EVALUATION OF RELIABILITY OF FULL HEMOGRAM AND URINALYSIS IN RELATION TOURINE CULTURE AS DIAGNOSTIC TOOLS FOR URINARY TRACT BACTERIAL INFECTIONS IN TRANS-NZOIA COUNTY REFERRAL HOSPITAL, KENYA

BY

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DECLARATION

I, Julius Kipkoech Serem, declare that this thesis is my original work and has never been presented for any academic award in any other university.

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DEDICATION

To my daughters, Judy Chelagat Serem, Joan Cherono Serem and Janelle Cherop Serem

ABSTRACT

In routine clinical practice, urinary tract bacterial infections (UTBIs) are diagnosed using urinalysis assay and urine culture is used as a confirmatory test to determine the infecting bacteria. Urinalysis assay relies on the levels of leukocytes, proteins, nitrites and red blood cells in the urine sample. However, in public and private hospitals without blood and urine culture tests, the levels of neutrophils and monocytes from full hemogram assay are commonly interpreted as indications of bacterial infections upon which treatment is prescribed. Prescription of broad-spectrum antibiotics based on such non-specific diagnosis may result in multi drug resistance, human suffering and unnecessary expenses. The general objective of the study was to evaluate the reliability of full hemogram assay and urinalysis assay in relation to urine culture as diagnostic tools for urinary tract bacterial infections at Trans-Nzoia County Referral Hospital in Trans-Nzoia County, in Kenya. The specific objective of this cross-sectional study was to; determine the correlation between counts of neutrophils and monocytes from full hemogram assay, determine the correlation of counts of leukocytes, proteins, nitrites and red blood cells from urinalysis assay and determine the extent of reliability of counts of full hemogram assay and urinalysis assay in relation to urine culture test. The study obtained Informed Consent from the participants prior to participation and through Simple random sampling technique, 173 participants were recruited from both in-patient and out-patient departments at the hospital. About 2-4ml of urine sample was obtained from the patients for urinalysis and urine culture tests. Urine culture in this case was used as a gold standard diagnosis for urinary tract bacterial infections. About 1ml of blood was drawn by venipuncture from the same study participants for full hemogram assay to determine the counts of monocyte and neutrophils. Out of 173 study patients, 122 patients were confirmed positive for urinary tract bacterial infections by urine culture test. Out of these positive cases, 55 patients were neutrophilic, 24 patients presented with monocytosis and 39 patients had leukocytosis. On contrast, 51 samples tested negative by urine culture and were used as control group in the study. The study used a desired confidence level of 95% and an acceptable margin error of 5%. There was no correlation between the counts of neutrophils and monocytes from full hemogram assay with value of r=0.0794 and p-value of 0.299.However, there were correlations between the variables of urinalysis assay and their means had a significant relationship with a p-value of <0.0001on one way Analysis of Variance test. Urinalysis assay was not reliable tool with an overall score of 0.17 on Cronbach's Alpha scale while full hemogram was not reliable tool with the best score of 45% in relation to urine culture test. On ranking of reliability of the variables from both assays, Neutrophils were most reliable with 45.08%, Leukocytes 31.97%, Proteins 20.49%, Monocytes 19.67% and RBCs at 9.84% in relation to confirmatory urine culture technique. In conclusion, full hemogram assay and urinalysis assay are not reliable tools for diagnosis of urinary tract bacterial infectionsin relation to the confirmatory test of urine culture technique. From the study outcome, it is recommended that hospitals should use urine culture techniques in diagnosis of urinary tract bacterial infections as opposed to rapid tests that include urinalysis and full hemogram assays.

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LIST ABBREVIATIONS AND ACRONYMS

ANC Absolute Neutrophil Count

ANOVA Analysis of variance

CD Cluster of Designation

CT Computer Tomography

EDTA Ethylene diamine tetra-Acetic Acid

FHG Full Hemogram

IL Interleukin

MHC Major Histocompatibility Complex

ML Milliliter

NETS Neutrophil Traps

PMN Polymorphonuclear

RBCs Red Blood Cells

SPSS Statistical Packages for Social Scientists

TNF Tumour Necrosis Factor

UTBIs Urinary Tract Bacterial Infections

UTIs Urinary Tract Infections

WBCs White Blood Cells

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CHAPTER ONE
INTRODUCTION

1.1 Background Information

A urinary tract infection (UTI) is an infection of any section of the urinary tract system. This system includes the urethra, bladder, ureters and kidneys. Most of these infections affect the lower part of the urinary tract comprising of the bladder and the urethra (Partin, 2021). Urinary tract infection is the second most prevalent disease after gastrointestinal tract infections affecting hundreds of thousands of people globally and accounts for over 8.3 million hospital visits each year (Oliveira, 2016).

In Kenya, the overall UTI prevalence is 54.1 %, but women have a higher prevalence rate of 59.3% compared to men with 31.3%. UTI prevalence is highest in the age range between 25-30 years with 16.2% and children below five years have a prevalence rate of 1.2%. This is according to research conducted in Nairobi region hospitals, KEMRI Centre for Microbiology Research Lab, Nanyuki Teaching and Referral Hospital, and Makueni Level 5 Hospital (Susan et al., 2022). The prevalence of bacterial infections in Western Kenya, Trans-Nzioa County according to the research carried out in 2019 on anti-microbial resistance is estimated at 67.7% (Susan et al., 2022). UTI prevalence rate among adults between 20 to 50 years of age is about fifty times more common in females than males (Gordon & Mermel, 2013) while young and sexually active women between the age of 18 to 24 years have the highest incidence rate of UTIs (Warkulwiz et al., 2017). In Tanzania and Uganda, the prevalence rate was reported at 28% and 32.2% respectively, while in the Unites states the overall prevalence is estimated at 11% (Chu & Lowder, 2018). The prevalence increases in patients above 50 years, but the ratio of female to male tend to decrease because of the increased prevalence of prostate disease in males. Approximately 95% of UTIs manifest when bacteria move up the urethra to the bladder and may lead to acute uncomplicated pyelonephritis if the bacteria ascend to the ureter and finally to the kidney while about 5% of UTIs occur as hematogenous (Gupta et al., 2011). Elderly people are susceptible to urinary tract infections due to age-predisposition risk factors that include high incidences of diabetes mellitus, weak bladder leading to urine retention, frequent hospital visits leading to nosocomial infections, vaginal atrophy, prostate cancers, weakened immunity and cases of malnutrition. It is estimated that 6.5% of cases of nosocomial bacteremia are attributed to UTI (Foxman, 2014).

Most bacterial UTIs are caused by colonic gram-negative aerobic bacteria, particularly strains of *Escherichia coli (E. coli)* which specifically affect the epithelium of the bladder and ureteral tubes (Hannan, 2012). Other gram-negative urinary bacteria are *enterobacterial* pathogens that include *Pseudomonas aeruginosa, Klebsiella and Proteus mirabilis*. Gram-positive pathogens implicated in UTI include *Enterococci* group particularly group D of *streptococci* while *staphylococci* group infections are related to *staphylococcus saprophyticus* species. *Escherichia coli* causes about 75% of UTIs across all age groups while *staphylococcus saprophyticus* causes about 10% of the cases. From the hospitalized records, *E. coli* accounts for approximately 50% of the total cases and about 40% is caused by *Enterobacter* group, *Proteus* group, *Klebsiella* group and *Serratia* group. About 10% of the cases are caused by the gram-positive bacterial *cocci* that include *S. saprophyticus, E. faecalis and S. aureus* (Tong & Fowler, 2015).

UTI infection may be symptomatic or asymptomatic. Symptomatic UTI is characterized by the presence of significant bacterial growth with counts of $\geq 10^5$ colony forming units of bacteria per milliliter (CFU/ml) per urine sample (Arinzon, et al., 2012). The common symptoms for UTI include frequent and urgent urination, painful urination, back and pelvic pain while septic symptoms may occur in cases of kidney infections (Hamouda, 2014).

Usually in routine clinical practice, urinary tract bacterial infections (UTBIs) are diagnosed using urinalysis assays and urine culture technique as a confirmatory test to identify the specific bacterial pathogen. Urinalysis assay (test of urine parameters) relies on the examination of the levels of white and red blood cells, nitrites and proteins in a urine sample as indicators of UTIs (Delanghe & Speeckaert, 2016). During urinalysis assay, urine sample is obtained through a clean catch method by collecting urine specimen from the urinary tract mainstream into a sterile container. The genital area is washed, and the first urine is not collected to ensure that the test sample is free from any external contamination (Magiorkinis, 2015).

Complete blood counts from full hemogram assay are used in diagnosis of bacterial infections in the human body. These are mainly neutrophil and monocyte counts. Neutrophils account for about 70% of all white blood cells (WBCs) in the human body and about 1011 neutrophil counts are produced per day (Beyrau & Nourshargh, 2012). The count of neutrophils in human blood varies between 2.5-7.5 x 10^9 /L and this is regarded the conventional standard normal range while any count above this level is termed as neutrophilia (Hellebrekers, 2018). Neutrophils form

critical part of the innate immunity and together with eosinophils and basophils they form the larger component of polymorphonuclear cell family (PMNs). Neutrophils are usually found in the blood circulation system. In the acute phase of inflammatory reaction to bacterial infections, environmental exposures, and some form of cancers, neutrophils are the essential primary-responders towards the site of inflammation. They extravasate through the blood vessels to the interstitial tissue, in response to the chemical signaling by immune components such as interleukin-8 (IL-8) or C5a in a process of chemotaxis. Neutrophils directly kill pathogens through phagocytosis process, or release of soluble anti-microbials for example granule proteins or by generating neutrophil extracellular traps (NETs) (Leben, 2018).

