Preanalytical requirements: Focus on urine culture

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Abstract:
In a microbiology laboratory, the urine cultures rank among the major tests ordered by doctors. Urine culture using both automated and traditional manual methods are commonly used. The health-care services delivered in India are mainly patient-centered, and laboratory services are under increasing pressure to deliver test results within the stipulated time. Naturally, quality control methods have mainly focused attention only toward analytical aspects of the total testing process (TTP). The TTP includes the preanalytical, analytical, postanalytical phases, and laboratory errors can occur at any stage of the TTP. However, errors in the preanalytical phase are generally overlooked and as many as 46%–68.2% errors can occur. This paper reviews the sources of error in preanalytical phase of urine culture and steps to minimize such errors, which are crucial for laboratory diagnostics and patient safety.

Keywords:
Automation, errors, laboratory diagnostics, preanalytical phase, urine cultures

The laboratory total testing process (TTP) is an elaborate process, comprising three phases; the preanalytical, analytical, and postanalytical phases. George Lundberg originally referred TTP to, as “brain to brain cycle” rightly stating, “a laboratory test begins when a person’s brain, usually a physician, or it could be a patient himself or some other healthcare professional, decides that it would be a good idea to have a lab test and orders it. After that, a process results in collection of the specimen, identification of the patient and the specimen, and transportation to the laboratory, where it is received and prepared for analysis. The result is then reported to the stakeholder (i.e., the receiver’) who is hopefully the person who placed the original order and who interprets the results and takes some action on it.” Total quality assures an errorless TTP providing valuable insights to diagnosis and patient care.[1] In the TTP, factors associated in analytical and postanalytical phases have been steadily addressed by automation, but the preanalytical phase has witnessed few changes. During this intricate TTP process, a major proportion of errors; 46%–68.2% occur in the preanalytical phase.[2]

Urine is the most common sample sent for culture and sensitivity testing to the microbiology laboratory, which attributes up to 24%–40% of submitted cultures, with 80% of these urine cultures being submitted from the outpatient departments.[3] Automation in preanalytical urine processing is not much employed. Some larger clinical microbiology laboratories in Western Europe, Australia, and Middle Eastern nations utilize urine plating instrumentation, while the rest have minimal automation.[4] In this article, we review variables contributing to preanalytical errors and methods to reduce these in urine specimens submitted for culture.

The preanalytical phase is manually intensive and involves multiple steps. Laboratory errors can arise from faults within the system and inadequate checks on the operators involved in specimen
collection, handling, and transport. However, not all preanalytical errors cause adverse events because many of these “upstream” errors are detected during “downstream” procedures by laboratory personnel or physicians or are minor enough that if undetected, they do not impact patient outcomes. It has been reported that in around one-fifth of cases, if not detected right in time can lead to inappropriate investigations and unreliable laboratory results. This not only results in increase the health-care cost but also inappropriate modifications to therapy and also inappropriate care in nearly 6.4%. Inappropriate and random modifications in antibiotic therapy are an important cause of the emergence of drug-resistant uropathogens.

The major preanalytic variables affecting urine culture and sensitivity testing for the occurrence of preanalytical errors are:
1. Patient preparation and sample collection
2. Collection containers
3. Specimen transportation
4. Prior antibiotic use
5. Other errors.

**Patient preparation and sample collection**

The correct interpretation of urine culture depends largely on the patient preparation and optimal sampling. Although guidelines exist for the collection of urine samples for bacteriological examination, the significance of a proper preanalytical method for collecting urine specimens is usually not known to the patients. Besides, these factors are usually not directly under laboratory supervision. The laboratory staff, clinician, and patients need to be educated in sample collection procedures, and laboratory personnel should encourage the proper preanalytical procedures. In case of an improper urine sample collection procedure, the urine collection should be repeated. The quality of reporting directly depends on the proper collection of the urine sample. A random collection of urine samples is generally not recommended. The urine samples usually get contaminated from the distal flora of the urethra in both sexes and the introital flora in females. The contamination rate for females is twice that for males. Contamination is usually more than 10,000 CFU/ml of two or more microorganisms, but based on the collection and transport methods used the laboratory may define its own criteria.

Although the ideal sample is the suprapubic sample but being invasive is not a preferred sample. Similarly, catheterization is not preferred as it is invasive and can lead to iatrogenic infection of the bladder. Hence, the preferred method of sampling is the midstream urine specimen to reduce cellular and microbial contamination. The first-void urine is discarded as it is rich in urethral flora. Cleaning the introital region with water reduces the chances of contamination of specimens. The number of false-positive urine cultures can be reduced up to 20% by washing the genitalia. Therefore, both in males and females, standardized instructions for urine collection, both written and verbal, should be given before collecting the urine sample. Using soap and antiseptics are not advised as it can affect the viability of bacteria and lead to artificial reduction of colony counts. Several authors, however, question periurethral cleaning both in males and females and also cleaning of the urethral meatus routinely in males irrespective of circumcision status.

