Manuscript draft: UTid+ results.

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Introduction

Urinary tract infections (UTI) commonly affect dogs and cats and are one of the most common reasons for antimicrobial therapies in companion animals. The most common UTIs manifest by a bacterial infection of the bladder implying in clinical signs such as dysuria, pollakiuria, and/or increased urgency of urination along with presence of bacteria in urine (Warren et al., 1999). Improper antimicrobial administrations are often attributed to erroneous diagnosis of the pathogens present in the urinary tract and it can lead to failure to resolve infection, antimicrobial resistance, and economic losses. Proper and timely diagnosis of UTI pathogens is crucial to a successful treatment. The correct diagnosis allows the proper decision of the antimicrobial, increasing the chances of solving the case.

The clinical signs commonly observed are nonspecific and do not give enough information for a correct UTI diagnosis. Instead, these abnormalities should lead to further investigations. The complete urinalysis, which includes urine-specific gravity, urine glucose level determination and the examination for crystalluria are considered as a minimum necessary for the evaluation of a potential UTI (Weese et al., 2011). Furthermore, for a complete and trustworthy diagnosis of UTIs,

bacteriologic culture of urine samples should be performed in order to identify the pathogens that may be causing the disorder.

Bacterial culture methods are needed to identify the pathogens present in the urine samples. Commonly, urine samples are collected and submitted to the laboratory, and the recommendation is to refrigerate and ship the samples as soon as possible to reduce risks of deterioration and contamination of the samples. However, delays in receiving the culture results or in the transportation process are often a problem that can imply in wrong diagnosis or in the health of the patient. Therefore, different culture methods are needed to improve the diagnosis process of UTIs. New culture-based tools for the identification of UTI pathogens that can be performed on the practice by personnel with low experience in microbiological training, without the necessity of submitting the samples to a specialized laboratory would be ideal to improve the quality and accuracy of the diagnosis.

Herein, we aimed to evaluate the use of a selective chromogenic on-site culture system designated for identification of specific bacterial pathogens associated with UTI, constituted by a single plate containing four selective chromogenic media (UTid+, FERA Diagnostics and Biologicals, College Station, TX). The accuracy, specificity, sensitivity, negative and positive predictive values of this testing product were evaluated based on the results from a standard laboratory culture.

Materials and methods

Statistical methods

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated based on true positives, true negatives, false positives and false negatives

as stated by (Dohoo et al., 2003) comparing results from UTid cultured samples and reference laboratory results. In addition, accuracy was calculated by dividing the number of true positives and true negatives by the total number of tests. The simple Cohen's kappa coefficient (κ) was calculated using the FREQ procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc., Cary, NC). This parameter assumes that the two response variables (UTid and gold standard culture) are independent ratings, and the coefficient equals 1 when there is complete agreement between the two tests. The null hypothesis for this test is that if agreement happens due to chance the Kappa coefficient is equal to zero. Under this null hypothesis, *P* values associated with this test equal or smaller than 0.05 were considered significant.

Results

In total 212 urine samples were collected. The prevalence of pathogens reported herein was calculated based on results from standard laboratory culture methods which was considered the gold standard and is known as the true prevalence (Table 1). The most prevalent pathogens in the urine samples collected were *Escherichia coli* (9.45%) followed by *Enterococcus faecalis* (4.5%) and *Staphylococcus pseudintermedius* (3.15%).

Regarding the test characteristics of UTid plates for identification of urinary tract infectious pathogens, the overall sensitivity, specificity, PPV, NPV, accuracy and κ coefficient are presented in Table 2. Among the Gram-negative bacteria detected in the urine samples, the overall sensitivity, specificity, PPV and NPV was 85.2%, 99.5%, 95.8% and 97.9%, respectively, with an accuracy of 97.6% and kappa coefficient of 0.88 ($P<0.001$). Additionally, when analyzing test characteristics of UTid for detection of *E. coli* infections, higher values of sensitivity (95.2%) and NPV (99.5) were observed (Table 3).

Furthermore, when analyzing the UTid test characteristics for gram-positive pathogens, the overall sensitivity, specificity, PPV and NPV were 96.6%, 98.4%, 90.3% and 99.4%, respectively,

and the accuracy and kappa coefficient for this test were 98.1% and 0.92 (P<0.001), respectively. In addition, among the gram-positive pathogens, we observed 100% in sensitivity and NPV in the 13 detected infections of *Enterococcus spp.* in the UTid plates. High values of test characteristics of UTid to identify *Streptococcus* spp. and *Staphylococcus* spp. were also observed (Table 4).