Monocytes are WBCS that proliferate to macrophages and dendritic cells in the immune system. Monocytes, their progeny macrophages and dendritic cells have three main roles in the immune system. These are mainly phagocytosis function, participation in antigen presentation and production of cytokines (Tak, 2017). Monocytes carry out phagocytosis either using intermediary components such as antibodies or the complement that coat the pathogen for compatibility, but they can bind the pathogen directly through pattern-recognition receptors (Jakubzick et al., 2017). Monocytes can destroy infected cells via antibody-mediated cellular cytotoxicity. In human blood count, any level of monocytes above 950/μL is termed as monocytosis.

More often than not, clinicians use complete cell blood count (CBC) commonly the counts of neutrophils and monocytes from full hemogram assays to determine treatment for UTBIs (Hellebrekers et al., 2018). It is reported that some deficiencies that include excess use of non-UTI laboratory tests such as CBC have affected most research studies and diagnosis in the hospitals (Mbatia et al.,2023).

Lack of adequate laboratories, lack of diagnostic capacity, lack of diagnostic and laboratory equipment, common stockouts of crucial laboratory reagents and consumables, requesting for anti-microbial sensitivity testing and incorporating results from diagnostic testing into clinical decision-making is not common practice. These challenges in turn may contribute to the overuse and misuse of antimicrobials (Government of Kenya, 2017).

On the other hand, urinalysis assay and urine culture are often used regardless of the prior probability of UTI, and their precision role in the diagnosis of acute uncomplicated UTI remains controversial (Hooton, 2012).

Therefore, the outcome of this study provide evidence that Trans-Nzoia County Referral Hospital and by large the Ministry of health (department of laboratory services) should use Urine culture test as opposed to urinalysis assays and full hemogram assays in routine diagnosis of UTBIs. This in turn addresses the gaps in diagnosis of UTIs and minimizes chances of wrong treatments.

1.1.1 Study Rationale

In routine hematologic and immunologic diagnostic procedures, full hemogram (FHG) parameters that include the absolute levels of neutrophils, monocytes, lymphocytes, eosinophils and basophils are usually analyzed to derive the medical implication of the patient. In most cases, elevated or low levels of any of these parameters may be non-specific for definitive diagnosis and subsequent treatment (Pagana, 2019). UTIs are routinely diagnosed by urinalysis assay, which is a rapid test method as opposed to the most reliable test of urine culture technique (Delanghe & Speeckaert, 2016). However, most hospitals lack urine culture tests.

The outcome of this study was necessary to guide Trans-Nzoia County Referral Hospital and by large the Ministry of health (department of laboratory services) on the extent of reliability of urinalysis assays and full hemogram assays in routine diagnosis of UTBIs as compared to urine culture test. This in turn would address the gaps in diagnosis of UTIs and minimize chances of wrong treatments.

1.2 Statement of the Problem

Urinary tract bacterial infection (UTI) is the second most prevalent bacterial disease in the human body after gastrointestinal tract infections, with over 8.3M hospital annual visits globally. The prevalence of bacterial infections in Western Kenya, Trans-Nzioa County according to the research carried out in 2019 on anti-microbial resistance is estimated at 67.7% which is higher than the reported overall national prevalence rate of 54.1%. UTIs are routinely diagnosed by urinalysis assay, which is a rapid test method as opposed to the most reliable test of urine culture technique.

Clinicians oftenly rely on counts of neutrophils and monocytes from full hemogram assays as an indication of bacterial infections. However, most of the health facilities lack urine culture tests (confirmatory test), and therefore rely on rapid tests which are urinalysis assay and full hemogram assay. Relying on non-specific diagnosis leads to wrong treatment and inappropriate antibiotic prescription may lead to multi-drug resistance, human suffering and unnecessary expenses. With this gap, there was a need to ascertain the extent of reliability of urinalysis and full hemogram assays in relation to the gold standard method of urine culture technique.

1.3 General objectives

The general objective of the study was to evaluate the reliability of full hemogram assay and urinalysis assay in relation to urine culture as diagnostic tools for urinary tract bacterial infections at Trans-Nzoia County Referral Hospital in Trans Nzoia County, Kenya.

1.4 Specific Objectives

i. To determine correlation between the counts of neutrophils and monocytes from full hemogram assay in patients with confirmed urinary tract bacterial infections.
ii. To determine correlation among the counts of leukocytes, proteins, nitrites and red blood cells from urinalysis assay in patients with confirmed urinary tract bacterial infections.
iii. To determine the extent of reliability of counts of full hemogram assay and urinalysis assay in relation to urine culture test.

1.5 Study Questions

i. There is no correlation between the counts of neutrophils and monocytes from full hemogram assay in patients with confirmed urinary tract bacterial infections.
ii. There is no correlation among the counts of leukocytes, proteins, nitrites and red blood cells from urinalysis assay in patients with confirmed urinary tract bacterial infections.
iii. The extent of reliability of counts of full hemogram assay and urinalysis assay in relation to urine culture test is not acceptable.

1.6 Delimitations, Limitations and Assumptions

1.6.1 Delimitations

The research focused only on patients above 20 years as participants noting that UTI prevalence is highest in the age range of between 25-30 years with 16.2% and children below five years

have a prevalence rate of 1.2% (Susan et al., 2022). UTI prevalence rate among adults between 20 to 50 years of age is about fifty times more common in females than males (Gordon & Mermel, 2013) while young and sexually active women between the age of 18 to 24 years have the highest incidence rate of UTIs (Warkulwiz et al., 2017). Expectant mothers were also excluded. The prevalence of UTIs at 13.9% was a subjective average with respect to all bacterial types, patient gender, and the status of health services. The study chose to focus on urinary bacterial infections which are the second most prevalent bacterial infections after gastrointestinal bacterial infection and easy to sample urine as specimen. The choice to carry out this study at Trans- Nzoia County Hospital was based on high bacterial prevalence rate of 67.7% which was higher than the overall national prevalence rate of 54.1% (Susan et al., 2022), availability of hemogram assays, urinalysis assays and urine cultures.

1.6.2 Limitations

Some of the limitations included inadequate support from the hospital's laboratory personnel, limited supply of reagents and test kits and hesitation of some patients to enroll in the study due to fear of the unknown outcome of the study. These were minimized by offering small personal incentives to hospital staff for their extra work, personally meeting the extra costs incurred on additional reagents and test kids and use of informed consent forms to demystify the unknown outcome of their participation.

1.6.3 Assumptions

Since the study design incorporated urine cultures as confirmatory tests as opposed to rapid tests, the data obtained was accurate and reliable and its interpretation could be generalized in the conclusion and recommendation. An assumption that neutrophilia and monocytosis in the study patients with UTIs as confirmed by urine culture tests were not related to other exposures since there were no clinical manifestations and the chances of probability were determined by use of control study group. The study assumed that there would be positive enrolment of participants without much hesitation, panic or fear.

1.7 Conceptual Framework

For every participant, the tests were subjected to specific diagnostic tools (independent variables) and the results (dependent variables) were analyzed against the corresponding results from the standard gold test of urine culture (Intervening variable).

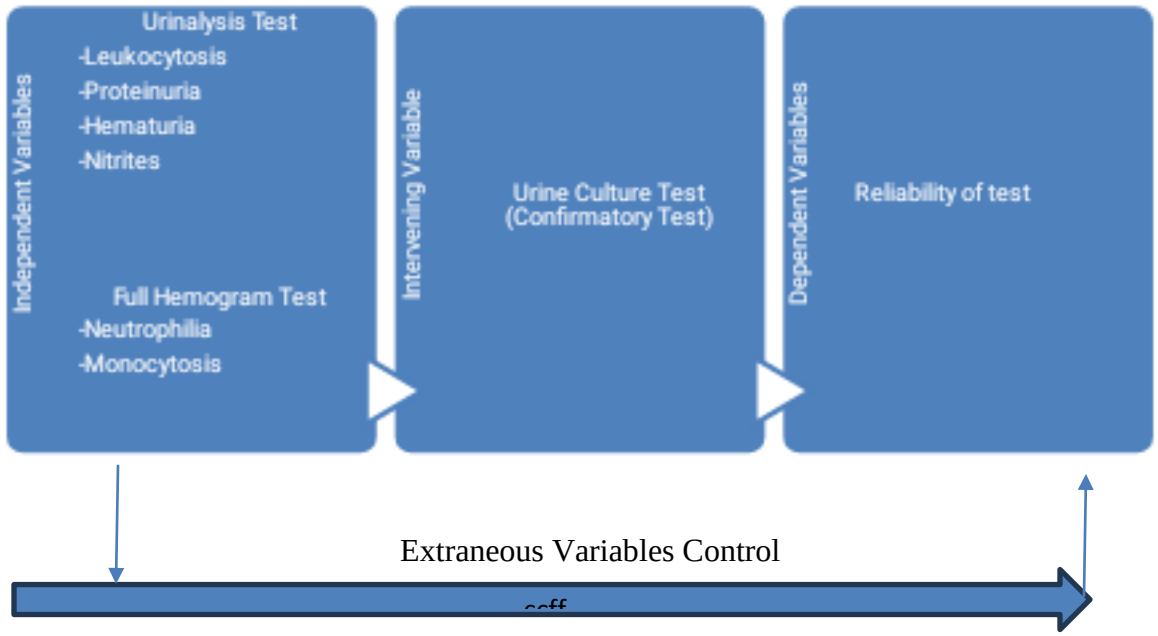


Figure 1.1: Conceptual framework

CHAPTER TWO
LITERATURE REVIEW

2.1 Urinary Tract Bacterial Infection and Its Prevalence

A urinary tract infection (UTI) is an infection of any section of the urinary tract system. This system includes the urethra, bladder, ureters and kidneys. Most of these infections affect the lower part of the urinary tract comprising of the bladder and the urethra (Partin, 2021). Urinary tract infection is the second most prevalent disease after gastrointestinal tract infections affecting hundreds of thousands of people globally and accounts for over 8.3 million hospital visits each year (Oliveira, 2016).

