



Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Comparison of Uriswab to alternative methods for urine culture collection and transport: confirmation of standard culture methodology for investigation of urinary tract infections

Robert P. Rennie^{a,*}, Lee-Ann Turnbull^a, Kaylee Gauchier-Pitts^a, Tracy Bennett^b,
Debbie Dyrland^b, Susan Blonski^c

^a Laboratory Medicine and Pathology, University of Alberta Hospital, Edmonton, AB, Canada

^b Red Deer Regional Hospital, Red Deer AB, Canada

^c Banff Mineral Springs Hospital, Banff, AB, Canada

ARTICLE INFO

Article history:

Received 5 February 2016

Received in revised form 8 April 2016

Accepted 4 May 2016

Available online xxxx

Keywords:

Urine preservation

Uriswab

BD vacutainer

Urine culture paddle

ABSTRACT

The ability to isolate and identify causative agents of urinary tract infections relies primarily on the quality of the urine sample that is submitted to the microbiology. The most important factors are the method of collection, the maintenance of viability of the potential pathogens during transport, and standardization of the culturing of the urine sample. This report is a composite of several investigations comparing collection and transport on urine culture paddles, with a preservative urine sponge (Uriswab), and a comparison of Uriswab with the BD preservative transport tube as methods of preservation of urinary pathogens. Primary studies showed that Uriswab maintained significantly more urinary pathogens than the urine culture paddle with fewer mixed or contaminated cultures. The two preservative transport systems were comparable for maintenance of viability of the pathogens, but there were fewer mixed cultures when samples were collected with Uriswab. This study confirms the importance of a standard volume of 1 µL of urine for culture.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Elucidation of the true pathogens associated with a urinary infection is often difficult. Quantitation, identification and antimicrobial susceptibility testing is predicated on initial appropriate collection, ensuring that transport is performed in such a way as to preserve the right pathogen at the correct concentration, and third, that what is assumed to be the pathogen is actually causing an infection.

Recent literature suggests that laboratory results on what was previously considered to be the causative agent in a particular sample may not be the case. Based on outcome studies in hospitalized patients, Kwon and colleagues (Kwon et al., 2012) have indicated that methods now used may overcall urinary infections by nearly 40%. The result of this is inappropriate therapy, additional antimicrobials that are not required and potential contribution to antimicrobial resistance.

At the forefront are two major issues. The first is the collection and transport of the specimen. The second is ensuring that standardized culture methods are being used so that bacterial numbers in the sample are not artificially enhanced and thus over estimated.

Aside from the bedside issues of collection of mid-stream urines, samples from catheterized patients, and infants, there are concerns for

transport of the sample to the laboratory to avoid overgrowth (or loss) of the causative organism. It is standard practice (Garcia, 2010) that samples without preservative need to be transported within two hours of collection or refrigerated. There is some controversy about how long a urine sample may be refrigerated before it can be cultured. Most microbiologists would suggest no longer than 24 h; indeed the time may actually be shorter.

Many laboratories have, in the past, bypassed the need for refrigeration by direct inoculation of the urine near the bedside to a urine culture medium paddle (Guttmann and Naylor, 1967; MacLean et al., 1971; Rennie et al., 2012). This involves either dipping or pouring the urine over the culture medium contained on both sides of the paddle and sending the paddle at ambient temperature to the laboratory (Starplex, 2014). The other option is direct inoculation of urine to a system that will allow preservation of bacteria and allow the sample to be sent at ambient temperature to the laboratory for standard culture work up (Bourbeau and Swartz, 2007; Guenther and Washington, 1981; Lauer et al., 1979; Eriksson et al., 2002; Hilt et al., 2014). There are two devices available that accomplish this method of transport; the Becton Dickinson Urine Vacutainer (BD, Towson, MD - sometimes called a "Grey-Vac"), and the Copan Uriswab (Copan Innovations, Brescia, Italy). In the Grey-Vac, urine is poured or transferred into the tube that contains a preservative pill. Urine volume must reach the line on the tube (approximately 4 mL). In the Uriswab method, preservative

* Corresponding author. Tel.: +1-7809043293; fax: +1-7804645380.

is impregnated into the sponge, and the sponge is either dipped into the urine to saturate the sponge, or the patient may urinate directly onto the sponge. The Uriswab sponge holds approximately 1.5 mL.