Among the 212 tested urine samples, 119 were collected through cystocentesis, 33 by catheter and 32 were collected using a free catch method, the other 28 samples were not specified. The lower values for sensitivity, specificity PPV, NPV, accuracy and kappa coefficient were observed when samples were collected through a free catch method (Table 5), suggesting a higher risk of contamination in those samples.

Conclusions

The on-site culture system evaluated in the present study is suitable for use in companion animal clinics and presented satisfactory overall accuracy for detection of common UTI pathogens. In addition, when tests characteristics were performed to evaluate the sensitivity, specificity, PPV and NPV for important gram-negative and gram-positive bacteria associated with UTI, high values were also observed. Furthermore, the identification of bacteria based on colors allows for easy interpretation of the results by individuals who are not experienced with microbiological training.

Table 1. Prevalence of pathogens associated urinary tract infection identified by standard laboratory culture from 222 tests.

Pathogen	**Number**	**Prevalence (%)**
Beta Streptococcus	3	1.35
Corynebacterium auriscanis	1	0.45
E. coli	21	9.45
Enterococcus casseliflavus	1	0.45
Enterococcus faecalis	10	4.50
Enterococcus faecium	1	0.45
Enterococcus gallinarum	1	0.45
Klebsiella oxytoca	1	0.45
No growth	163	73.42
Proteus mirabilis	5	2.25
Staphylococcus coagulase -	2	0.90
Staphylococcus pseudintermedius	7	3.15
Staphylococcus schleiferi	3	1.35
Staphylococcus sciuri	1	0.45
Streptococcus alactolyticus	1	0.45
Streptococcus gallolyticus	1	0.45
Total	222	100

Table 2. Overall test characteristics of selective chromogenic culture plates (UTid+) to identify bacteria associated with urinary tract infection determined by standard laboratory culture.

Parameter	**UTid+**	**95% Confidence Interval**
Number of Tests	212	
True Prevalence, % (n/n)	20.3 (43/212)	(15.4 – 26.2)
Sensitivity, %	93	(81.4 – 97.6)
Specificity, %	95.3	(90.9 – 97.6)
PPV[1], %	83.3	(70.4 – 91.3)
NPV[2], %	98.2	(94.8 – 99.4)
Accuracy, %	94.8	(90.9-97.1)
K[3], %	0.84	(0.75 – 0.93)
k P-value	<0.001	

[1] Positive predictive value.
[2] Negative predictive value.
[3] Cohen's kappa coefficient. *k* = 0 denotes poor agreement; 0.01 to 0.20 denotes slight agreement; 0.21 to 0.40 denotes fair agreement; 0.41 to 0.60 denotes moderate agreement; 0.61 to 0.80 denotes substantial agreement and 0.81 to 1.00 denotes almost perfect agreement.

Table 3. Test characteristics of selective chromogenic culture plates (UTid+) to identify gram-negative bacteria associated with urinary tract infections determined by standard laboratory culture.

Parameter	UTid+ results			
	Overall Gram-negative	***E. coli***	***Proteus* spp.**	***Klebsiella* spp.**
True Prevalence, % (CI[1]) (n/n)	12.7 (8.9–17.9) 27/212	9.9 (6.6-14.7) 21/212	2.4 (1.0-5.4) 5/212	0.5 (0.1-2.6) 1/212
Sensitivity, %	85.2 (67.5-94.1)	95.2 (77.3–99.2)	40 (11.8-76.9)	100 (20.7-100)
Specificity, %	99.5 (97-99.9)	99.5 (97.1-99.9)	100 (98.2-100)	100 (98.2-100)
PPV[2], %	95.8 (79.8-99.3)	95.2 (77.3-99.2)	100 (34.2-100)	100 (20.7-100)
NPV[3], %	97.9 (94.7-99.2)	99.5 (97.1-99.9)	98.6 (95.9-99.5)	100 (98.2-100)
Accuracy, %	97.6 (94.6-98.9)	99 (96.6-99.7)	98.5 (95.9-99.5)	100 (98.2-100)
K[4], %	0.88 (0.79-0.98)	0.60 (0.44-0.75	0.56 (0.12-1.00)	1.00
k P-value	<0.001	<0.001	<0.001	0.004

[1] 95% confidence interval.
[2] Positive predictive value.
[3] Negative predictive value.
[4] Cohen's kappa coefficient. *k* = 0 denotes poor agreement; 0.01 to 0.20 denotes slight agreement; 0.21 to 0.40 denotes fair agreement; 0.41 to 0.60 denotes moderate agreement; 0.61 to 0.80 denotes substantial agreement and 0.81 to 1.00 denotes almost perfect agreement.

