

# Sensitivity Test and Specificity Invention Gram Negative Rods with Gram Staining for Screening Test of Urinary Tract Infection (UTI)

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**ABSTRACT**— *Screening test is not for diagnose, but to determine whether the one who involved in this ailment sick or not. Then for those with positive result will be threatening intensively. In order to prevent the infection. Because screening test is not for diagnose, there for it is requires direct follow up and appropriate treatment. The formulation of this research was the sensitivity and specificity's values of gram negative rods invention with Gram staining as a screening test of urinary tract infection (UTI). This research was a cross sectional study, the samples of this research was 18-20 years old students of laboratory technician program in Polytechnic of Health Ministry of Health Mataram taken with consecutive sampling method. The urine culture as a Gold standard of urinary tract infection (UTI) test. The result from this research shows that 8 samples with bacteria positive and 69 samples with negative result from 77 samples. The Gram staining from samples shows that 9 samples with positive result and 76 are negative. Urine microscopy test show 15 samples are positive leukocyturia and 62 are negative. Sensitivity and specificity value present from the 2 x 2 chi – square table. Sensitivity value is 75.05% and specificity is 80,51%. Gram staining of urine without centrifugation result is higher than leukocyturiaurine.*

**Keywords**—*Gram Staining, Sensitivity, Specificity, Urinary Tract Infection (UTI)*

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## 1. INTRODUCTION

Indonesia is a developing country with a high number of infectious diseases, dominated by respiratory tract infection followed by gastrointestinal infections, and other infections such as urinary tract infections, skin and even systemic infections. Urinary tract infections is one of the infectious diseases among the most prevailing infectious diseases with a substantial financial burden on society.

In the US, UTIs are responsible for >7 million physician visits annually (Foxman, 2002). Approximately 15% of all community-prescribed antibiotics in the US are dispensed for UTI (Mazzulli, 2002) and data from some European countries suggest a similar rate (UVI, 2007). In the US, UTIs account for >100,000 hospital admissions annually, most often for pyelonephritis (Foxman, 2002). These data do not account for complicated UTI associated with urological patients, the prevalence of which is not well known. At least 40% of all hospital acquired infections are UTIs and the majority of cases are catheter associated (Ruden, s1997).

Bacteriuria develops in up to 25% of patients who require a urinary catheter for one week or more with a daily risk of 5-7% (Maki DG, 2001; Tambyah P, 2010). The recent Global Prevalence Infection in Urology (GPIU) studies have shown that 10-12% of patients hospitalized in urological wards have a healthcare-associated infection (HAI). The strains retrieved from these patients are even more resistant (Johansen, 2007).

Urinary Tract Infection (UTI) or Tracts Urinary Infection (UTI) is a condition of the infection rate of microorganisms in the urinary tract. Urinary Tract Infection (UTI) is a condition in which was found a bacterial infection in the urinary tract. Women were more susceptible to this disease because the vessels of the urethra (the tube that connects the bladder to the outside of the body) of women is shorter (about 3-5 cm). In another case with the male urethra are longer than woman it makes the bacteria more difficult to enter the body. Common symptoms were characterized by more frequent to pee but the volume of urine was quite a bit, pain when urinating, pain like burning in the bladder. (Barbara, 1998).

Urinary tract infection is one of the infectious diseases where the number of bacteriuria were multiplied by the number of germs in urine culture  $> 100,000/\text{ml}$  urine (Morgan, 2003). Asymptomatic bacteriuria is defined as a positive urine culture without complaint, while symptomatic bacteriuria defined as a positive urine culture accompanied by complaints (Sukandar, 2006). UTI is caused by different bacteria including both gram positive and gram negative bacteria. Among the negative gram bacteria *Escherichia coli* cause approximately 75% of UTIs worldwide (Beyene & Tsegaye, 2011). *Escherichia coli* has recognized virulence factors which aid in the persistence of bacteria in the urinary tract and induce inflammation. The factors include the presence of pili or K antigen in bacterial capsule, fimbriae, hemolysin and colicin production and the ability to acquire iron etc. (Rushton, 1997). *Klebsiella*, *Staphylococci*, *Enterobacter*, *Proteus*, *Pseudomonas* and *Enterococci* species are more often isolated from penitents show UTIs symptoms (Bronsema et al., 1993). Some reports mentioned that coagulase negative *Staphylococci* are a common cause of urinary tract infection. *Staphylococci saprophyticus* tends to cause infection in young women of a sexually active age (Schneider & Riley, 1996). There is a notable increase in the prevalence of antimicrobial resistance among pathogens that cause UTI worldwide due to extensive misuse of antibiotics in practice (Grude et al., 2001)