The information reported here is a compilation of several studies comparing Uriswab to either the urine culture paddle method or to the Grey-Vac for the ability of urinary pathogens to survive transport and grow urinary pathogens from clinically significant infections. In addition the studies revealed that standard methods (Mc Carter et al., 2009) are appropriate for the quantitation and identification of these uro-pathogens.

2. Material and methods

2.1. Uriswab collection

Collection of urine with Uriswab utilizes a sponge impregnated with preservative (boric acid and sodium formate), the sponge on the handle is removed from the container. The sponge can either be dipped into urine collected in a sterile standard urine container (used for performing dip tests (leukocyte esterase, nitrate, etc.) or the patient can hold the sponge directly in the stream of urine once the flow of urine has started and thus ensure collection of a mid-stream sample without stopping the flow of urine. Contact time of 2–3 s will saturate the sponge without reducing the preservative ratio. The sponge is then placed back in the transport tube and sent to the testing laboratory at ambient temperature in a biohazard bag. Leakage from the tube will not occur because the sponge cannot be super-saturated. Once at the testing laboratory, urine is removed from the sponge by either squeezing the soft-sided transport tube, or centrifugation for 3 minutes at 3000 rpm. Urine is then plated directly on appropriate medium with a standard 0.001 mL (1 µL) calibrated loop (Mc Carter et al., 2009). Previous in-house studies (data not shown) have identified no differences in quantitative cultures whether the Uriswab is centrifuged quickly at low speed or the tube is squeezed to remove the culture from the sponge. After overnight incubation at 35 °C, quantitation is performed and identification and susceptibility testing are done as required.

2.2. BD urine vacutainer (Grey-Vac) collection

For collection of urine into the Grey-Vac container, urine is collected into the blue sample cup provided with the device. The urine is then transferred via the needle into the transport tube that contains a preservative pill (boric acid and sodium formate). The urine must fill the Vacutainer tube to the line so that the appropriate ratio of urine to preservative is maintained (approximately 4 mL). Urine may also be transferred directly to the Vacutainer tube by removing the cap and pouring the sample into the tube. The urine is then transported at ambient temperature to the testing laboratory, the cap is removed from the Vacutainer tube, and the urine is plated, incubated and read in the same manner as described above for the Uriswab.

2.3. Urine culture paddle collection

For collection of urine on the culture paddle, urine is collected into a sterile container. The urine is then poured onto each side of the culture paddle and allowed to drain. The culture paddle is then screwed back into the transport container and sent to the testing laboratory at ambient temperature. Once at the testing laboratory the tube containing the culture paddle is incubated at 35 °C overnight, quantitation is performed based on morphotypes observed, and organisms are then picked for identification and susceptibility testing as appropriate. For samples which had apparent confluent growth of a single morphotype, a sweep gram smear and subculture was made to ensure that other species were not present in those samples. It is essential that the paddle is not allowed to freeze during transport, or that excess urine does not remain in the transport tube during transit to the testing laboratory. In

this study the Starplex Dip-Count device (Starplex Scientific Inc. Etobicoke, Ontario) was used. The paddles have MacConkey agar on one side and CLED (Cysteine, Lactose, Electrolyte Deficient) agar on the other.

2.4. Evaluations

2.4.1. Uriswab versus culture paddle

Two evaluations were performed in different settings to evaluate quality and quantitation of the methodology. A total of 370 samples were collected in each device from the same patients to give the evaluations sufficient power since different methods and urine volumes applied to the transport devices. Samples were collected from outpatients attending physician clinics with suspected symptoms of a urinary infection. The samples were all collected in the outpatient laboratories of the local hospitals to ensure that the sample collection and inoculation of the transport devices was standardized. Transport occurred between 2 h and 24 h, depending on the distance of the collection site to the testing laboratory. The collection sites were specifically selected to provide a real world opportunity for either short distance transport (in the same city as the testing site) or long distance transport (several hours driving distance from the testing laboratories). The samples in Uriswab were centrifuged for 3 min at 3000 rpm to remove urine from the sponge. Alternatively the Uriswab tube was squeezed to release the urine from the sponge. Urine was inoculated with a 1 µL standard loop onto Blood agar and MacConkey agar using the standard urine culture inoculation method (Garcia, 2010). Both the Uriswab sample and the culture paddle were incubated at 35 °C overnight. Morphotypes were quantitated and identification of uro-pathogens was made using either Vitek 2 (bioMérieux) GNI or GPI cards or with other validated non-automated methods.

