Journal of Indonesian Medical Laboratory and Science
pISSN 2775-0108 || eISSN 2774-2504

Relationship between urinary track infection screening tests and urine culture results in patients with suspect urinary track infection

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Received: 2023-06-01
Revised: 2023-10-07
Accepted: 2024-04-16
Available online: 2024-04-19

DOI: 10.53699/joimedlabs.v5i1.150

Citation
Indriati, E., & Silviani, Y. (2024). Relationship between urinary track infection screening tests and urine culture results in patients with suspect urinary track infection. Journal of Indonesian Medical Laboratory and Science, 5(1), 66–77. https://doi.org/10.53699/joimedlabs.v5i1.150

Abstract
Background: Urinary tract infection (UTI) is a common disease found in the world. Gold standard for the diagnosis of UTI is urine culture, but this examination has the disadvantage of taking longer results. As a screening test, urine dipstick and urine flowcytometry provide more rapid diagnosis of UTI. *Objectives:* The purpose of this study was to determine the relationship between the results of the dipstick test and UF4000 with urine culture. *Materials and Methods:* This study used an analytic observational method with a cross sectional approach, with accidental sampling. The samples obtained was 38 samples from patients at the Clinical Laboratory of Prodia Diponegoro and Prodia SDP. *Results:* The results showed that there was 84.2% concordance between leukocyte esterase results and culture results, and 31.6% concordance between nitrite results and culture results, 97.4 5% concordance between urine flowcytometry results and urine culture results. *Conclusions:* Based on the Chi square statistical, it was concluded that there were relationship between Urine culture and leukocyte esterase (p-value 0.000), nitrite (p-value 0.004, urine flowcytometri (p-value 0.000). Both of these screening tests can be used to help diagnose UTI, but the gold standard for UTI still uses urine culture result.

Keywords
Leukocyte esterase, Nitrites, Urine flowcytometry, UTI.

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1. Introduction

Urinary tract infection (UTI) is a common disease found in world, with an estimated number of sufferers around 150 million people per year. This disease has been reported to have been discovered as early as 1550 BC by the ancient Egyptians. This disease is more common in female than male. More than 50% of all female in the world have experienced this infection in their lives, of which 20% experienced a recurrence. A person is categorized as experiencing re-infection or relapse if they experience recurrent events ≥ 2 times in a 6 month period or ≥ 3 times in 1 year (Yang & Foley, 2020).

Urinary tract infection is a condition where the urinary tract is infected by pathogenic

bacteria, causing the presence of bacteria in the urine produced. Urinary tract infections begin with the colonization of pathogenic bacteria in the periurethral area. This can be caused by the transfer of bacteria from the anus (digestive bacteria), bacteria from the vagina, or caused by sexual intercourse. Urinary tract infections can be caused by several types of bacteria (Yashir & Apriani, 2019).

The bacterial cause of UTI can be identified through urine culture examination, which is currently the gold standard test for diagnosing UTI (Rinawat & Aulia, 2022). This examination uses a urine sample. The recommended procedure for shelter is to clean the genital area with water first. The recommended urine collection technique is mid-stream urine, this aims to minimize the possibility of bacterial contamination from the skin or genitals. The container requirement for urine culture examination must be a sterile container (Yang & Foley, 2020).

Diagnosis of UTI is carried out by taking anamnesis, physical examination and supported by laboratory examination. The laboratory examination carried out is urine culture which is the gold standard for diagnosing UTI, however this test has the disadvantage of being expensive and time consuming and can produce 60-80% negative results. However, urine culture examination has the advantage that if a positive result is obtained, information will be obtained regarding the type of bacteria causing the infection, the number of bacteria and resistance to antibiotics, making it easier for clinicians to provide appropriate treatment (Pratistha et al., 2018). Some hospitals carry out UTI screening using a dipstick analysis test (nitrite and leukocyte esterase), this test has low sensitivity and specificity (Sari & Muhartono, 2018).

There is an automatic urinalysis examination using the Flowcytometry method which has the ability to provide possible urinary tract infections more quickly and cheaply. This tool informs suspected UTIs through flagging tools based on the results of leukocytes and the number of bacteria analyzed automatically. Many studies report that the use of urine flow ytometry results can reduce the number of urine samples cultured resulting in a significant reduction in workload and costs, especially laboratory costs. However, from the current literature there are wide variations in determining cut off, sensitivity and specificity in determining urinary tract infections using urine flow cytometry results (Delanghe, 2019). Based on previous research, there are variations in results regarding the relationship between UTI screening tests using dip strip tests (Tuntun & Aminah, 2021) and urine flow

cytometry with urine culture results (Monsen, 2017).