In Kenya, the overall UTI prevalence is 54.1 %, but women have a higher prevalence rate of 59.3% compared to men with 31.3%. UTI prevalence is highest in the age range between 25-30 years with 16.2% and children below five years have a prevalence rate of 1.2%. This is according to research conducted in Nairobi region hospitals, KEMRI Centre for Microbiology Research Lab, Nanyuki Teaching and Referral Hospital, and Makueni Level 5 Hospital (Susan et al., 2022). The prevalence of bacterial infections in Western Kenya, Trans-Nzioa County according to the research carried out in 2019 on anti-microbial resistance is estimated at 67.7% (Susan et al., 2022). UTI prevalence rate among adults between 20 to 50 years of age is about fifty times more common in females than males (Gordon & Mermel, 2013) while young and sexually active women between the age of 18 to 24 years have the highest incidence rate of UTIs (Warkulwiz et al., 2017). In Tanzania and Uganda, the prevalence rate was reported at 28% and 32.2% respectively, while in the Unites states the overall prevalence is estimated at 11% (Chu & Lowder, 2018). The prevalence increases in patients above 50 years, but the ratio of female to male tend to decrease because of the increased prevalence of prostate disease in males. Approximately 95% of UTIs manifest when bacteria move up the urethra to the bladder and may lead to acute uncomplicated pyelonephritis if the bacteria ascend to the ureter and finally to the kidney while about 5% of UTIs occur as hematogenous (Gupta et al., 2011). Elderly people are susceptible to urinary tract infections due to age-predisposition risk factors that include high incidences of diabetes mellitus, weak bladder leading to urine retention, frequent hospital visits leading to nosocomial infections, vaginal atrophy, prostate cancers, weakened immunity and cases of malnutrition. It is estimated that 6.5% of cases of nosocomial bacteremia are attributed to UTI (Foxman, 2014).

About 34% of persons over 20 years self-reported having had a urinary tract infection at least once in their lifetime and about 14% of adults between 20-74 years who self-reported were males while 53.5% were females (NHNS, 2003).

2.2 Common Causes of UTIs

Most bacterial UTIs are caused by colonic gram-negative aerobic bacteria, particularly strains of *Escherichia coli (E. coli)* which specifically affect the epithelium of the bladder and ureteral tubes (Hannan, 2012). Other gram-negative urinary bacteria are *enterobacterial* pathogens that include *Pseudomonas aeruginosa, Klebsiella and Proteus mirabilis*. Gram-positive pathogens implicated in UTI include *Enterococci* group particularly group D of *streptococci* while *staphylococci* group infections are related to *staphylococcus saprophyticus* species. *Escherichia* coli causes about 75% of UTIs across all age groups while *staphylococcus saprophyticus* causes about 10% of the cases. From the hospitalized records, *E. coli* accounts for approximately 50% of the total cases. About 40% is caused by *Enterobacter* group, *Proteus* group, *Klebsiella* group and *Serratia* group. About 10% of the cases are caused by the gram-positive bacterial *cocci* that include *S. saprophyticus, E. faecalis and S. aureus* (Tong & Fowler, 2015). Some UTIs are associated with catheterization, diabetes, pregnancy, diabetes, obstructions due neurogenic bladder, tumors, prostatic hyperplasia and calculi (Lo, 2014).

2.3 Routine Tests of UTIs

In routine practice UTIs are tested commonly by urinalysis assays and urine culture technique (Levey, 2015). Urinalysis assay (test of urine parameters) relies on the examination of the levels of white and red blood cells, nitrites and proteins in a urine sample as indicators of UTIs (Delanghe & Speeckaert, 2016). The common components tested for indication of bacterial infections include; nitrites which should be negative, leukocyte esterase which should be negative, proteins with normal value of <150mg/d, red blood cells with normal value of <2 RBCs/hpf, white blood cells with normal value of <2-5 WBCs/hpf, glucose with normal value < 130mg/d and bilirubin which should be negative (Sharp et al., 2014). During urinalysis assay, urine sample is obtained through a clean catch method by collecting urine specimen from the urinary tract mainstream into a sterile container. The genital area is washed, and the first urine is not collected to ensure that the test sample is free from any external contamination (Magiorkinis, 2015). From the urine sample bacteria are inoculated and grown in a culture media and may be

tested against different antibiotics, in a process called sensitivity test. Other tests for UTIs are microscopic tests, x-rays, ultrasound examination and cystoscopic test that uses light source to examine the bladder through the urethra (Magiorkinis & Diamantis, 2015). However, urinalysis and urine culture are commonly used though in some cases, full hemogram assays which determines elevated levels of neutrophil and monocyte counts are interpreted as indicators of bacterial infection. Ultrasonography or helical computer tomography (CT) are commonly used for diagnosis of obstructive complications of the urinary tract system.

2.4 Neutrophil Granulocytes and Their Anti-microbial Function

Neutrophil granulocytes, also called neutrophils or polymorphonuclear neutrophils (PMNs) are further sub-classified classified into two groups; segmented neutrophils (segs) and banded neutrophils (bands) (Leben, 2018). Neutrophils are the majority type of leucocytes in human body and form a critical part of the innate immunity. In the acute phase of inflammatory reaction to bacterial infections, environmental exposures, and some form of cancers, neutrophils are the essential primary-responders towards the site of inflammation. They extravasate through the blood vessels to the interstitial tissue, in response to the chemical signaling by immune components such as interleukin-8 (IL-8) or C5a in a process known as chemotaxis (Oliveira et al., 2016).

Neutrophils directly kill pathogens through phagocytosis process, or release of soluble anti-microbials for example degranulation of granule proteins or by generating neutrophil extracellular traps (NETs) (Leben R, 2018). They engulf and kill many pathogens, through phagocytic event that involves use of reactive oxygen species and hydrolytic enzymes. Consumption of oxygen through the process of respiratory or oxidative burst, involves the activation of the enzyme called NADPH oxidase, that then produce large quantities of superoxide. Superoxide is converted to hydrogen peroxide by superoxide dismutase, and finally hydrogen peroxide is converted to hypochlorous acid HClO by myeloperoxidase. The bactericidal properties of the HClO radical kill the phagocytosed bacteria through the activation of proteases. Neutrophils have ability to express and release various cytokines e.g IFN, IL-4 and IL-10 that act by amplifying the inflammatory reactions by several other cell types of immunity that include activation of adaptive immunity response (Beyrau et al., 2015).

2.5 Monocytes and Their Anti-microbial Function

Monocyte is a white blood cell that is part of the essential defense mechanism in the human body immune system. Monocytes have multiple functions in the immune system that include, firstly replenishing of macrophages and dendritic cells secondly responding to inflammation signals such as cytokines that trigger monocytes to sites of infection in the tissues where they immediately under division and differentiate into resident macrophages and dendritic cells to mount an immune response within approximately 8-12 hours (Yona & Breker, 2013). They are produced by the bone marrow from stem cell precursors known as monoblasts through a series of haematopoietic processes. Monocytes circulate within the blood system for about 1-3 days before migrating into tissues throughout the body in readiness for immune defense (Jakubzick et al., 2017). Monocytes form approximately 3-8% of the leukocyte count in the blood and about 50% of them are usually stored as a reserve pool in the spleen in cluster form in the red pulp's Cords of Billroth. While residing in the tissues, monocytes mature and differentiate into different types of resident macrophages depending on the anatomical location (Patel, 2017). Monocytes in the human blood fall in three categories namely, (1) the classical monocytes, usually with high level expression of the CD14 cell surface receptors also known as (CD14++ CD16- monocyte), (2) the non-classical monocytes, characterized by low level expression of CD14 but with additional co-expression of the CD16 receptor also known as (CD14+CD16++ monocyte), (3) the intermediate monocytes, with high level expression of CD14 and expression of low levels of CD16 hence termed as (CD14++CD16+ monocytes). Usually, a high level of CD14+CD16+ monocytes are found in severe infections (sepsis stage) indicating that this this is the most mature version of a monocyte (Rua & McGavern, 2015).

Monocytes and their progeny macrophages and dendritic cells elicit immune response mainly through three mechanisms that include phagocytosis of pathogen, presentation of antigen and production of cytokines. Monocytes carry out phagocytosis process mainly using the intermediary proteins e.g antibodies or complement factors that opsonize the pathogen for internalization (Yona & Breker, 2013). Monocytes can bind the pathogen directly through pattern-recognition receptors that identify pathogens. They are also capable of destroying infected host cells through the process of antibody-mediated cellular cytotoxicity (Jakubzick et al., 2017). The microbial portions resulting from the digestion by proteolytic enzymes serve as antigens. These antigens are incorporated into MHC11 molecules and moved to the cell surface

of macrophages, monocytes, and dendritic cells. These molecules are finally presented to the activated CD_4^+T lymphocytes which then elicit a specific immune response to destroy the antigen fragment. Monocytes can react directly to some microbial products by eliciting production of pro-inflammatory agents and anti-inflammatory cytokines. Typically, monocytes produce cytokines that include interleukin-12, IL-1 and tumor necrosis factor (TNF) (Rivollier & Kelsall, 2012).

2.6 Neutrophil and Monocyte Counts

The laboratory test results of monocytes and neutrophils largely vary depending on several factors that include age, sex, health exposure history and method of test (Tan et al., 2013). However, the standard range of results considered to be normal has been approved for conventional use. Adults normally have average absolute monocyte count of between 0.2-0.95 x 10^3 cells/microL, with relative count of 4%-11% to total white blood cell count while persons below 21 years have absolute monocyte count range of between 0-0.8 x 10^3 cells/microL with relative count of 4% to total white blood cell count.