2.4.2. Uriswab versus Grey-Vac

This evaluation was performed in two hospital outpatient laboratories. A total of 200 samples were collected in this part of the evaluation. Similarly, patients presented who were suspected by their physicians of having a urinary tract infection. The urine was collected in a sterile cup and then transferred to the Grey-Vac tube (4 mL), or the Uriswab was dipped directly into the urine to saturate the sponge. (approximately 3 s). The two tubes were then sent to the testing laboratory at ambient temperature. Transport time varied between 1 hour and 24 hour depending on the collection site.

Then, urine was removed from the sponge (still within the transfer tube) by centrifugation or squeezing the tube, and both the Uriswab urine and the Grey Vac tube urine were plated directly to chromogenic agar (UriSelect, Bio-Rad, Montreal, Canada) and a blood agar plate using standard inoculation with a 1 µL loop. After overnight incubation at 35 °C, morphotypes were quantitated, identified and compared for the two methods.

2.4.3. Quantitation of uro-pathogens

For all transport methods, quantitation of potential uro-pathogens was considered significant at 10^7 (10×10^6 CFUL) or 10^8 (100×10^6 CFUL) CFU/L. With an established historical percentage of approximately 30% positive cultures, the sample sizes for these studies were considered sufficient to detect significant differences between collection techniques, if present.

3. Results

3.1. Uriswab vs. culture paddle

The combined results of this investigation are shown in Tables 1 and 2. There were 25 more potential pathogen isolates recovered from the Uriswab than the culture paddle. Particularly for *E. coli*, 10 additional isolates were identified from Uriswab, and there were no beta-haemolytic streptococci recovered from the culture paddle. We did

Table 1

Comparison of culture results for Uriswab and the urine culture paddle.

Transport Device	Number of samples	Not evaluable	No growth (%)	Mixed growth Number (%)	Potential pathogens ^a Number (%) that grew at 10 – 100 × 10 ⁶ CFU/L
Uriswab	370	1	139 (38)	132 (36)	98 (43)
Culture Paddle	370	5	97 (27)	195 (53)	73 (27)

^a These percentages do not include those with low numbers (1–2 colonies) of a single isolate.

not observe a large increase in enterococci from the Uriswab, although other data (Bourbeau and Swartz, 2007) and information from another midstream collection device (The Whiz, JBOL, UK; Jackson et al., 2005) have suggested that phenomenon. However, review of those data does not conclusively support that conjecture. There were five urine culture paddles that were not evaluable because of dried medium or urine remaining in the container that did not permit quantitation. One Uriswab did not have sufficient urine on the sponge.

3.2. Uriswab versus Grey-Vac

The results of this investigation are shown in Tables 3 and 4. There were no significant differences in the number of different species recovered from both transport devices. An additional three *E. coli* were recovered at significant concentrations from Uriswab that were not observed in the Grey-Vac portion of the same urine sample. There was also one sample where both *Klebsiella pneumoniae* and *E. coli* were found in the Uriswab sample (Table 4) but only the *E. coli* was identified from the Grey-Vac portion. Otherwise both devices gave similar results. There did not appear to be any differences in survival of bacterial species during transit.

4. Discussion

Understanding of the patho-physiology of urinary tract infections is a crucial component of determining whether the organisms isolated in the microbiology laboratory are true pathogens or are merely colonizers in the system. There is a body of evidence that indicates that on the bladder walls, in the ureters and even in the kidney (for upper tract infections), the common pathogens form biofilms. (Nickel et al., 1994). The concentrations of bacteria in those biofilms are significant and may be as great as 10⁹–10¹⁰ CFU/L (10⁶–10⁷ CFU/mL). It is in that state that altered physiological processes take place, from which symptoms are derived – dysuria, frequency, flank pain, fever, etc. Depending on the state of the biofilm, when urination occurs prior to treatment, a relatively small proportion (1 – 10%) of the organisms will be sluffed with the stream. Since the numbers of bacteria required to create significant symptoms is large, there will be greater numbers of cells that are then collected in the sample that is tested.