Table 4. Test characteristics of selective chromogenic culture plates (UTid+) to identify Gram-negative bacteria associated with urinary tract infections determined by standard laboratory culture.

	UTid+ results			
Parameter	**Overall gram-positive**	***Streptococcus* spp.**	***Staphylococcus* spp.**	***Enterococcus* spp.**
True Prevalence, % (CI[1]) (n/n)	13.7 (9.7-19.0) 29/212	2.4 (1.0-5.4) 5/212	5.7 (3.3-9.6) 12/212	6.1 (3.6-10.2) 13/212
Sensitivity, %	96.6 (82.8-99.4)	80.0 (37.6-96.4)	91.7 (64.6-98.5)	100 (77.2-100)
Specificity, %	98.4 (95.3-99.4)	98.6 (95.8-99.5)	98.5 (95.7-99.5)	98.5 (95.7-99.5)
PPV[2], %	90.3 (75.1-96.7)	57.1 (25.0-84.2)	78.6 (52.4-92.4)	81.3 (57.0-93.4)
NPV[3], %	99.4 (96.9-99.9)	99.5 (97.3-99.9)	99.5 (97.2-99.9)	100 (98.1 – 100)
Accuracy, %	98.1 (95.2-99.2)	98.1 (95.2-99.3)	98.1 (95.2-99.3	98.5 (95.9-99.5)
K[4], %	0.92 (0.84-0.99)	0.65 (0.34-0.97)	0.83 (0.67-0.99)	0.88 (0.76-1.00)
k P-value	<0.001	<0.001	<0.001	<0.001

[1] 95% confidence interval.
[2] Positive predictive value.
[3] Negative predictive value.
[4] Cohen's kappa coefficient. *k* = 0 denotes poor agreement; 0.01 to 0.20 denotes slight agreement; 0.21 to 0.40 denotes fair agreement; 0.41 to 0.60 denotes moderate agreement; 0.61 to 0.80 denotes substantial agreement and 0.81 to 1.00 denotes almost perfect agreement.

Table 5. Overall test characteristics of selective chromogenic culture plates (UTid+) by method of urine collection to identify bacterial pathogens associated with urinary tract infections determined by standard laboratory culture. Percentage values and 95% CI are shown. 28 samples were not included in this analysis because of lack of information about the collection method.

	Collection Method		
Parameter	**Cystocentesis**	**Catheter**	**Free Catch**
Number of tests	119	33	32
True Prevalence, % (n/n)	21.8 (15.4-30.1) 26/119	12.1 (4.8-27.3) 4/33	25.0 (13.3-42.1) 8/32
Sensitivity, %	92.3 (75.9-97.9)	100 (51.0-100)	87.5 (52.9-97.8)
Specificity, %	97.8 (92.5-99.4)	100 (88.3-100)	79.2 (59.5-90.8)
PPV[1], %	92.3 (75.9-97.9)	100 (51.0-100)	58.3 (32.0-80.7)
NPV[2], %	97.8 (92.5-99.4)	100 (88.3-100)	95.0 (76.4-99.1)
Accuracy, %	96.6 (91.7-98.7)	100 (89.5-100)	81.25 (64.6-91.1)
K[3], %	0.90 (0.80-0.99)	1.0	0.57 (0.27-0.86)
k P-value	<0.001	<0.001	0.001

[1] Positive predictive value.

[2] Negative predictive value.

[3] Cohen's kappa coefficient. $k = 0$ denotes poor agreement; 0.01 to 0.20 denotes slight agreement; 0.21 to 0.40 denotes fair agreement; 0.41 to 0.60 denotes moderate agreement; 0.61 to 0.80 denotes substantial agreement and 0.81 to 1.00 denotes almost perfect agreement.

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