On average, the standard range for monocyte count is between 0.12 – 0.80 x 10^9/L and any count of monocytes in the blood circulation above 950/μL is termed as monocytosis (Eo et al., 2015).
The total number of neutrophil granulocytes circulating in the human blood is referred to as absolute neutrophil count (ANC). The neutrophil range values are categorized into three, normal value if ≥ 1500 cells/ mm^3, mild neutropenia if ≥1000 but <1500/ mm^3, moderate neutropenia if ≥500 but <1000/mm^3, and severe neutropenia if < 500/mm3.On average, the standard neutrophil count range is between 2.00-7.00 x 10^9/L and any count above this upper limit is termed as neutrophilia.

In adults, the total count of white blood cell (WBC) range between 4,400 to 11,000 cells/microL given as (4.4 to 11.0 x 10^9/L), and mature neutrophils are abundant, being the majority with about 60% of the total count. However, the white blood cells are compost of several differentiated cell types that include neutrophils with relative count of 50-60%, lymphocytes with relative count of 20-40%, basophils with relative count of 0.5-2%, eosinophils with relative count of 1-4%, monocytes with relative count of 2-9%.

More often clinicians use monocytosis and neutrophilia from full hemogram assays parse as an indication of bacterial infection (Hellebrekers et al., 2018). It is reported that some deficiencies that include excess use of non-UTI laboratory tests such as CBC have affected most research studies and diagnosis in the hospitals (Mbatia et al.,2023).

CHAPTER THREE
METHODS AND MATERIALS

3.1 Study Area and Population

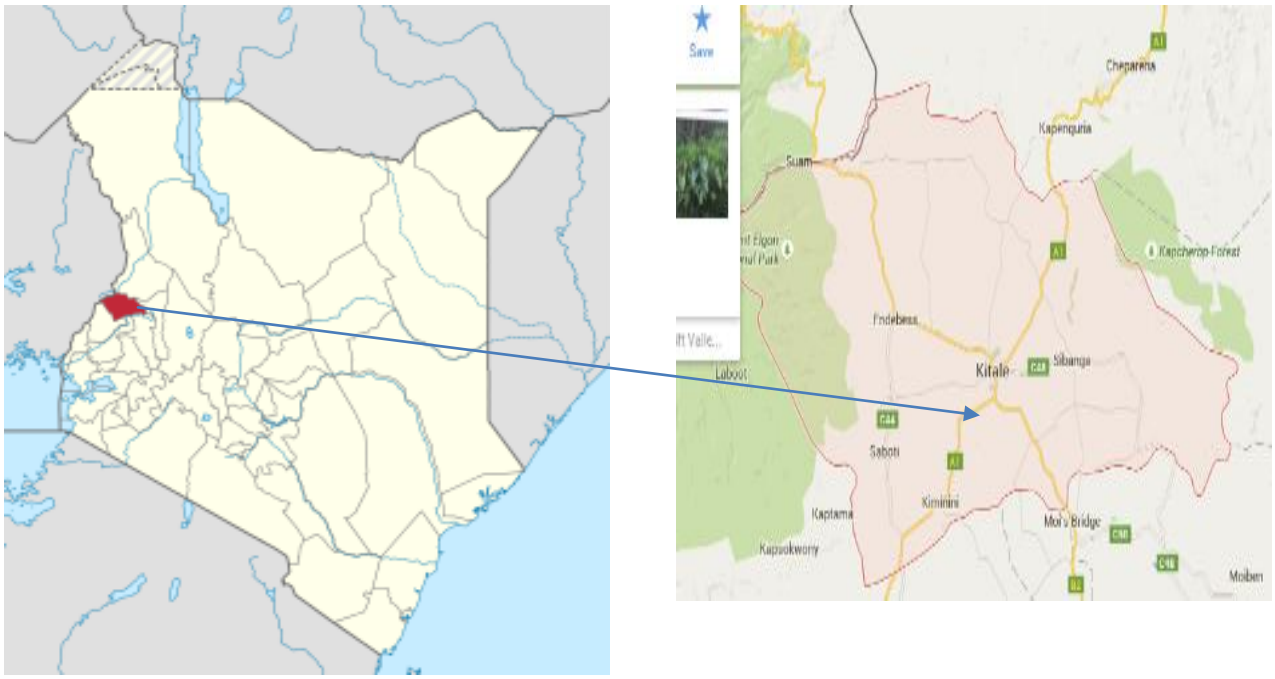
The study was carried out at Trans-Nzoia County Referral Hospital at the center of Kitale town in Trans-Nzoia County, situated 380 km North-west of Nairobi, lying on latitude 1.0567° N and longitude 34.9507° E, in Kenya. The county has a total population of 990,341 according to the national census data for the year 2019.

The prevalence of bacterial infections in Western Kenya, Trans-Nzioa County according to the research carried out in 2019 on anti-microbial resistance is estimated at 67.7% which is higher than the overall national prevalence rate of 54.1% (Susan et al., 2022). UTI prevalence is highest in the age range between 25-30 years with 16.2% and children below five years have a prevalence rate of 1.2%. This was in comparison to the research conducted in Nairobi region hospitals, KEMRI Centre for Microbiology Research Lab, Nanyuki Teaching and Referral Hospital, and Makueni Level 5 Hospital (Susan et al., 2022). UTI prevalence rate among adults between 20 to 50 years of age is about fifty times more common in females than males (Gordon & Mermel, 2013) while young and sexually active women between the age of 18 to 24 years have the highest incidence rate of UTIs (Warkulwiz et al., 2017).

Trans-Nzoia County Referral Hospital is equipped with adequate laboratory diagnostic equipment and kits for various procedures that include Auto-Hemato Analyzer for full hemogram, dip sticks for urinalysis, microscopy, cultures, and imaging. This made it possible for the study to realize the required sample size and acquire reliable data for analysis of the proposed thesis research.

Study was carried out between May 2018- March 2020 inclusive of one month pretest period to test validity of the methods, instruments and equipment of the research.

After the clinical examination stage, the study participants were selected by simple random sampling method to allow generalization about the population and eliminate any chances of biasness.



Extract of Trans-Nzoia County map

KEY

Location of Trans-Nzioa County on latitude
1.0567° N and longitude 34.9507° E

Figure 3.1: Location of Trans Nzoia County in Kenya.

3.2 Study Design

The study design was a cross-sectional study which involved both outpatient and inpatient participants. The patients underwent clinical examination by a clinician to determine possible signs and symptoms of UTI that included frequent and urgent urination, painful urination, back and pelvic pain and medical history of the patient. Symptomatic and asymptomatic patients over 20 years of age who presented with manifestations of UTI were referred to the microbiology laboratory without any costs. Before the participants went to the laboratory for sampling and testing, they were taken through the importance of the study and only those who accepted to participate were guided to fill the structured questionnaire forms and attestations of informed consent of voluntary participation. Laboratory investigations for full hemogram assay, urinalysis assay and urine culture were performed with the support of hospital's laboratory personnel.

About 2-4ml of urine sample was obtained from the patients for urinalysis test and urine culture. Urine culture in this case was used as a gold standard diagnosis for urinary tract bacterial infections and a method for identifying specific pathogenic bacterial species involved in the infection. About 1ml of blood was drawn by venipuncture from the same study participant for full hemogram assay to determine the counts of monocyte and neutrophils. Urine cultures with significant growth (counts of $\geq 10^5$ (CFU/ml) per urine sample were considered for further analysis while urine cultures without growth were used as control study group.

The study used structured data entry form (Appendix 2) to manage the results obtained from the laboratory tests.