Table 2Pathogens isolated at putative significant concentrations (10–100 × 10⁶ CFU/L) from Uriswab and a urine culture paddle.

Microbial species.	Uriswab	Culture Paddle
<i>Escherichia coli</i>	63	53
<i>Klebsiella pneumoniae</i>	3	3
<i>Enterococcus sp.</i>	15	11
<i>Enterobacter species</i>	3	3
<i>Pseudomonas aeruginosa</i>	1	1
beta-Haemolytic streptococci	6	0
<i>Proteus mirabilis</i>	3	0
<i>Hafnia alvei</i>	1	1
Non-lactose fermenter – not speciated	2	0
<i>Aerococcus urinae</i>	1	1
Total	98	73

The consideration of collection method must also consider the ability of the patient to provide a suitable sample and issues of rapid sample transport. (Gauchier-Pitts et al., 2014; Harrington, 2014; Hilt et al., 2014; Hoban et al., 2007; Hudyn et al., 2013; Rennie et al., 2008; Rennie et al., 2012). Use of screening for significant bacteriuria may allow reduction in the numbers of cultures that require collection (Gutierrez-Fernandes et al., 2012), but it is still imperative, as these studies have shown, that appropriate collection captures and maintains uropathogen(s) at significant concentrations in the sample.

The comparative isolation of the types of uro-pathogens in both parts of this evaluation was similar, but the overall numbers of uropathogens were greater from Uriswab when compared to both the dip slide and to the Grey-Vac. In our own laboratory the dip slide culture is no longer used because of concerns about missing uro-pathogens, particularly *E. coli*. This investigation, which was performed in entirely separate locations, has confirmed those concerns. In both studies, the urine was the same sample inoculated to the transport devices at the same time. The number of *E. coli* recovered from Uriswab was considerably greater than the culture paddle. Further, there were three more *E. coli* and one additional *Klebsiella* recovered from Uriswab compared to the Grey-Vac. This was not a significant difference, but the numbers in this part of the evaluation were smaller. The preservatives on the Uriswab and in the Grey-Vac tablet are similar but the concentrations are not identical. The interaction of the preservative with the microorganism on the Uriswab sponge and in the Grey-Vac tube are likely different, and the microorganisms can readily adhere to the sponge material whereas in the Grey-Vac they are in fluid (Nickander et al., 1982). We don't know why there are such differences between Uriswab and the culture paddle cultures, but the volume of culture medium on the culture paddle is small compared to a regular petri dish and that may play a role in survival of these uro-pathogens. It is also possible that the other bacteria (as in the mixed cultures) have an inhibitory effect on growth of some uro-pathogens.

These suppositions are of course speculative, but it is clear that Uriswab is a better transport system for preserving the growth of the most important urinary tract pathogens. It was also interesting to note that some common contaminants (at low concentrations) did not survive well on the Uriswab. This may additionally enhance the recovery of the causative urinary pathogen.

These studies were performed in clinic outpatients for who a urine culture was indicated by the attending physician. As expected in these samples the most common species were *E. coli* and *Enterococcus species*. Isolates of *P. aeruginosa* were infrequent. A different study of long term care patients may well increase the number of this species recovered. However, as stated previously, early in-house development studies with Uriswab did not show a decrease in *P. aeruginosa* and an increase in enterococci recovered from this transport device. These observations suggest that the sponge matrix of Uriswab reduces the opportunity for either enhancement or inhibition of growth of particular species of uropathogens.

It was also noted in these studies that there were few Gram-positive species isolated other than beta-haemolytic streptococci. This is in part due to the patient population studied. Small number of *Staphylococcus aureus* were not seen but coagulase – negative staphylococci were often recovered but at concentrations not considered significant, and in the context of mixed cultures.

The standardization of plating using a 1 µL sample size was originally developed for two reasons based on the patho-physiology described

Table 3

Recovery of pathogens from Uriswab and BD Vacutainer (Grey-Vac).