Another test conducted to support the diagnose of UTI are urinalysis leukocyturia or Puria: are one marker of UTI. Bacteriological examination are microscopic examination and bacterial culture multistrip dipstick test for WBC (leukocyte esterase test) and nitrite (Griess test for nitrate reduction). Positive leukocyte esterase test: if the patients had pyuria. Nitrate reduction test positive: if there are bacteria reducing nitrate to nitrite in normally urine. UTI can be caused by many kinds of microorganisms, mostly are bacteria. Another cause of UTI are rarely found by fungus, virus, chlamydia, parasites, microbacterium. Based on the results of urine culture examination most of UTI are caused by gram-negative aerobic bacteria that is normally found in the gastrointestinal tract (*Enterobacteriaceae*) (Ryu, et.al, 2007).

The most Examination performed in outside, especially in the health center of observation by researchers is the examination of urinary sediment leukocyte namely leukocyturia (discovery of leukocytes in urine) there are five or more leukocytes (white blood cells) per area of view in the urine sediment. If it turns out the examination of this sediment is not good enough to detect the presence of UTI in children, there have been so many cases of children with undiagnosed UTI so will increase the risk of long-term complications. Therefore it is important to know the sensitivity and specificity the examination of the urinary sediment. Is another urinalysis checks better than the examination? Which examination are best for detecting UTI's or which examination that can eliminated the UTI? By knowing the answers of the questions, it's expected to be able to use the best urinalysis examinations, so that will be used as reference in therapy and to determine whether it needed or not for doing the other examination like urine culture. This methods was a cross sectional study, conducted in the Department of Health Analyst of Polytechnic of Health Ministry of Health Mataram. By using the formula for a diagnostic test, it needs a total of 77 students.

In this research, there will be some test; sensitivity and specificity test of Gram Stained by making a direct preparation of unspun urine by putting two drops of urine on the glass object, dried it and then stained with Gram stain. The Preparats will be read with oil immersion on it (oil immersion field = oil). It is become positive if found one or more bacteria per are of view, this method has used by the researched of Arslan, et al., (2002). As a comparison to urine culture, the media are using endo agar and blood agar, which is then incubated for 48 hours at a temperature of 35-37°C. Gold Standard urine culture is called positive when colony growth of pathogenic bacteria found  $\geq 100,000/\text{mL}$  urine. On the results of research there will be some test: diagnostic test (by entering into the examination table 2 x 2) to assess the sensitivity, specificity.

Screening is a process which is intended to identify diseases of unknown / not detected by using various test / test that can be applied appropriately in a correct scale. Screening or filtering of cases (screening tests) is a way to identify a disease that has looked through a test or examination or other procedure that can quickly split between people who may suffer from the disease with people who may not have suffered. Screening tests is not to diagnose but to determine if they are sick or not. For patients who are positive there will be some intensive treatment in order not contagious others. Screening is generally not a diagnostic test, it requires a study (follow-up) were quick and appropriate treatment. The purpose of screening is for the early detection of asymptomatic disease or with symptoms which are not typical of the people who seem healthy, but may suffer from the disease, those who have a high risk of disease (Population at risk). Discovered asymptomatic patients with treatment can be done thoroughly so as not to endanger himself or the environment and does not become a source of disease transmission. Target filtering are chronic diseases such as bacterial infections (Leprosy, Tuberculosis etc.), viral infections (Hepatitis) non-infectious diseases (Hypertension, Diabetes Mellitus, Coronary Heart Disease, Cervical Ca Prostate Ca, glaucoma) HIV-AIDS. Sensitivity is the ability of a test for identify individuals with the precise, with a positive result and really sick. Specificity is the ability of a test for identify individuals with the appropriate, with negative results and was not sick. (Arikunto, Suharsimi, 2010). This study aims to

find the sensitivity and specificity of the invention of gram-negative bacilli with gram staining as a test filter diagnostic of urinary tract infection.