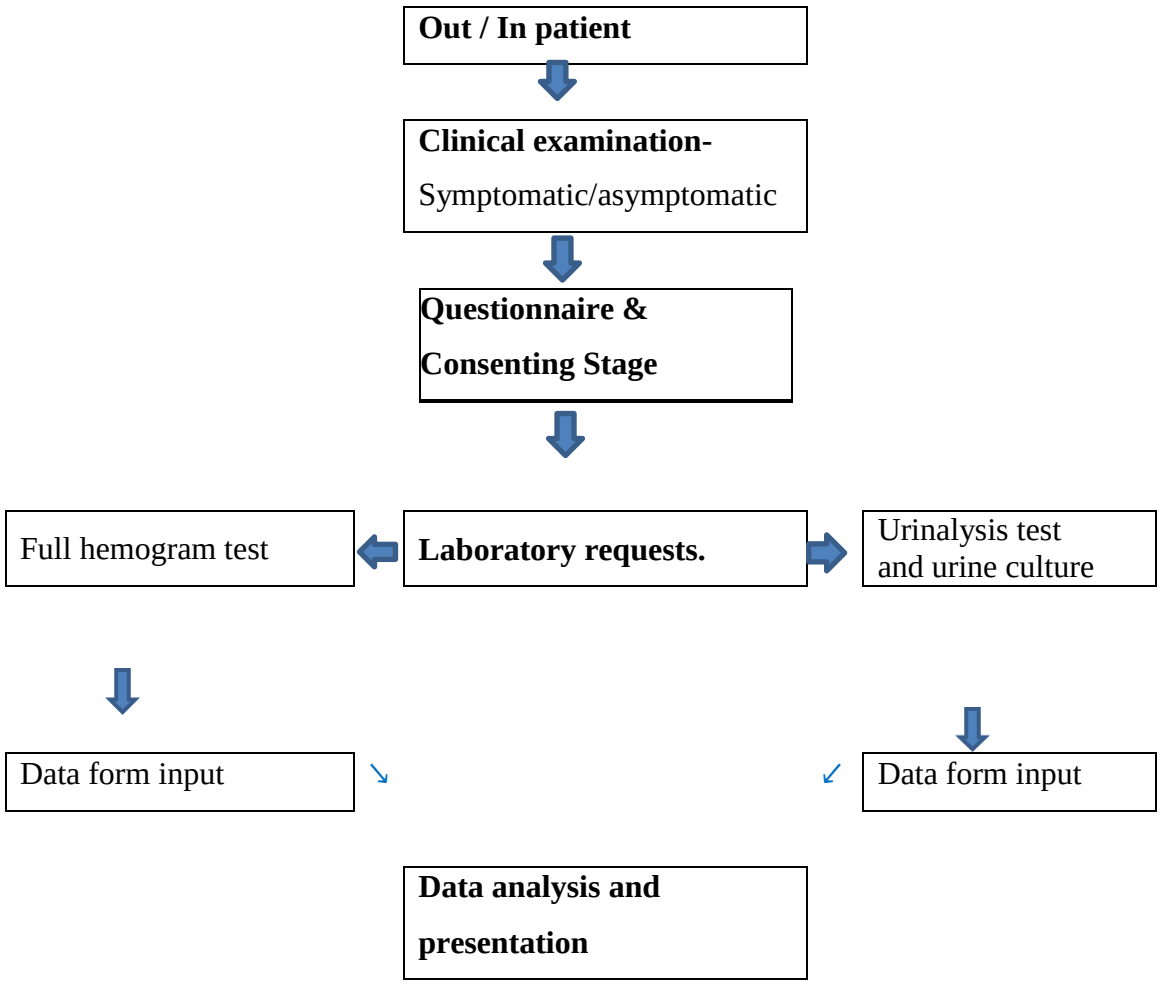


Figure 3.2: Study Design Flow Chart

3.3 Sample Size Determination

Using the estimated prevalence of urinary tract bacterial infections of 13.9% in adults ≥20years (National Health and Nutrition Survey, 2003), the desired level of confidence of 95%, the acceptable margin error of 5% and the formula by (Magnani, 1997), the study was carried out on 173 patients recruited from both in-patient and out-patient departments at the hospital.

Magnani formula $n = \frac{t^2 \times p(1-p)}{m^2}$

Where:

n = being the required sample size

t = being the confidence level at 95% i.e (standard value of 1.96)

p = being the estimated prevalence of urinary tract infections from a referenced survey.

m = being the margin of error at 5% i.e (standard value of 0.05), was used.

Computed as below,

$n = \frac{1.96^2 \times 0.13(1-0.13)}{0.05 \times 0.05} = 173$

Desired sample size (n) = 173

3.4 Inclusion Criteria

Adults, both male and female ≥ 20 years from in-patient and out-patient departments at the hospital were recruited as the study participants. UTI prevalence is highest in the age range between 25-30 years with 16.2% and children below five years have the lowest prevalence rate of 1.2% (Susan et al., 2022). UTI prevalence rate among adults between 20 to 50 years of age is about fifty times more common in females than males (Gordon & Mermel, 2013) while young and sexually active women between the age of 18 to 24 years have the highest incidence rate of UTIs (Warkulwiz et al., 2017).

3.5 Exclusion Criteria

Expectant mothers were exempted from the study participation since they commonly present with cases of pyuria and hematuria which is deemed clinically normal due to increased pressure on the kidneys. Patients who were under antibiotic administration two weeks prior to this recruitment were also excluded from the study.

3.6 Ethical Considerations

The study sought ethical review approvals from Maseno university institutional ethics review committee (reference MSU/DRPI/MUERC/00360/16) and Trans Nzoia county Hospital's Ethical review committee based on study validity, sample collection and management, data confidentiality and patient protection. The study obtained formal and documented informed consent from every study participant. The laboratory test orders were requested by trained and qualified clinical officer or medical officer hospital as per the hospital's protocol and this was done free of charge for the consenting participants. Blood collection was done by trained and qualified phlebotomists at the hospital to reduce risks of bleeding incidences. The specimens were kept within safeguards of confidentiality and disposal was handled under the biohazard protocol and standard operating procedures of the hospital. To ensure data confidentiality, the hospital numbers (patient number) were entered in the study data entry form, instead of the patients' official names.

3.7 Collection and Processing of Urine and Blood Specimens

3.7.1 Urine Sample collection

The study participants were advised by the study Lab technologist on how to collect mid-stream urine and they were provided with a sterilized screw capped urine container. In this `clean-catch' method the participants were advised to wash the genital area to ensure that the test sample is free from any external contamination. About the first 4 ml of urine was not captured, but the next 5 to 10 ml was collected into the container. The urine sample per participant was aliquoted into two labelled containers to allow for urinalysis test and urine culture test. The test was done immediately after urine sample collection but in cases where delays were experienced the sample was kept stored at 4° c.

3.7.2 Urinalysis test

A urinalysis is a quick test that relies on a small sample of urine to determine any possibility of UTI infections or kidney problems. Urinalysis test involves physical examination, chemical test and microscopic investigation. Visual investigation was done on urine to determine the color and clearness. Presence of blood makes the urine reddish or 'tea color' while an infection including UTI may make urine appear cloudy or foamy.

Microscopy examinations were carried to check for red blood cells, white blood cells (or pus cells) and bacteria under high power focus. Dipstick tests were carried out determine abnormalities in urine arising from the elevated levels of PH, leukocyte esterase, proteins, reb blood cells, nitrites, glucose and bilirubin. A dipstick (ACON Labs type, plastic stick with strips chemicals impregnated on it) was dipped into the urine sample. The chemical strips changed the color intensities depending on the substances present in the urine sample and if their levels were above the normal range. Presence of protein and red blood cells may indicate kidney infections that affect glomerular filtrations. Leukocyte esterase is an indication of presence of white blood cells in the urine and presence of pus cells is an indication of death neutrophil cells arising from phagocytosis of bacterial pathogens. Presence of nitrites is an indication bacteria breaking down nitrates in the urine.

Under normal circumstances, nitrites should be negative, leukocyte esterase should be negative, proteins with normal value of <150mg/d, red blood cells with normal value of <2 RBCs/hpf, white blood cells with normal value of <2-5 WBCs/hpf, glucose with normal value < 130mg/d and bilirubin which should be negative (Sharp et al., 2014).

3.7.3 Urine Culture Test

The second aliquot of urine sample of about 0.002 was inoculated using calibrated loop onto cysteine lactose electrolyte deficient (CLED) agar media and incubated aerobically at 37˚C for about 2 to 3 days. CLED is an all-purpose and differential medium that supports the growth of all potential uro-pathogens. Isolated microorganisms with significant growth of $\geq 10^5$ colony forming units of bacteria per milliliter (CFU/ml) per urine sample was considered positive for urinary tract infection (Arinzon, et al., 2012). Urine culture in this case was used as a gold standard diagnosis for urinary tract bacterial infections.

3.7.4 Full Hemogram Test

A sample of blood was drawn by venipuncture from the same study participant for full hemogram assay to determine the counts of monocyte and neutrophils. About 1ml of venous blood in ethylene di-amine triacetic acid (EDTA) tube was obtained by a qualified and practicing phlebotomist. The specimen was run in Auto-Hemato Analyzer machine (Analyzer YR05120) which uses optical and electronic technologies to enumerate, classify and determine the morphology formations of the blood components. Computer results printed-out on complete

blood count (CBC) was obtained and this contained the counts of red blood cell, hemoglobin concentration, hematocrit, white blood cell, platelet count, red blood cell indices, and leukocyte differential count. The normal range of counts of neutrophils in adults is between 2.5-7.5*10^9/L (Hellebrekers P, 2018) while the normal range of counts of monocytes in adults is between 0.12-0.8*10^9/L (Chang HJ, 2015).

3.8 Data Management and Analysis

The data generated from urinalysis, full hemogram assays and urine culture was extracted from the questionnaires and consolidated into an Excel spread sheet (Appendix 2). The first objective on determination of the correlation between the counts of neutrophils and monocytes from full hemogram assay was analyzed using Pearson correlation coefficient method with a statistical significance of $P<0.05$. The second objective on determination of the correlation among the counts of leukocytes, proteins, nitrites and red blood cells from urinalysis assay was tested using Pearson correlation coefficient method with statistical significance of $P<0.05$ to determine the level of correlations and one way analysis of variance (ANOVA) at $p< 0.05$ was used to test for statistical differences among the means of the urinalysis variables.

The third objective on determination of the extent of reliability of counts of full hemogram assay and urinalysis assay in relation to urine culture test was analyzed using percentages of elevated levels of full hemogram assay and urinalysis assay compared to positive urine culture test. The reliability of urinalysis assay was further tested using the qualitative test of Cronbach's Alpha tool and the rating was determined as per the scale given by George and Mallery (2003). From the urine culture test, the culture cases that have no significant growth, $\leq 10^5$ (CFU/ml), were further analyzed as a study control group to determine effects of potential confounding factors (external independent variables). The statistical results were illustrated and presented using tables, line and bar graphs.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic Characteristics and Diagnosis of UTI

Out of 173 patients who were clinically suspected of UTIs, 122 (70%) confirmed positive for UTIs by urine culture while 51 (30%) were negative. 87 (50.2%) were females while 86 (49.8%) were males. The youngest participant was 20 years while the oldest was 84 years with mean age of 37.7 years. Out of 122 positive cases 98 (80%) were inpatient while 24 (20%) cases were outpatient. Majority of the positive cases about 76% were between the age of 20-44 years.