Transport device	Number of samples	No growth (%)	Mixed cultures number (%) of those that grew	Potential pathogens: ^a Number (%) that grew (10 – 100 × 10 ⁶ CFU/L)
Uriswab	200	121 (61)	49 (62)	30 (38)
Grey-Vac	200	115 (58)	58 (68)	27 (32)

^a These percentages do not include those with low numbers (1–2 colonies) of a single isolate.

above and on methodology described in commonly used procedure manuals (Garcia, 2010; Mc Carter et al., 2009). Those authors indicate that 1 µL should be inoculated for standard voided specimens and for samples from catheterized patients which constitute the large majority of urine cultures collected. First, it was observed that 1 µL in a significant bacteriuria would result in the delivery of approximately 200 colonies from the large number sluffed from the bladder biofilm. That would permit both the ability to count individual colonies on the plate, and to differentiate different species if there was more than one morphotype present. Second, the 1 µL delivered would not falsely augment the number of colonies coming from the stream such that a non-significant culture suddenly appeared to be a significant bacterial infection.

It has been suggested that culture of larger volumes (e.g., 10 µL) might be useful in certain clinical situations (Mc Carter et al., 2009). These included so-called asymptomatic bacteriuria in pregnancy, and the discovery of pathogens from massage samples in male patients with prostatitis. For all other urinary infections, the culture of 1 µL has been and continues to be the standard.

In the current evaluation of urine transport systems, urine from both Uriswab and the Grey-Vac were plated using a 1 µL volume. For the culture paddle cultures it is recognized that 1 µL would not be sufficient to flood even one side of the culture paddle. Likely, at least 10 µL is necessary to flood both sides of the medium paddle. In the package inserts there is an indication that confluent growth is consistent with a concentration of 1 × 10⁹ CFU/L. At that concentration, separation of individual organisms becomes difficult and there is an increased likelihood of finding non-pathogenic contaminants. This changes the reporting characteristics of the entire procedure. Further it may well result in delays in sorting pathogens from non-uropathogens, and either overcalling a urine as significant or missing a true pathogen. In either of these events, responsible antimicrobial therapy becomes a concern.

In the investigations presented here, where the culture paddle was compared to Uriswab (Table 1), we discovered 63 more mixed cultures from the culture paddle. For the Uriswab vs. Grey-Vac evaluation (Table 3) seven more samples were reported as mixed from the Grey-Vac tube. This may be due to the occasional low colony skin contaminant that does not grow from the Uriswab sample. None of these from either transport system had potential uro-pathogens in the mixed cultures.

In summary, the isolation of urinary pathogens from sites both remote and close to the testing laboratory was enhanced by collection on the Uriswab compared both to culture paddle cultures and to the Grey-Vac preservative transport device. Costs for the culture paddle method become greater than either the Uriswab or the Grey-Vac because of the number of mixed cultures on the dip slides that need to be sub-cultured before determining if the isolates are significant to the

infectious process. There is little difference in the cost of the Uriswab and the Grey-Vac, possibly only in a number of additional pathogen isolations that could prevent re-culturing and more rapid appropriate antimicrobial treatment at the outset.

Acknowledgments

This study was supported by provision of materials by Alere, Canada, Copan Innovations, Brescia, Italy, and by Becton Dickinson, Towson, Maryland. The authors thank the staff of participating facilities and the technologists at the testing laboratories.