The aim of this study was to see the relationship between the UTI screening test, namely dip strips (leukocyte esterase and nitrite parameters), and the relationship between the UF 4000 results and the flag "UTI?" with urine culture results. The difference between the previous research and the one carried out by the current researchers is that they looked at the relationship between two UTI screening tests (dip strip test and urine flow cytometry) using the same samples and the results of the two tests were compared with the results of urine culture, apart from that in the research. This time, a flowcytometry urine examination using a UF 4000 instrument.

2. Materials and Methods

The research design in this study is observational analytic with cross sectional approach. Sampling was carried out at the Prodia Diponegoro Surabaya and Prodia Simpang Darmo Surabaya clinical laboratories. The research location was carried out at the Prodia Diponegoro Surabaya clinical laboratory. The research period is September 2022 to March 2023. The population in this study is all patients who undergoing a urine culture examination with suspected urinary tract infection. Samples were obtained from all patients with suspected UTI who undergoing urine culture, dipstick urine and urine flow cytometry simultaneously. The sample inclusion criteria were all patients with suspected UTI who undergoing urine culture examination. The sampling technique in this study used the accidential sampling method. The reagent used was a dip strip test (combur 10 Test® M), UF 4000 reagent (UF-cellsheath, UF-cellpack SF, UF-cellpack CR, UF-fluorocell CR, UF-fluorocell SF, cellclean, UF- control), gram stain covering (gram A (crystal violet), gram B (lugol), gram C(alcohol 70%), gram D(safranin), vitek 2 reagent (identification card),sensitivity test card/ /antimicrobial susceptibility tests vitek® 2, antimicrobial susceptibility tests GN93 vitek® 2, antimicrobial susceptibility tests GP67 titek® 2,anti-microbial susceptibility tests ST-03).

The examination stages begin with the patient being asked to collect urine aseptically. The urine material is collected in a sterile container and processed for a maximum of 2 hours at room temperature. The urine sample is then carried out by urine culture examination, urine dipstick and urine flowcytometry.

In the dipstick examination, the urine specimen is mixed homogeneously and must not be centrifuged. A total of 10 or 12 mL of sample is poured into a test tube that has been given a patient identification/barcode label. The strip is dipped into the sample until all parts are submerged, immersion is carried out for a maximum of 1 second. Excess sample is wiped on the edge of the tube or by touching it to filter paper. Strip reading is carried out on a Cobas U411 device or visually (Prodia, 2022).

In Flowcytometry urine examination, the specimen is homogenized by turning the sample container upside down or the sample is stirred using a glass stir bar in a zig-zag direction and then inserted into the Flowcytometry tool (Prodia, 2023).

Urine culture examination using Chromagar Orientation media. The urine sample is homogenized, the urine pot is opened and using a sterile 1 µl (0.001ml) or 10 µl (0.01ml) calibrated loop, the calibrated loop is dipped vertically. iInoculate the urine sample aseptically using a calibrated loop of 1 µl (0.001m) for midstream urine while a calibrated loop of 10 µl (0.01ml) for catheter urine, suprapubic aspiration or cystoscopy into Chromagar media. Orientation by making a line in the middle of the Chromagar media. Orientation is from top to bottom, then urine is streaked evenly from the top of the media over the entire surface of the agar. The media was incubated at 37°C for 18-24 hours. Whether there is growth on Chromagar Orientation media is known by making observations. If there is growth, the number of germs growing on the agar is counted and the bacteria are inoculated into blood and Mac Conkey agar media. The media was incubated at 37°C for 18-24 hours.Gram staining, identification and sensitivity testing were carried out on suspect colonies from Mac Conkey/blood agar media. The sample used was an inoculum suspension with a turbidity of 0.5-0.63 McFarland originating from a bacterial culture aged 18-24 hours which was incubated at temperature 35-37 ^{o}C in the medium blood agar/CBA/MacConkey.The system is kinetic, depending on the metabolic rate of the bacteria. The reaction results will be read photometrically at a wavelength of 430-568 nm (colorimetry) or 660 nm (turbidimetry). The antimicrobial susceptibility test was carried out using a 0.5-0.63 McFarland turbidity inoculum suspension derived from bacterial cultures aged 18-24 hours which were incubated at a temperature of 35-37 ^{o}C in blood agar/CBA/MacConkey media. In this antimicrobial susceptibility test, several series of antibiotic concentrations are used.the results are reported as resistant (R), intermediate (I) or sensitive (S) in accordance with manufacturing interpretation

standards which are updated at least once a year and a maximum of once every three years based on certain breakpoints (Prodia, 2021).

The collected data is processed and presented in the form of a descriptive table. Followed by analytical tests using Chi square with a 2x2 table to test the relationship between two categorical variables with SPSS program. The criteria for formulating the hypothesis are if the p value is > 0.05 then there is no significant relationship between the dip strip screening test (leukocyte esterase and nitrite) and urine flow cytometry and urine culture, conversely if the pvalue < 0.05 then there is a significant relationship between the tests. Strip screening (leukocyte esterase and nitrite) and urine flowcytometry with urine culture.

This research received approval for research ethics permission from the Health Research Ethics Commission of the Muhammadiyah University of Purwokerto with registration number: KEPK/UMP/15/XI/2022 and approval was obtained through informed consent from the patient before being taken as a sample.

3. Results and Discussion

Research has been carried out and provided results as shown in Table 1-4. In this study, 38 samples were obtained. The number of male patients was 23 patients or 60.5% and 15 female patients or 39.5%. In this study, 13 samples or 34.2% of urine culture results were positive and 25 patient samples (65.8%) had negative urine culture results. In this study the number of male patients was greater than female patients. The ages of the patients in this study varied from the youngest being 3 months and the oldest being 91 years. All patients who came were patients with a clinical diagnosis of urinary tract infection. The characteristics of the research subjects can be seen in Table 1.

All patients undergoing a dipstick urine examination using a Cobas U411instrument. In Table 1, the majority of respondents were men, 23 patients or 60.5% and women, 15 or 39.5%. Meanwhile, based on Tables 2 and 3, it is known that the number of positive urine culture results was 13 people or 34.2%, with 9 male results (69.2%) and 4 female results (30.8%). One possible reason why more men were diagnosed with UTI in this study was because the number of male respondents was greater than that of women, there is 60.5%. In previous research conducted by Mayangsari, it was found that 66.67% of UTI sufferers

were female and 33.33% were male. One of the reasons female suffer from UTIs is that the anatomical structure of female has a shorter urethra than male (Mayangsari et al., 2021). The age of most respondents was ≥ 60 years as many as 26 patients or 68.4%. In this study, 13 respondents or 34.2% had positive urine culture results and all of them were over 45 years old. The results of this study are similar to those carried out previously at Sanglah Hospital where 36.7% were 36.7% aged 40-59 years (Putra, 2022). The incidence of UTI increases in adults and the elderly due to sexual activity, chronic diseases, genitourinary functional disorders, and drug use (Pratistha et al., 2018). Apart from that, the ability of a person's bladder organ to empty urine in the bladder also plays a role in the risk of UTI, because when the bladder is completely empty, it will release pathogenic bacteria in the urine (Yang & Foley, 2020).

Based on this research, it can be seen that the age range of patients is from babies to the elderly with more female patients than male patients. The distribution of dip and culture strip results for men and women can be seen from Table 2 and Table 3.

Table 1. Subject characteristics

Characteristics	Frequency (n)	Percentage (%)
Age (year)		
Baby (0-1)	2	5.3
Toddlers and preschool age (1-6)	1	2.6
School age children and teenagers (6-18)	1	2.6
Adults (18-45)	4	10.5
Pre elderly (45-59)	4	10.5
Elderly (≥ 60)	26	68.4
Gender		
Male	23	60.5
Female	15	39.5
Total	2	5.3

Table 2. Distribution of Dipstick Examination Results (Leukocytes, Nitrite Esterase), Urine Flowcitometry and Culture in Male Patients

	Positive Results		Negative Results	
Frequency (n)		Percentage (%)	Frekuensi (n)	Percentage (%)
Leukocytes esterase	10	26.3	13	34.2
Kultur	9	69.2	14	56
Urine Flowcytometri	8	21.1	15	39.4
Kultur	9	69.2	14	56

Based on the research results in Table 2, it can be seen that the number of positive UTIs on culture was found to be the highest compared to other methods

Tabel 3. Distribution of Dipstick Examination Results (Leukocytes, Nitrite Esterase), Urine Flowcitometry and Culture in Female Patients

Positive Results			**Negative Results**	
Frequency (n)		**Percentage(%)**	**Frequency (n)**	**Percentage(%)**
Leukosit esterase	7	18.4	8	21.1
Nitrit	2	5.3	13	34.2
Urine Flowcytometri	8	21.1	7	18.4
Kultur	4	30.8	11	44

Based on the research results in Table 3, it can be seen that the number of positive UTIs on leukocyte esterase was found to be the highest compared to other methods.

Chi square analysis was carried out to see the relationship between leukocyte esterase and urine culture. Based on statistical tests, the p-value or sig value is 0.000, using an alpha of 0.05, the p-value <0.05 means that it can be concluded that there is a significant relationship between the urine culture results and the results of the leukocyte esterase dip strip. Fisher's exact test was carried out to see the relationship between nitrite and urine culture. Based on statistical tests, the p-value or sig value was 0.004. By using an alpha of 0.05, the p-value is <0.05, meaning it can be concluded that there is a significant relationship between the urine culture results and the results of the nitrite dip strip.

The relationship between urine flow cytometry and urine culture was analyzed using the Fisher's exact test as an alternative chi square test. Based on the statistical results, a p-value or sig value of 0.000 was obtained. By using an alpha of 0.05, it can be concluded that a p-value <0.05 exists significant relationship between urine culture results and UF urine results that have the flagging "UTI?

In this study, 12 samples or 31.6% were obtained with equally positive leukocyte esterase and urine culture results. There were 20 samples or 52.6% of the results of leukocyte esterase and urine culture which were both negative. Meanwhile, there were discrepancies in the results of a total of 6 results or 15.7%, of which 5 results or 13.2% were positive for leukocyte esterase and negative urine culture and 1 result or 2.6%

were negative for leukocyte esterase and positive urine culture. A similar study was also carried out by Guspa *et al.*, (2018) who conducted research to see the relationship between the leukocyte esterase dipstick and urine culture in 42 respondents, getting 31 samples or 73.8% with equally positive leukocyte esterase and urine culture results. There were 3 samples or 7.1% of the results of leukocyte esterase and urine culture which were both negative. Meanwhile there was 1 result or 2.5% positive leukocyte esterase and negative urine culture, 7 results or 16.7% negative leukocyte esterase and positive urine culture. Apart from that, previous research conducted by Malau & Adipireno (2019) concluded that there was a weak positive relationship between leukocyte esterase results and urine culture results. Some of the reasons why leukocyte esterase results are positive while urine culture results are negative are the presence of leukocytes in the urine (pyuria) indicating an inflammatory process that can occur in the urine secretion system, starting from the kidneys to the urethra, but this inflammation is notIt is always caused by the presence of bacteria so this could be one of the factors causing the difference in results between leukocyte esterase results and urine culture. Apart from that, other causes of positive leukocyte esterase include fungi, Trichomonas and trauma (Yang & Foley, 2020). Meanwhile, the possible cause of negative leukocyte esterase results and positive urine culture is that the leukocyte cells read by the Cobas U411 instrument are leukocyte esterase or leukocytes that only have granules (neutrophils, basophils and eosinophils) so not all types of leukocytes are read. Another possible cause is because the number of leukocytes in the urine is below the reading limit of the tool. The Cobas U-411 tool has a sensitivity of 20 - 25 leuko/µL, so that if the results are below the cut off the reading will be reported as negative (Brunzel, 2013).

In 6 samples or 15.8% the nitrite and urine culture results were both positive. There were 6 samples or 15.8% with both negative nitrite and urine culture results. Meanwhile, 1 patient or 2.6% was positive for nitrite and negative urine culture, 25 patients or 65.8% were negative for nitrite and positive urine culture. Meanwhile, research conducted by Guspa, *et al.*, (2018) who conducted research to see the relationship between the nitrite dipstick and urine culture in 42 respondents obtained results from 11 samples or 26.2% with both positive nitrite and urine culture results. There were 4 samples or 9.5% with both negative nitrite and urine culture results. Meanwhile, zero patients or 0%

were positive for nitrite and negative urine culture, 27 patients or 64.3% were negative for nitrite and positive urine culture. The difference in results between nitrite and urine culture in detecting the presence of bacteria is caused by several factors, including that the nitrite test has a sensitivity of 35 - 85% in detecting UTI-causing bacteria which are unable to convert nitrate to nitrite. Meanwhile, previous research by Malau & Adipireno (2019) concluded that there was no significant relationship between nitrite results and urine culture results. Some of the causes of differences in results between nitrite and urine culture depend on how long the nitrate reducing bacteria infects the bladder, apart from that it takes a minimum of 4 hours for the bacteria to be in the bladder so they can convert nitrate into nitrite. Another factor is that some drugs also affect nitrite results, such as phenazopyridine (Brunzel, 2013).

The relationship between urine flow cytometry results and urine culture was seen from 12 patients or 31.6% had UF results flagging "UTI?" and urine culture were both positive, 25 patients or 65.8% with UF results flagging "UTI?" and urine cultures were both negative. Meanwhile, there was only 1 result or 2.6% where the UF flagging "UTI" result was negative and the urine culture was positive. The cut off for the UF-4000 device in this study was to issue the flagging "UTI?" based on the reading of leukocyte cells (WBC) 40/µL and bacteria 125/µL. In this study, there was 1 different result between the UF urine results and urine culture, where the UF-4000 did not produce the flagging "UTI?" or negative while the urine culture results were positive. The patient's results showed that leukocyte cells (WBC) were 69.4/µL and bacteria were 99/µL. one of the criteria for the appearance of the flagging "UTI?" if bacteria < 125/µL is not met, then the UF 4000 device cannot produce the flagging "UTI?". This patient's urine culture results had a colony count of 80,000 CFU/mL. A similar study to look at the relationship between urine flowcytometry and urine culture was carried out where there was a concordance of results between urine flowcytometry and culture of 51.8% (Marta García-Coca, Ignacio Gadea, 2017).

Urine flow cytometry method for detecting leukocytes and for reading bacteria where bacteria are stained with a new RFID dye reagent and bacteria that have been stained are then read based on cell size or height, refractive index, degree of staining, complexity of the surface thencalculated quantitatively, the UF-4000 tool has better results with urine culture results when compared to the dipstick screening test which is

based on chemical reactions, where the measurement principle is based on color changes. The results of this study are similar to those conducted by Gehringer, where from this study it was concluded that the results of Flowcytometry urine examination had a better diagnostic rate when compared to dip-strip urine (Gehringer, 2021). Flowcytometry is an effective detection method, especially in children (Maarten, B, 2018). The bacteria obtained in this study are shown in Table 4:

Table 4. Bacteria that cause UTI based on positive urine culture results

	Frequency (n)	Percentage (%)
Escherichia coli	11	78.6
Pseudomonas aeruginosa	2	14.3
Enterococcus faecalis	1	7.1

In this study it was also found that the most common bacteria causing UTI was *Escherichia coli* in 11 patients or 78.9%. The results of this study are similar to those conducted by Sumolang et al. In the research conducted, it was found that the most common cause of urinary tract infections was *Escherichia coli* at 16.7% (Sumolang *et al.*, 2013). *Escherichia coli* is a coliform bacteria belonging to the Enterobacteriaceae family. Enterobacteriaceae are enteric bacteria or bacteria that can live and survive in the digestive tract. Some strains of these bacteria provide benefits to humans, for example preventing the colonization of pathogenic bacteria in human digestion. However, there are several other groups that can cause disease in humans, known as *E. coli* pathogens (Rahayu *et al.*, 2018). *Escherichia coli* can enter the urinary tract due to inappropriate sexual hygiene and personal hygiene. In addition, the high possibility of exposure to pathogenic germs from the anus increases the risk of urinary tract infections (Yang & Foley, 2020).

4. Conclusions

Based on this research, a p value of 0.00 was obtained, so it can be concluded that there is a relationship between the results of the dip strip test (leukocyte esterse and nitrite) and urine flow cytometry with the results of urine culture as the gold standard test for diagnosing urinary tract infections.

Acknowledgments: This research can run well with support from management Prodia Clinical Laboratory and Undergraduate Study Program in Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Nasional.

Funding: PT Prodia Widyahusada tbk, Sekolah Tinggi Ilmu Kesehatan Nasional.

Conflicts of Interest: There is no conflict of interest in the research that has been conducted.

Author Contributions: EL: performed laboratory work, analyzed the data. YS: designed the research, analyzed data and discussion, designed the research.

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