4.2 Correlation between the counts of neutrophils and monocytes from full hemogram assay in patients with confirmed urinary tract bacterial infections

Determination of the correlation between the counts of neutrophils and monocytes from full hemogram assay was analyzed using Pearson correlation coefficient method with a statistical significance of $P<0.05$. The outcome indicated a correlation coefficient $R=0.0794$ and P-Value of 0.299078 at $p < 0.05$. Mean for neutrophil counts at 6.745 was within the normal range with 55 cases (45.08%) being neutrophilic while the mean for monocyte counts at 0.848 was slightly above normal range with 24 cases (19.67%) having monocytosis. Table 4.1 illustrates the mean and standard deviations of neutrophils and monocytes.

Table 4.1: Illustration of mean and standard deviations of neutrophils and monocytes.

	Mean	Std Deviation	Median	High	Low	Normal range
Neutrophils	6.745	5.32	5	30.5	0.7	2.0 - 7.0
Monocytes	0.84	3.29	0.43	6	0.1	0.12 - 0.80

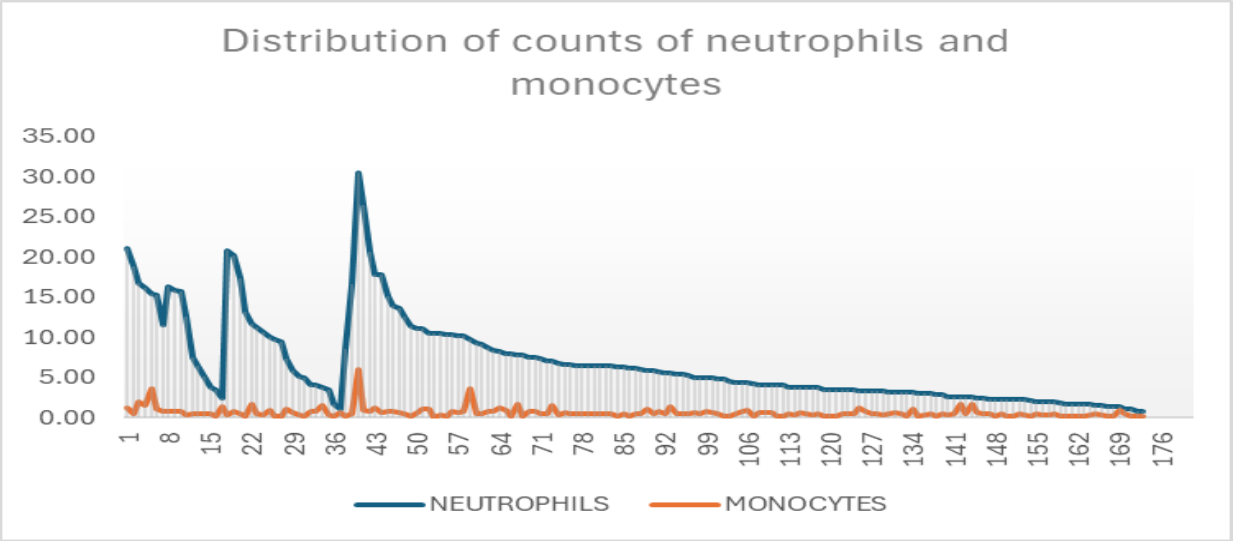


Figure 4.1: Illustration of distribution of counts of neutrophils and monocytes

4.3 Correlation among the counts of leukocytes, proteins, nitrites and red blood cells from urinalysis assay in patients with confirmed urinary tract bacterial infections

Determination of the correlation among the counts of leukocytes, proteins, nitrites and red blood cells from urinalysis assay was tested using Pearson correlation coefficient method with statistical significance of $P<0.05$ to determine the levels of correlations. One way analysis of variance (ANOVA) at $p< 0.05$ was also used to test for statistical differences among the means of the urinalysis variables including leukocytes, proteins and red blood cells. Using Pearson correlation coefficient at $p<0.05$, all the three variables were correlated with strong relationship between leukocytes and proteins with $R=0.411$ and p value of 0.00001.

Table 4.2: Illustration of correlation among the variables of urinalysis assay

Pearson Correlations at p < 0.05	**R-Value**	**P-Value**
Red blood cells vs Leukocytes	0.176	0.02
Leukocytes vs Proteins	0.411	0.00001
Proteins vs Red blood cells	0.116	0.013

One way analysis of variance (ANOVA) was used to analyze statistical differences among the means of the urinalysis variables. The probability of the result was less than 0.0001 at $p < 0.05$ and degree of freedom (d.f) of 2. This was statistically significant meaning there was a statistical difference among the means of the urinalysis variables. Table 4.3 illustrates ANOVA results among the variables of urinalysis assay.

Table 4.3: Illustration of ANOVA results among the variables of urinalysis assay.

The results of a ANOVA statistical test

Source of Variation	Sum of Squares	d.f.	Mean Squares	F
Between	7788.	2	3894.	27.10
Error	7.4137E+04	516	143.7	
Total	8.1924E+04	518		

The probability of this result is less than 0.0001 at $p < 0.05$

Out of 122 participants that tested positive for urine culture, 39 (31.9%) cases had leukocytosis, 25 (20.5%) cases had proteinuria while 12 (9.84%) cases had hematuria. Under normal circumstances proteins should be <150mg/d, red blood cells should be <2 RBCs/hpf while leukocytes should be <2-5 WBCs/hpf. Figure 4.2 illustrates the distribution of Leukocytes, RBCs and Proteins from Urinalysis assay.

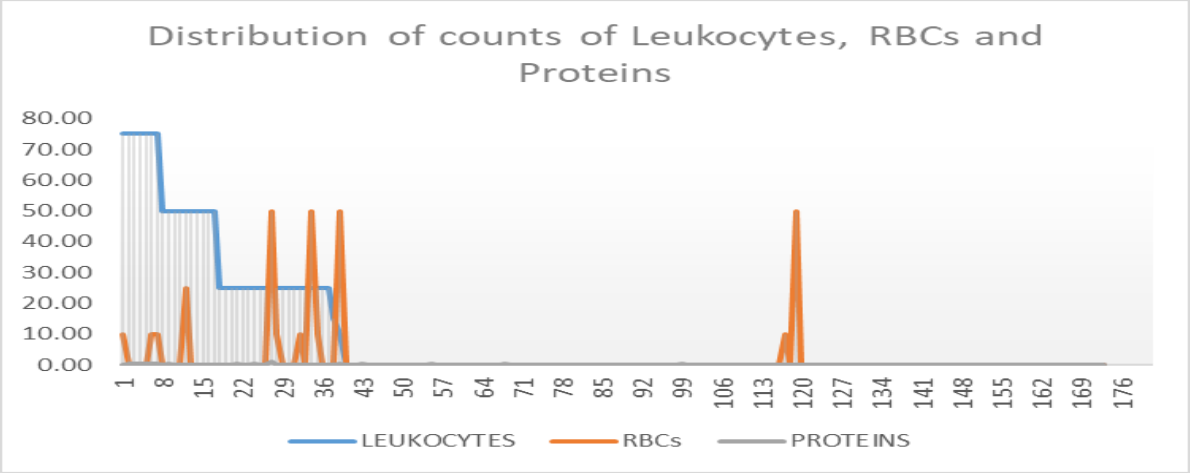


Figure 4.2: Illustration of distribution of counts of Leukocytes, RBCs and Proteins from Urinalysis assay

4.4 The extent of reliability of counts of full hemogram assay and urinalysis assay in relation to urine culture test

The third objective on determination of the extent of reliability of counts of full hemogram assay and urinalysis assay in relation to urine culture test was analyzed using percentages of elevated levels of full hemogram assay and urinalysis assay compared to positive urine culture test. The

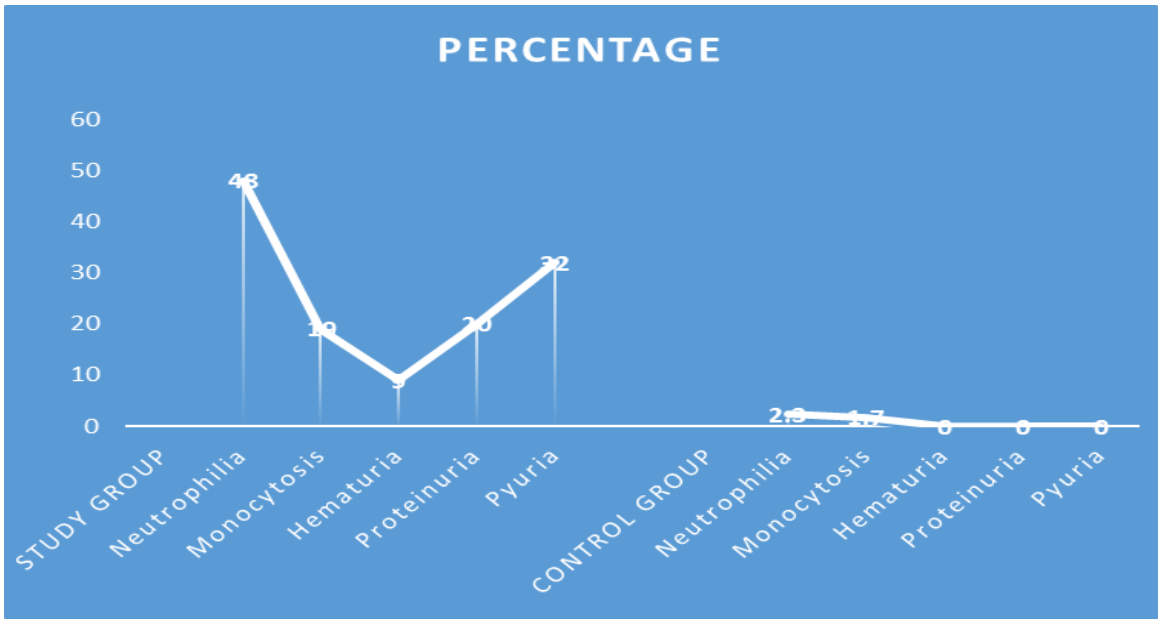
reliability of urinalysis assay was further tested using the qualitative test of Cronbach's Alpha tool and the rating was determined as per the scale given by George and Mallery (2003).