References

- Bourbeau P, Swartz B. Comparative evaluation of Uriswab urine collection transport and preservative and BD Vacutainer C & S Preservative Plus plastic tube for preservation of urine specimens. Abstract C-141. Toronto, Canada: American Society for Microbiology; 2007.
- Eriksson I, Lindman R, Thore M. Microbiological evaluation of a commercial transport system for urine. Scand J Clin Lab Invest 2002;62:325–35.
- Garcia L, editor. Clinical Microbiology Procedures Handbook. Washington, DC: ASM Press; 2010. p. 3–12.
- Gauchier-Pitts K, Rennie RP, Turnbull L, Oosterhoff C. Comparison of urine collections systems that are designed to preserve urinary pathogens during transport. Effect of system and transport on bacterial survival. Presentation I-01. AMMI-CACMID, Victoria, British Columbia Canada. Apr. 2014; 2014.
- Guenther KL, Washington II JA. J Clin Microbiol 1981;14:628–30.
- Gutierrez-Fernandes J, Lara A, Bautista MF, de Dios Luna J, Polo P, Miranda C, et al. Performance of the Sysmex UF1000i system in screening for significant bacteriuria before quantitative culture of aerobic/facultative fast-growth bacteria in a reference hospital. J Appl Microbiol 2012;113:609–14.
- Guttmann D, Naylor GRE. Dip-slide: an aid to quantitative urine culture in general practice. Br Med J 1967;3:343–5.
- Harrington S. If specimen collection and processing guidelines fall, does anyone hear them? Pre-analytical conundrums in clinical microbiology. Clin Microbiol News 2014;36:105–14.
- Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol 2014;52:871–6.
- Hoban S, Buchanan Y, Iarokos M, Alfa, Harding G. The challenge of midstream urine culture in elderly patients. AMMI-CACMID Annual Meeting, Halifax, NS., May 2007, 18. Can J Infect Dis Med Microbiol; 2007. p. 35–92.
- Hudyn E, Blonski S, Turnbull L, Rennie RP. Use of Uriswab in a community hospital setting. P-28. AMMI-CACMID, Quebec City, PQ. Apr 2013; 2013.
- Jackson SR, Dryden M, Gillett P, Kearney P, Weatherall R. A novel midstream urine-collection device reduces contamination rates in urine cultures amongst women. Br J Urol Int 2005;96:360–4.
- Kwon JH, Fausone MK, Du H, Robicsek A, Peterson LR. Impact of laboratory-reported urine culture colony counts on the diagnosis and treatment of urinary tract infection for hospitalized patients. Am J Clin Pathol 2012;137:778–84.
- Lauer BA, Reller LB, Mirrett S. Evaluation of preservative fluid for urine collected for culture. J Clin Microbiol 1979;10:42–5.
- MacLean DW, McCallum FM, Davies BI. Dip-slide urine cultures in general practice. J R Coll Gen Pract 1971;21:710–3.
- Mc Carter YS, Burd EM, Hall GS, Zervos M. Laboratory Diagnosis of urinary tract infections. Cumitech 2C. Washington, D.C.: ASM Press, American Society for Microbiology; 2009.
- Nickander KK, Shanholtzer CJ, Peterson LR. Urine culture transport tubes: Effect of sample volume on bacterial toxicity of the preservative. J Clin Microbiol 1982;15:593–5.
- Nickel JC, Costerton JW, McLean RJ, Olson MJ. Bacterial biofilms: influence on the pathogenesis, diagnosis and treatment of urinary tract infections. J Antimicrob Chemother 1994;33(Suppl. A):31–41.
- Rennie RP, Turnbull L, Brosnikoff C. Clinical evaluation of a preservative impregnated sponge to stabilize urine samples transported for microbiological culture. Abstract P 1371. 18th European Conference on Clinical Microbiology and Infectious Diseases. Barcelona Spain. Apr. 2008; 2008.
- Rennie RP, Turnbull L, Fuller J, Tyrrell G, Bennett T, Dyrlund D. Real-time evaluation of Uriswab vs. a dip-slide method for enumeration of urinary pathogens in outpatient settings. Abstract P1754. 22nd European Conference on Clinical Microbiology and Infectious Diseases. London U; 2012.
- Starplex Information for Use, Dip - N-Count. Dip slide method for the detection of urinary tract infection Starplex Scientific Inc. Ontario: Etobicoke; 2014.

Table 4Pathogens recovered from Uriswab and BD Vacutainer (Grey-Vac) at 10–100 × 10⁶ CFU/L.

Microbial species	Uriswab	BD Vacutainer (Grey-Vac)
<i>Escherichia coli</i>	17	14
<i>Enterococcus species</i>	7	8
<i>Klebsiella pneumoniae</i>	3	2
<i>Proteus mirabilis</i>	1	1
<i>Enterobacter cloacae</i>	1	1
beta-Haemolytic streptococci	1	1
Total	30	27