Benefits: This study is a theoretical application of microbiology laboratory and diagnostic examination of urinary tract infections and implementing science of urinalysis examinations in the areas of microbiology. Related Institutions of microbiological examination to establish urinary tract infection can apply the appropriate screening examination.

## 2. MATERIALS AND METHODS

### 2.1 Conceptual Framework

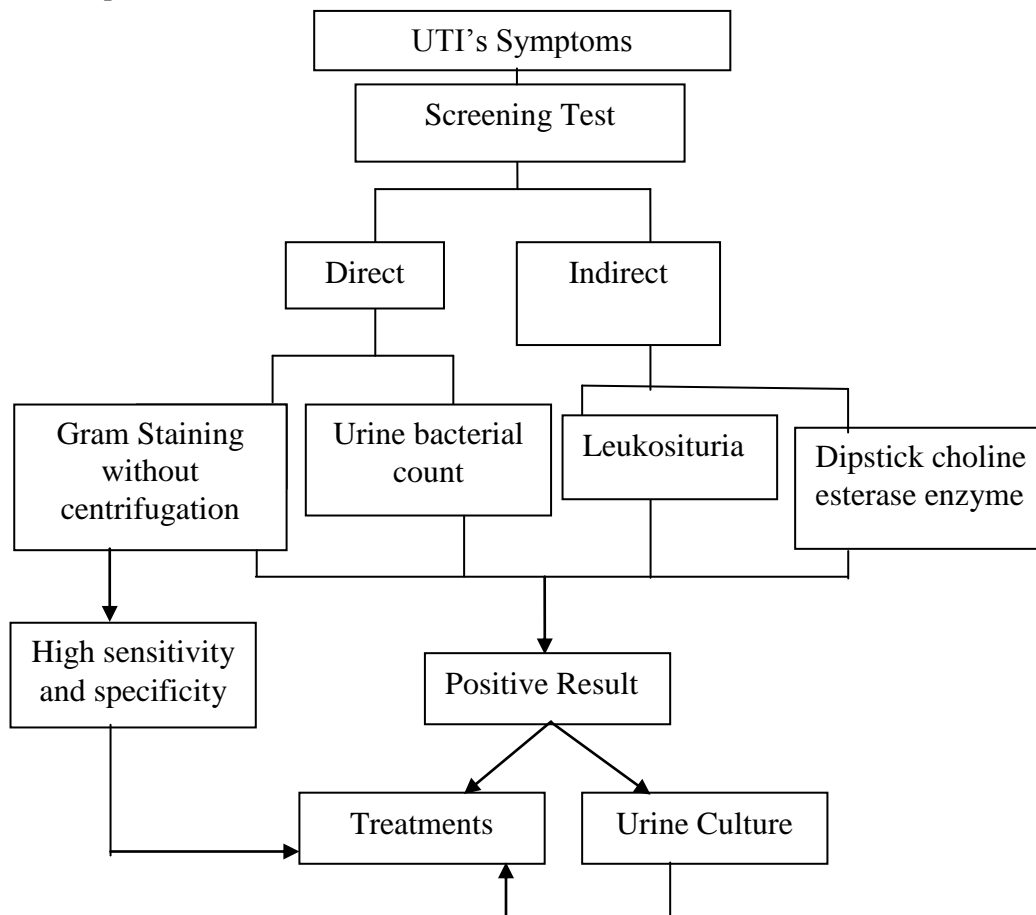


Figure 1 Framework Concepts

### 2.2 Place and Location of Research

The experiment was conducted at the Department of Health Analyst Mataram Poltekkes Kemenkes Mataram during March to August 2014.