4.4.1 Urine culture test

Out of 173 patients who were clinically suspected of UTIs, 122 (70%) confirmed positive for UTIs by urine culture while 51 (30%) were negative. Out of 122 positive cases, 55(45.08%) had neutrophilia, 24 (19.67%) had monocytosis, 39 (31.9%) cases had leukocytosis, 25 (20.5%) cases had proteinuria while 12 (9.84%) cases had hematuria. 51 cases that had no growth were used as a study control group to eliminate any chances of confounding independent variables that may affect generalized interpretation of the study results. From the study control group, the incidence of neutrophilia at 2.31% and monocytosis at 1.73% are within the assumed margin of error of 5% that was adopted in sampling of 173 study participants. Table 4.4 illustrates the counts of neutrophilia, monocytosis, hematuria, proteinuria and pyuria from urine culture. Figure 4.4 illustrates the distribution of counts of neutrophilia, monocytosis, hematuria, proteinuria and pyuria from urine culture.

Table 4.4: Illustration of counts of neutrophilia, monocytosis, hematuria, proteinuria and pyuria from urine culture

Urine Culture Outcome=173		
Positive growth ≥10^5 (CFU/ml) (Study group)	**122 Cases**	**% of counts above normal range**
✓ Neutrophilia	55	45.08%
✓ Monocytosis	24	19.67%
✓ Hematuria	12	9.84%
✓ Proteinuria	25	20.49%
✓ Pyuria	39	31.97%
Null growth ≤10^5 (CFU/ml) (Control group)	**51 Cases**	
✓ Neutrophilia	4	2.31%
✓ Monocytosis	3	1.73%
✓ Hematuria	0	0.00%
✓ Proteinuria	0	0.00%
✓ Pyuria	0	0.00%



*Graph 1 is an illustration of a study group while graph 2 illustrates the study control group.

Figure 4.3: Illustration of the distribution of counts of neutrophilia, monocytosis, hematuria, proteinuria and pyuria from urine culture

4.4.2 Cronbach Alpha test

Cronbach Alpha method was used to test reliability of qualitative variables from urinalysis assay.

Construct reliability assessment using Cronbach's Alpha, allows the evaluation of the extent to which a variable or set of variables is consistent in what it intends to measure (Straub, Boudreau, & Gefen, 2004). It is a statistical index that is used to evaluate the internal consistency or reliability of an assessment. Cronbach's Alpha = 0.17 for all 3 variables is rated as unacceptable on the scale given as;

(< 0.5 Unacceptable, > 0.5 Poor, > 0.6 Questionable, > 0.7 Acceptable, > 0.8 Good, > 0.9 Excellent). From values of R there was weak positive correlations, an indication of low convergent validity.

Table 4.5: Illustration of the statistical indices of neutrophils, RBCs and proteins on Cronbach's Alpha scale.

Cronbach Alpha and Related Statistics				
Items	**Cronbach Alpha**	**Std. Alpha**	**G6(smc)**	**Average R**
All items	0.1727	0.6003	0.5261	0.3336
LEUKOCYTES excluded	0.0028	0.582	0.4104	0.4104
RBCs excluded	0.0096	0.586	0.4144	0.4144
PROTEINS excluded	0.2211	0.2993	0.176	0.176

Std*- Standard deviation, G6*- Guttman's Lambda 6, smc*- Squared multiple correlation.

CHAPTER FIVE
DISCUSSION

5.1 Introduction

This study established that the prevalence rate of UTI in Trans-Nzoia County was about 70.52% derived from 122 positive cases out the study sample of 173 cases. This was in concurrence with research by Susan et al. (2020) that the prevalence rate of bacterial infections in Western Kenya, Trans-Nzioa County according to the research carried out in 2019 on anti-microbial resistance was estimated at 67.7% which was higher than the overall national prevalence rate of 54.1%. This also supported the earlier research by Gordon and Mermel (2013) that UTI was the second most prevalent bacterial disease in the human body after gastrointestinal tract infections, with over 8.3M annual hospital visits globally.

From the study control group of 51 cases, the incidences of neutrophilia at 2.31%, monocytosis at 1.73%, RBCs at 0%, proteins at 0% and leukocytes at 0% were well within the assumed margin of error of 5% that was adopted in sampling of 173 study participants implying that any chances of confounding independent variables that could have affected generalized interpretation of the study results were eliminated.

The study found that full hemogram assay and urinalysis assay were not reliable tools for diagnosis of UTI with the best ratings of 45.08% and 31.9% respectively in relation to urine culture test. Urinalysis assay was further rated as an unacceptable tool for UTI diagnosis with a score of 0.17 on Cronbach's Alpha scale with a baseline of less 0.5. These findings agreed with the research as reported by Hellebrekers et al. (2018) stating that UTIs were routinely diagnosed by rapid test method of urinalysis assay as opposed to the most reliable test of urine culture technique. Clinicians more often rely on the counts of neutrophils and monocytes from full hemogram assays as an indication of bacterial infections. Relying on non-specific diagnostic tools lead to wrong treatment and inappropriate antibiotic prescription may lead to multi-drug resistance, human suffering and unnecessary expenses. From the study results it was confirmed that there was a knowledge gap in the extent of reliability of urinalysis and full hemogram assays in relation to the gold standard method of urine culture technique and this was addressed by the findings of this study.

5.2 Correlation between the counts of neutrophils and monocytes from full hemogram assay in patients with confirmed urinary tract bacterial infections

From the reported results, it was concluded that neutrophils with 45.08% were more reliable diagnostic indicators for UTIs as compared to monocytes with 19.67% and this concurred with the findings by Leben & Rakhymzhan (2018).

The results indicated that there was no correlation between the counts of neutrophils and monocytes with p-value of 0.299078 at $p < 0.05$ and the level of relationship was very weak with $r=0.0794$ which was close to zero. Out of 122 cases that tested positive for UTI as confirmed by urine culture test, 45.08% were neutrophilic, which supports the previous research findings by Oliveira et al. (2016) that neutrophils are the majority type of leucocytes in the human body and form a critical part of the innate immunity. Neutrophils are the essential primary responders towards the site of inflammation and they extravasate through the blood vessels to the interstitial tissue, in response to the chemical signaling by immune components such as interleukin-8 (IL-8) or C5a in the process of chemotaxis. From the same full hemogram assay 19.67% of the cases had monocytosis indicating that monocytes are also essential defense mechanism in the human body immune system responding to inflammation signals such as cytokines that trigger movement to the sites of infection in the tissues where they immediately undergo division and differentiate into resident macrophages and dendritic cells to mount an immune response within approximately hours as cited in (Yona & Breker, 2013).

5.3 Correlation among the counts of leukocytes, proteins, nitrites and red blood cells from urinalysis assay in patients with confirmed urinary tract bacterial infections

The study results confirmed that there was correlation among the red blood cells, leukocytes and proteins but with strong relationship between leukocytes and proteins with $r=0.411$ and p value of 0.00001 at $p < 0.05$. Out of 122 participants that tested positive for urine culture, 31.9% cases had leukocytosis, 20.5% cases had proteinuria while 9.84% cases had hematuria. This implied that leukocytes were more reliable indicators of UTI as compared to RBCs and proteins as asserted by Levey (2015). The presence of protein and red blood cells indicated kidney infections that affect glomerular filtrations hence allowing large molecules to permeate. Leukocyte esterase was an indication of presence of white blood cells in the urine and the

presence of pus cells was an indication of death neutrophil cells arising from phagocytosis of UTI bacterial pathogens.

One way analysis of variance (ANOVA) showed that there were statistical differences among the means of the urinalysis assay variables. The probability of the result was less than 0.0001 at $p < 0.05$ and degree of freedom of 2. This was statistically significant, meaning there was a statistical difference among the means of urinalysis variables. However, large F value of 27.10 indicated that the between group variation was larger than the within group variation in the urinalysis variables.

5.4 The extent of reliability of counts of full hemogram assay and urinalysis assay in relation to urine culture test

The study found that out of 173 patients who were clinically suspected of UTIs, 70.52% confirmed positive for UTIs by urine culture while 29.48% tested negative for bacterial growth. Isolated microorganisms with significant growth of $\geq 10^5$ colony forming units of bacteria per milliliter (CFU/ml) per urine sample was considered positive for urinary tract infection as guided by Arinzon, et al. (2012). However, urine culture technique in this case was used as a gold standard diagnosis for urinary tract bacterial infections. From both assays, neutrophilia was rated the most reliable tool for UTI diagnosis while hematuria was the least.

On reliability and validity of the qualitative variables of urinalysis assay, construct reliability was assessed using the Cronbach's Alpha method to evaluate the extent to which a variable or set of variables was consistent in what it intends to measure as stated by Straub et al. (2004). The overall Cronbach's Alpha score was at 0.17 for all the three variables of urinalysis assay, and this was rated as unacceptable tool for UTIs according to George and Mallery (2003). Leucocytes with 0.0028 and RBCs with 0.0096 were ranked as more reliable diagnostic tools for UTI while proteins were the least with 0.2211. This interpretation was based on the exclusion-impact as computed by the Cronbach's Alpha model. From the multivariate results on the Cronbach's Alpha model, there was weak positive correlation, an indication of low convergent validity.