In cases of catheterization, urine is collected from the catheter tube using a syringe and then transferred to an appropriate sterile container. Suprapubic aspiration (SPA) can be done in patients who cannot be catheterized, in coma or in infants to obtain the urine specimen. Although it is the “gold standard” only reserved for special circumstances such as interpretation of equivocal results from voided urine in infants and small children. In case SPA is attempted, a 20 ml sample is sufficient for urine culture, and it is preferable to use ultrasound guidance to determine the presence of urine in the bladder.

The collection of urine samples in pediatric patients is challenging and requires cooperation from parents as well. SPA in infants being invasive is unacceptable to parents. Different methods have been studied; child with nappy, toilet trained, and collection from urine bag. In the DUTY study on nappy pad urine samples, more than 10% of samples obtained by nappy pads were grossly contaminated; as compared to 2% of the clean catch urine samples obtained from older children. Nappy pad urine culture results can be clinically helpful with dipstick though the results need to be interpreted carefully. This method of urine sample collection has been used by the National Institute for Health and Care Excellence (NICE) in young children in nappies when a clean-catch sample cannot be obtained. Urine samples obtained from sterile bags remains questionable and are not advised as it has unacceptably high false-positive and contamination rates.

Many novel methods have been devised. These are the vibrating bladder stimulator for infants and lumbar/bladder stimulation for neonates in the neonatal intensive care unit but have its own limitations. Although the clean catch urine collection method is recommended by NICE guidelines, these can be time-consuming or may even result in a futile attempt. Recently, a novel method, the “Quick-Wee” method; suprapubic cutaneous stimulation method, using gauze soaked in cold fluid to trigger voiding for clean catch urine from infants in an acute care setting has shown to be quick and faster.
method and can significantly increase the 5 min voiding and success rate of clean catch urine collection.\textsuperscript{[23,24]} Urine specimens can also be collected during a surgical procedure from cystoscopy, nephrostomy, and urostomy, or by a bladder washout.

**Urine collection containers**

Various types of urine collection containers have evolved during the years and a wide variety of collection containers are available in the market; however, the most common is the urine collection cup with a screw-capped lid. The issues associated with the urine collection containers are possible leakage and exposure to contaminants while opening the cup. Bacterial contamination can occur from hands, skin, clothing, exposure to antiseptics from hands, traces of bacteriostatic or bactericidal agents in the collection container. This will affect the final colony count. To avoid these, the basic requirements for a urine collection container should be, (i) transparent high-quality leak-proof container with a main screw cap, (ii) integrated user-friendly flip-cap for using the bacteriological loop or any other urine culture device (dip slide) or for transfer of specimen, (iii) fill volume marking, (iv) instruction leaflet for intended use, (v) ease in using by the patient, and (vi) label and barcode for noting the details of the urine specimen. These features will protect both the specimen from possible contamination and protect the laboratory worker from exposure to the specimen. The closed system will provide a safe and convenient processing of urine specimens and enhance the quality of laboratory reporting.\textsuperscript{[2]} The collection cup should be reviewed for ease in handling before being used and put in specimen collection urine protocol by the hospital.

**Transportation**

Urine being a good culture medium undergoes bacterial overgrowth leading to falsely elevated colony counts.\textsuperscript{[25]} The average time of replication of \textit{Escherichia coli} is “n” minutes so that the number of bacteria increases exponentially with time (e.g., $2^n$ after $n$ minutes).\textsuperscript{[26]} Two major preanalytical variables contributing to errors are (i) length of time between specimen collection and analysis and (ii) lack of temperature control. In case of delay, the urine should be refrigerated (2°C–8°C) but not for more than 48 h or have a bacteriostatic preservative, especially urine samples collected from satellite locations. Such samples should be transported in coolers. The refrigerated specimens should be allowed to reach room temperature before testing.\textsuperscript{[27]}

The common preservatives used are boric acid, hydrochloric acid, acetic acid, and oxalic acid. Boric acid 1%-2% maintains the urine specimen in a state comparable to that is equivalent to refrigeration so as to prevent the growth of organisms for 48–96 h and the other cellular components also remain intact.\textsuperscript{[28]} However, these preservatives should be used carefully. Buffered boric acid is a better preservative as it decreases the detrimental consequences of the preservatives on the organisms rather than non-pH buffered boric acid, which is harmful to certain organisms.\textsuperscript{[29]} Urine specimens that are nonrefrigerated but preserved can be tested up to 48 h after collection for culture and sensitivity. The specimen preservative ratio should be adhered to, and the manufacturer should provide the duration of preservative potency.\textsuperscript{[25]}

**Prior antibiotic use**

The use of antibiotics before sending the urine specimen for culture sensitivity can affect the bacterial growth and faulty interpretation of urine culture reports. There may be reduced bacterial growth, which results in false reporting. This can lead to inappropriate antimicrobial use and emergence to drug resistance. In case of antibiotic use the duration and type of antibiotic should be mentioned on the request form.

**Other errors**

These include; sample volume <0.5 ml, a boric acid container under- or overfilled; leaking container; discrepancy in the request form and sample label; unlabeled container. In these cases, the urine specimen should not be processed. A written acceptance/rejection criterion is beneficial.

**Preanalytical Process Improvement**

The laboratories are currently faced with increasing number of laboratory diagnostic tests. To meet the increasing load while maintaining quality, productivity, and error reduction, newer management models are being tried.\textsuperscript{[1]} Various approaches used for improving process flow and efficiency within industries are business process modeling, workflow mapping, Shewhart cycle, including Lean and Six Sigma. The selection of a particular process improvement methodology depends on the requirements, objectives, and resources available in the existing working environment. Business process modeling and workflow mapping are better suited for layout planning and product flow. Lean and Six Sigma have shown to be applicable to health-care settings. Authors state “By contrast, the pioneering laboratories in the United States that use LEAN and Six Sigma to redesign workflow in their high-volume core chemistry and hematology labs found that these quality management systems – in a 12 to 16-week project – could lead to a 50% reduction in average test turnaround time for a hospital lab, a 40% to 50% improvement in labor productivity and a comparable improvement in quality of results.”\textsuperscript{[30]} Similar benefits can be achieved by applying from Lean and Six Sigma principles to the preanalytical phase in the TTP of urine culture.
being managed manually in urine collection, handling, and processing areas. \cite{1} Lean, developed by Taiichi Ohno’s of the Toyota Production System, is based on a set of principles, practices, and methods for designing, improving, and managing processes. \cite{31} The main focus is to increase efficiency by eliminating particular kinds of waste which consume time and resources and do not add value. These steps need modification, including removal of unnecessary process steps, movement of materials or people without a purpose, unnecessary waiting times, and many more. \cite{32} Proper implementations of LEAN tools usually have an immediate and definite impact. The Six Sigma, is a metric methodology that focuses on reducing variability (i.e., counting and decreasing the number of defects in a process). A process that performs at a Six Sigma level realizes defects at a rate of 3.4 occurrences per million opportunities. \cite{33}

LEAN tools along with Six Sigma methodology can be utilized to monitor and improve quality performance. LEAN can reduce errors where possible, and Six Sigma will help to manage parts of a process that cannot be eliminated. This principle, in respect to the preanalytical process for urine culture, has been applied to achieve efficiency. In a hospital workflow analysis, all of the major process steps were captured from urine specimen collection to specimen disposal. Nonvalue adding steps and error-prone steps were identified. By applying the LEAN model, 11 nonvalue steps were removed from the process. In the preanalytical phase two error-prone steps were eliminated, and three nonvalue added steps were eliminated. Overall, the eliminated steps represented 64% of all steps in the process and increased the efficiency. \cite{34} Thus, a simple tool can lead to vivid quality improvements by simplifying the preanalytical process in the laboratory and reduce the frequency of errors without any added costs. However, to carry out these models, the hospital management should support and enforce the process change is required.

**Preanalytical Automation Methods**

1. Sample specimen input area: Barcoded labeled specimens are put into the loading module system. The input unit separates specimens into different trays or racks. The system controls are devised to determine the steps to be performed based on the specimen’s loading location.

2. Sample identification: Two methods of sample identification are available (i) multiple linear bar code readers, (ii) radio-frequency identification (RFID) of specimen carriers combined with 1 or more bar code readers. \cite{34} It is important to note that specimens that are identified by bar codes, barcode-label quality, and orientation are important while specimens that are identified by RFID system are fixed in their carriers, so tubes should not be removed manually from the carriers as it maintains the link between the tube bar code and the carrier’s identification. However, the RFID system possesses multiple bar code readers placed at critical sites to locate specimens and provide information for their transportation to the various stations in the processing system.

3. Tube types: The sizes of tube carriers or racks will vary depending on the size and type of tubes for processing.

4. Transport system: The specimens are placed in transport carriers with a variable holding capacity and move on a conveyor belt line to the appropriate destination. \cite{34}

5. Lean Six Sigma and automation was implemented in a microbiology laboratory to the TTP for urine analysis and outcomes assessed. Lean Six Sigma methodologies in the urine workflow resulted in significant improvements in both productivity and turn-around time. There was a 5000% overall reduction in urinalysis turnaround time. Lean improved the processes while automation standardized the processes. \cite{35}

**“Big data” technologies**

Novel methods have been devised using supervised machine learning algorithms to predict accurate results in laboratory medicine. Literature search shows that machine learning and artificial intelligence in laboratory medicine have shown promising results in a broad array of data sets, including bacterial colony morphology, urine flow cytometry, and to review test results for quality assurance. \cite{36-38} Artificial intelligence has accurately diagnosed positive urine culture results in an emergency department, thus reducing workload. By reducing the number of query samples to be cultured and enabling diagnostic services to concentrate on those in which there are true microbial infections, a significant improvement in efficiency can be possible. \cite{39,40}

**Conclusion**

The preanalytical phase of the TTP starts from ordering the test to the point of processing the specimen. It is mandatory to focus on the preanalytical subphases by complete mapping of the area of concern and identify potential sources of unnecessary variability leading to laboratory errors. By employing simple and systematic procedures laboratory performance can be improved, enhancing the quality of health care. Automation in the preanalytical phase can reduce sources of error.

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Conflicts of interest
There are no conflicts of interest.

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