### 2.3 Type and Design Research

This study was a cross sectional study, all study subject aged around 18-20 years old college student who is attending education in the Department of Health Analyst Mataram Poltekkes Kemenkes Mataram taken by consecutive sampling.

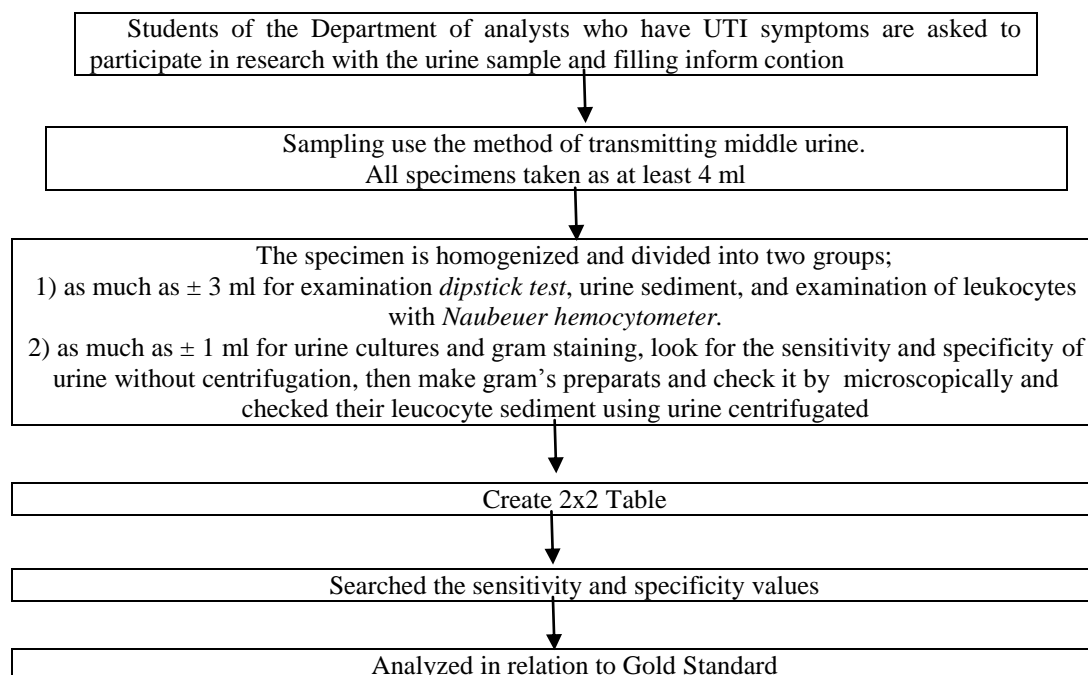
- Inclusion criteria were: All Students with a fever with no obvious cause, if the fever is accompanied by one of the the symptoms of dysuria, pungent smell of urine, or hematuria
- Exclusion criteria were: The students has excluded from the study, if the students are not willing to participate in the study or if there is a history of antibiotic usage in the last 3 days.
- By using the formula for some diagnostic test, it needs a total of 77 respondents.

### 2.4 Variables and Data Collection Techniques

- The independent variables in this study is a laboratory results of the examination of gram-negative bacteria, leukocyturia and results of bacterial culture.
- The dependent variable is the sensitivity and specificity values of the examination of gram-negative bacteria, leukocyturia

## 2.5 Data source

### Workflow



## 2.6 Research Instruments

### a. Tools and Materials

**Tools:** Petri dish, glass beaker, erlenmeyer, glass objects, cover glass, stir bar, knives, hot plate, micropipette, tip, incubator, inoculating loop, colony counter, test tube, tube rack, light spirits, oven, autoclave, lighters, rubber, cotton, tissue, paper pH

**Reagents:** Nutrient agar, gram stain.

## 2.7 Data collection

### a. Sampling method transmit middle urine (MSU)

All specimens taken as at least 4 ml, homogenized, and were divided into 2 groups; 1) as much as  $\pm 3$  ml for examination dipstick test, urine sediment, and examination leukocytes with Neubauer hemocytometer. 2) as much as  $\pm 1$  ml to 0.01 ml urine culture for as many as 0.01 ml gram staining. All specimens immediately sent and examined within 1-2 hours after taking the urine. If the examination cannot be done in 2 hours, then the urine is first stored in a refrigerator or a cold pack, a maximum of 24 hours.

### b. Examination of urine sediment carried perform urine centrifugation at a speed of 1500 rpm for 5 minutes. Test results obtained are reported positive when $\geq 5$ leukocytes / hpf.

### c. Gram staining is performed by making a direct preparation of unspun urine by putting two drops of urine on the glass object, dried and then stained by gram. If found one or more bacteria per field of view with oil immersion (oil immersion field) is called positive.

### d. For urine culture, media used endo agar and blood agar, which is then incubated for 48 hours at a temperature of 35-37°C. Called positive urine culture if found pathogenic bacteria colony growth $\geq 100,000$ / ml urine.

### e. In the research conducted diagnostic test (by entering into the examination table (2 x 2) to assess the sensitivity, specificity,

## 2.8 Data analysis

The data obtained were analyzed descriptively

## 3. RESULTS

Based on the results of urine examination painted gram then centrifuged made wet preparations for examinations leukocyturia and cultured as much as 0.1 ml then made the examination table like Table 3.1

**Table 1:** Urine examination painted gram then centrifuged made leukocyturia wet preparations for examination and culture.

No.	Basil Gram (-)	Leukocyturia	Bacteria Culture Leukocyturia
1.	Negative	Negative	Negative
2.	Negative	Negative	Negative
3.	Negative	Negative	Negative
4.	Negative	Positive	Negative
5.	Negative	Negative	Negative
6.	Negative	Negative	Negative
7.	Positive	Positive	Positive
8.	Negative	Negative	Negative
9.	Negative	Negative	Negative
10.	Negative	Negative	Negative
11.	Negative	Negative	Negative
12.	Negative	Negative	Negative
13.	Negative	Positive	Negative
14.	Negative	Negative	Negative
15.	Negative	Negative	Negative
16.	Negative	Positive	Negative
17.	Negative	Negative	Negative
18.	Negative	Positive	Negative
19.	Negative	Negative	Negative
20.	Negative	Negative	Negative
21.	Negative	Negative	Negative
22.	Positive	Positive	Positive
23.	Negative	Negative	Negative

24.	Negative	Negative	Negative
25.	Negative	Positive	Negative
26.	Negative	Negative	Negative
27.	Negative	Negative	Negative
28.	Negative	Positive	Negative
29.	Positive	Negative	Positive
30.	Negative	Negative	Negative
31.	Negative	Negative	Negative
32.	Negative	Negative	Negative
33.	Negative	Negative	Negative
34.	Negative	Negative	Negative
35.	Negative	Negative	Negative
36.	Negative	Negative	Negative
37.	Positive	Negative	Negative
39.	Negative	Negative	Negative
40.	Negative	Negative	Negative
41.	Negative	Positive	Negative
42.	Negative	Negative	Negative
43.	Negative	Negative	Negative
44.	Negative	Positive	Negative
45.	Negative	Negative	Negative
46.	Negative	Positive	Negative
47.	Positive	Negative	Negative
48.	Negative	Positive	Negative
49.	Negative	Negative	Negative
50.	Negative	Negative	Negative

51	Negative	Positive	Negative
52.	Negative	Negative	Negative
53.	Negative	Negative	Negative
54.	Negative	Negative	Negative
55.	Negative	Positive	Negative
56.	Negative	Negative	Negative
57.	Negative	Negative	Negative
58.	Negative	Positive	Negative
59.	Negative	Negative	Negative
60.	Negative	Negative	Positive
61	Negative	Negative	Negative
62.	Positive	Positive	Positive
63.	Negative	Negative	Negative
64.	Negative	Negative	Negative
65.	Positive	Positive	Positive
66.	Negative	Ngatif	Negative
67.	Negative	Positive	Negative
68.	Negative	Negative	Negative
69.	Negative	Negative	Negative
70.	Positive	Positive	Positive
71.	Negative	Negative	Negative
72.	Negative	Negative	Negative
73.	Positive	Positive	Positive
74.	Negative	Ngatif	Negative
75.	Negative	Positive	Negative
76.	Negative	Negative	Negative

77.	Negative	Positive	Negative
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Examination of urine culture as the Gold Standard there are 8 samples were positive and 69 negative bacteria, gram urine examination without centrifugation were 9 positive 76 negative. Examination of urine sediment leukocyturia were 15 positive and 62 negative.



Figure 1: *Escherichia coli* in Gram staining

From the results of the inspection made graph to find the value of sensitivity and specificity of each inspection (gram urine examination without centrifugation and examination of urine sediment leukocyturia)

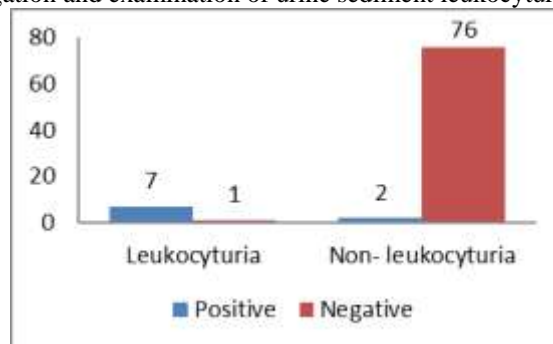


Figure 2: Specificity and Sensitivity with the Examination Gram staining of urine without centrifuge graph

Formula:

- Sensitivity =  $7 / (7 + 1) \times 100\% = 87,50\%$
- Specificity =  $76 / (2 + 76) \times 100\% = 97,43\%$

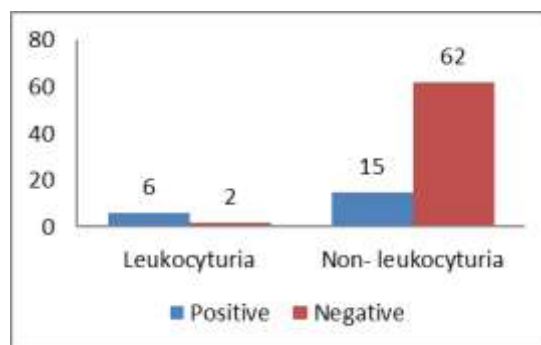


Figure 3: Specificity and Sensitivity Examination leukocyturia Graph

Formula:

- Sensitivity =  $6 / (6 + 2) \times 100\% = 75,05\%$
- Specificity =  $62 / (15 + 62) \times 100\% = 80,51,43\%$

#### 4. DISCUSSION

The method used in this research is descriptive method that analyzes the data at a given time without treatment. The researchers took the data as much as 77 samples of students who experience symptoms of Urinary Tracts Infection who



enroll in the Department of Medical Laboratory Technicians in Polytechnic of Health Ministry of Health Mataram in March through August.

Examination of urinary tract infection is one in which infectious diseases multiply the number of bacteriuria with urine culture germ count > 100,000 /ml urine. Screening Examination of gram staining is done by making the preparation directly from unspun urine by putting two drops of urine on the glass object, dried and then stained with Gram stain. with oil immersion (oil immersion field = oil) is called positive when found the bacteria or more per field of view of research. Staining gram is a differential staining technique used to characterize bacteria as Gram positive and Gram negative. The fixed bacterial smear is subjected to Crystal Violet, Iodine Solution, Alcohol (decolorizing agent) and Safranin respectively. Gram-positive bacteria retain crystal violet and hence appear deep violet in color, while Gram-negative bacteria lose the crystal violet and are counterstained by the Safranin they appear red in color (Korrapati et al, 2010).

The methods of leukocytes Examination from urinary sediment in examination the leukocyturia (discovery of leukocytes in urine) is by finding five or more leukocytes (white blood cells) per field of view in the urine sediment. Gold Standard urine culture is called positive if found colony growth of pathogenic bacteria. Urine samples constitute a major proportion of the samples tested in routine diagnostic laboratories. Although the urine culture is used as the reference standard to determine presence or absence of urinary tract infection, the culture is an expensive and time-consuming method (Christenson, 1985).

The objectives of the study were to compare screening test of UTI by using urine gram staining examination and leukocyturia examination. Based on the results that the sensitivity and specificity using methods Urine gram staining examination of Urinary Tracts Infection made preparations higher and better than leukocyturia examination. The number of false positives in the examination leukocyturia caused by several things that can be error factor must be considered in the examination of urinary sediment for leukocyturia. When scanning, all the material that settles not premixed with the liquid above by shaking the urine, the urine is not shaken the urine sediment will lag the bottom of the bottle container. Examination of the sediment that has been centrifuged, resuspended before being checked so that no sediment is mixed. Light entering the microscope too bright, subtle elements not seen. The tools used included the microscope must not clean. Little dirt on glass objects, glass cover or on a microscope lens that is not clean can be calculated elements of the sediment. Urine volume, speed centrifuges, not according to standards.

## 5. CONCLUSION

- a. Examination of Gram positive urine without actually centrifugation 7, 2 false positives and 76 true negatives and one false negative sensitivity specificity 87.45% 97.43%
- b. Examination of urine sediment leukocyturia 6 real positive, 15 false positives and 62 true negatives and 2 false negative sensitivity 80.51% 75.05% specificity.
- c. Examination of urine culture as the Gold Standard there are 8 samples were positive and 69 negative bacteria
- d. The sensitivity and specificity of Gram examination of urine without centrifugation higher than leukocyturia examination of urine

### Suggestion

- a. For Related Institutions like public health laboratories to diagnosed Urinarius Tractus Infection(UTI) quickly using gram staining in urine.
- b. For student in Department of Medical Laboratory Technicians in Polytechnic of Health Ministry of Health Mataram as study participants to better recognize the symptoms of a urinary tract infection and prevent disease with early inspection that can be done alone.

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**EXPLANATION OF RESEARCH AND APPROVAL**

**TO BE RESPONDENTS**

**(Information of Consent)**

Assalammukalaikum Wr.Wb/Best wishes

Mr. / Mrs. our Respect,

Iam, Siti Zaetun SKM, M.ked, The lecture in Health Analyst Department of Polytechnic of Health Ministry of Health Mataram want to do research about "Sensitivity Test and Specificity Invention Gram Negative Rods with Gram Staining for Screening Test of Urinary Tract Infection (UTI)". The purpose of this study is to determine how the sensitivity and specificity of the discovery of gram-negative bacilli with gram staining as a test filter diagnosis of urinary tract infections.

Considering the importance of this research to be a screening test to diagnose an urinary tract infections then I hope Mr / Mrs pleased to cooperate and participate in this study as the subject and be willing to take his/her own urine for the examination of urine culture, gram staining of urine and leukocyturia.

The cooperation of Mr. / Mrs. is very useful effort for screening urinary tract infections. In order to known that the results of the Mr/ Mrs. will be reserved only for the scope of this study so that the confidentiality of the identity of Mr. / Mrs. we'll guarantee concealed. For your attention and your willingness, I would say thankyou.

Wassalammukalaikum Wr.Wb/Best wishes

**INFORMED CONCENT**

After getting a description of the intent, purpose and significance of this research, I am willing / unwilling (\*) to conduct examination dealing with the need of research and are willing / unwilling (\*) action taking urine for urine's culture and examination of urine straining and leukocyturia.

Mataram, 11 Desember 2016  
Respondent

  
(Prita Deyi Matrisani)

(\*) = cross the unnecessary ones