In general, in relation to urine culture as a gold standard tool for UTIs, full hemogram and urinalysis assays are not reliable diagnostic tools for UTIs with their best ratings determined by this study as 45.08% and 31.9% respectively.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of the Findings

From the study it was found that out of 173 sampled patients 70.52% tested positive for UTIs while 29.48% tested negative. From the study control group of 29.48% cases, the incidences of neutrophilia at 2.31%, monocytosis at 1.73%, RBCs at 0%, proteins at 0% and leukocytes at 0% were well within the assumed margin of error of 5% that was adopted in sampling of 173 study participants implying that any chances of confounding independent variables that could have affected generalized interpretation of the study results were eliminated. It was established that the counts of neutrophils and monocytes from full hemogram assay were not correlated in diagnosis of urinary tract bacterial infections.

From urinalysis assay it was established that all the counts of leukocytes, proteins and red blood cells were correlated with strong relationship between leukocytes and proteins, however, there were statistical differences among their mean values. On the extent of reliability, it was found that full hemogram assay was not a reliable tool for diagnosis of UTI with the best rating of 45.08% in relation to urine culture test. It was also noted that urinalysis assay was not a reliable tool for diagnosis of UTI with the best rating of 31.9% in relation to urine culture test. The study further confirmed that urinalysis assay was an unacceptable tool for UTI diagnosis with a score of 0.17 on Cronbach's Alpha scale with a baseline of less 0.5.

6.2 Conclusions

In conclusion, the study found that;

i. Neutrophils with 45.08% are more reliable diagnostic indicators as compared to monocytes with 19.67% and their counts are not correlated.
ii. Leucocytes with 31.9% are more reliable indicators of UTIs while red blood cells are the least with 9.84%, but all the variables are correlated in urinalysis assay.
iii. In relation to urine culture as a gold standard tool, both full hemogram and urinalysis assays are not reliable tools for diagnosis of UTIs with the best ratings of 45.08% and 31.9% respectively. Urinalysis assay further rated as unacceptable with a score of 0.17 with a baseline of less than 0.5.

6.3 Recommendations from the Current Study

i. Hospitals without urine culture test may rely on the counts of neutrophils from full hemogram assay as opposed to counts of monocytes in in diagnosis of UTIs.

ii. Hospitals without urine culture test may rely more on the counts of leukocytes from urinalysis assay as opposed to proteins and red blood cells.

iii. From the study outcome, hospitals should use urine culture technique in diagnosis of the urinary tract bacterial infections as opposed to rapid tests. On the worst scenario where urine culture is not affordable or not available, a combination of neutrophils, leucocytes and protein counts would give more predictive diagnostic tool for UTBIs.

6.4 Recommendations for Future Studies

The study recommends further research to focus on evaluation of reliability of the existing diagnostic tools with the aim of establishing the best combinations of tools that can give better results.

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APPENDICES

APPENDIX 1

EVALUATION OF NEUTROPHILIA, MONOCYTOSIS AND URINALYSIS ASSAY AS DIAGNOSTIC TOOLS FOR URINARY TRACT BACTERIAL INFECTIONS IN PUBLIC AND PRIVATE HEALTH FACILITIES IN TRANS NZOIA COUNTY, KENYA															
RAW DATA SPREADSHEET															
	INDIVIDUAL PARTICULARS		**ADMISSION TYPE**		**CLINICAL EXAMINATION-UTI**		**LAB EXAMINATION (VALUES 10^9 /L)**							**MEDICATION**
							FULL HAEMOGRAM		***URINALYSIS***				***CONFIRMATORY TEST***	***ANTIBIOTIC PRESCRIPTION***
SERIAL NO.	SEX	AGE	INP NO.	OPD NO.	MATIC	TOMATIC	NEUT#	MONO#	LEUC#	RBCs	PROT#	NITR#	CULTURE	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX 2: MEDICAL RESEARCH INFORMED CONSENT FORM

Informed Consent form for adults, both male and female ≥ 20 years who attend Trans-Nzoia County Referral Hospital, and whom I'm inviting to participate in research on evaluation of neutrophils and monocytes as diagnostic tools for urinary tract bacterial infections in Trans-Nzoia County Referral Hospital

The title of my research project

"Evaluation of Neutrophilia and Monocytosis as Diagnostic Tools for Urinary Tract Bacterial Infections in Trans-Nzoia County Referral Hospital"

Name of Principal Investigator: Julius Kipkoech Serem

Name of Organization: Maseno University, Reg no. PG/MSC/082/08

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: INFORMATION SHEET

Introduction

I am Julius Kipkoech Serem, studying Master of Science in Biomedical science (medical immunology option) at Maseno University. I'm doing research on evaluation of neutrophils and monocytes as diagnostic tools for urinary tract bacterial infections in Trans Nzoia County referral hospital. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff

Purpose of the research

Urinary tract bacterial Infection is one of the most common and dangerous diseases in this in country. However, in public and private health facilities without blood and urine culture tests, the levels of neutrophils and monocytes in full hemogram assays are commonly interpreted as indications of bacterial infections upon which antibiotic treatment is prescribed. In most cases, elevated or low levels of any of these parameters may be ambiguous for definitive diagnosis and subsequent treatment, especially broad spectrum antibiotics that commonly result to multi drug resistance. Therefore, this cross sectional study is designed to evaluate the reliability of the counts of neutrophils and monocytes as diagnostic tools for urinary tract bacterial infections in

Trans Nzoia County referral hospital. The results of this study will help evaluate the reliability of neutrophil and monocyte counts in full hemogram assays in prescribing and administering antibiotics to patients with elevated counts of neutrophils and monocytes both in public and private health facilities that lack blood and urine culture procedures.

Type of Research Intervention

This research will involve a single venous puncture to draw about 1M of blood for full hemogram assay to determine the counts of monocyte and neutrophils and about 2-4ml of urine for urinalysis test and urine culture. There will be no follow-up.

Participant selection

We are inviting all adults, both male and female ≥ 20 years who attend Trans Nzoia county referral hospital to participate in the research on *"evaluation of neutrophils and monocytes as diagnostic tools for urinary tract bacterial infections in Trans Nzoia County referral hospital*".

1. Do you know why we are asking you to take part in this study? **YES** **NO**
2. Do you know what the study is about? **YES** **NO**

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at Hospital will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

1. If you decide not to take part in this research study, do you know what your options are? **YES** **NO**
2. Do you know that you do not have to take part in this research study, if you do not wish to? **YES** **NO**
3. Do you have any questions? **YES** **NO**

Procedures, Protocol and duration

This research will involve a single venous puncture to draw about 1M of blood for full haemogram assay to determine the counts of monocyte and neutrophils and about 2-4ml of urine for urinalysis test and urine culture. There will be no follow-up thereafter.

At the end of the tests, any left-over blood and urine sample will be destroyed immediately.

1. Do you have any questions? **YES** **NO**
2.

Side Effects and Risks

By participating in this research, there are no anticipated side effects or risks, these are normal procedures commonly used in hospitals to draw samples from patients for testing.

1. Do you have any other questions? **YES** **NO**

Benefits

If you participate in this research, there may not be any immediate benefit for you, but your participation is likely to help us find the answer to the research question. However, there may not be any benefit to the society at this stage of the research, but future generations are likely to benefit from precise diagnosis and appropriate prescription of drugs.

1. Do you have any questions? **YES** **NO**

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researcher will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except your clinician.

1. Did you understand the procedures that we will be using to make sure that any information that we as researchers collect about you will remain confidential? **YES** **NO**

2. Do you have any questions about them? **YES** **NO**

Who to Contact

If you have any questions, you may ask them now or later. If you wish to ask questions later, you may contact any of the following:

Julius Kipkoech Serem /0727603347/0716002895/juliasask@gmail.com

This proposal has been reviewed and approved by Maseno University Ethical Review Committee (MUERC) which is a committee whose task it is to make sure that research participants are protected from harm. For any questions pertaining to rights as a research participant, contact person is: The Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622/0722203411, 0721543976, 0733230878; Email address: muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

Other Remarks

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions? **YES** **NO**

PART II: CERTIFICATE OF CONSENT

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and the questions that I have asked have been

answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant_____

Signature of Participant _____

Date _____

Or/On behalf
I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____ AND Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Blood drawn for blood cells test

2. Urine collected for urine test

3. Confidentiality will be kept

4. Participation is voluntary

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Form has been provided to the participant.

Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

APPENDIX 3

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: PG/MSC/00082/2008

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 26th October, 2016

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR JULIUS KIPKOECH SEREM– PG/MSC/00082/2008

The above named is registered in the Master of Science Programme of the School of Public Health and Community Development, Maseno University. This is to confirm that his research proposal titled "Evaluation of Neutrophilia and Monocytosis Assays as Diagnostic Tools for Urinary Tract Bacterial Infections in Trans Nzoia County Refferal Hospital" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

MASENO UNIVERSITY RECEIVED 26 OCT 2016 GRADUATE STUDIES

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES

Maseno University *ISO 9001:2008 Certified*

APPENDIX 4

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 28th September, 2017

TO: Mr. Julius Kipkoech Serem
Department of Biomedical Sciences
School of Public health and community Development
P. O. Box Private Bag
Maseno University

REF: MSU/DRPI/MUERC/00360/16

RE: Evaluation of Neutrophilia and Monocytosis as Diagnostic Tool for Urinary Tract Bacteria Infection in Trans-Nzoia County Referral Hospital. Proposal Reference Number MSU/DRPI/MUERC/00360/17

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 28th day of September, 2017 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 27th September, 2018. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th August, 2018.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th August, 2018.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

DIRECTORATE OF RESEARCH PUBLICATION & CONSULTANCIES 28 SEP 2017 MASENO UNIVERSITY

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED