44@gmail.com	0735396547	
12		SNO	WAMBUA	THERESIA	MUMBUA	theresiawambua@yahoo.com	0726026962	
13		NO	Tojo	Esther	Enghoh	esthertojo@gmail.com	0725661723	Tojo
14		MR	OKWIRI	JOSEPH	JUMA II	josephokwiri@yahoo.com	0721688419	
15		Mr	Esther	MUITTA	WANJIKU	emuitta@NKY.AIC.KE	0738823270	
16		DR	OGONDO	JOACHUEM	OTIENO	jogindu@gmail.com	0702857277	(SUPERVISOR)
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MEDICAL SUPERINTENDANT PROVINCIAL GENERAL HOSPITAL NAKURU
14 MAY 2015
P. O. Box 71 NAKURU