

# Evaluation of the feasibility of the **VACUETTE**<sup>®</sup> Urine CCM tube for microbial testing of urine samples

## Background

The **VACUETTE**<sup>®</sup> Urine CCM tube is for the collection, transport and storage of urine samples for urine culture and urinalysis in the laboratory. The evacuated tube contains a stabilizer to preserve the urine sample at room temperature (20 – 25°C) for up to 48 hours. **VACUETTE**<sup>®</sup> Urine CCM tubes are made of PET with a pre-defined vacuum for exact draw volumes. They are fitted with yellow **VACUETTE**<sup>®</sup> Safety Caps. The tube interior is sterile.

In various studies rapid transportation of urine samples or preservative measurements of the samples are of critical importance to achieve reliable test results. Delay in delivery to the laboratory may lead to an increase in microbial counts generating false results. Therefore, if processing delay of the urine sample is unavoidable, either refrigeration at 4 °C or the use of a preservative is beneficial. [1, 2, 3, 4, 5, 6, 7]

Urinary tract infections (UTI) result from the presence and growth of microorganisms in the urinary tract. The incidence of UTI is influenced by age, sex or by predisposing factors that may impair the wide variety of normal host defense mechanisms. [3]

Urine culture results showing microbial counts of  $\geq 10^5$  CFU/ml using midstream urine are indicative for an infection and counts below usually indicate contamination of the urine sample. In specific patient groups, counts between  $10^5$  CFU/ml and  $10^2$  CFU/ml may also be significant, depending on the type of microorganisms detected. [3, 8]

## Study objective

A clinical evaluation of the **VACUETTE**<sup>®</sup> Urine CCM tube was carried out to evaluate the feasibility for microbial testing of urine samples. The objective was to investigate the stability of microorganisms and microbial counts including bacteria and yeasts when using the **VACUETTE**<sup>®</sup> Urine CCM tube up to 48 hours in comparison to **VACUETTE**<sup>®</sup> Urine Culture tubes both stored at room temperature (20-25°C), and the **VACUETTE**<sup>®</sup> Urine No Additive tube stored at 4°C-8°C in the refrigerator.

## Study design

The gold standard for the diagnosis of UTI is urine culture testing, mostly using midstream urine. [8]  
In a first step, testing of urine samples obtained from healthy donors was performed. Samples were spiked with known concentrations of pathogens including bacteria and/or yeasts.  
In a second step, testing of clinical urine samples obtained from routine setting was performed. Results obtained were evaluated separately.

For classification of results, the standard clinical protocol using the log transferred microbial counts/ml was applied and a change of one log was regarded as clinically significant.

## Material and Methods

The following materials were used:

**VACUETTE**<sup>®</sup> Urine CCM tubes (item # 455052)

**VACUETTE**<sup>®</sup> Urine Culture tubes (Boric Acid) (item # 455003)

**VACUETTE**<sup>®</sup> Urine No Additive tubes (item # 455210)

Dip slide UrinAX CL/MC/E from Axonlab (CLED, MacConkey, enterococcus agar) (item # AL0203)

## Testing of samples from clinical setting

A total of 48 urine samples from patients with suspected UTI were collected within daily routine procedure in a clinical setting. Urine samples were collected and filled in 3 different urine collection tubes: The **VACUETTE**<sup>®</sup> Urine CCM tube, **VACUETTE**<sup>®</sup> Urine Culture tube and the **VACUETTE**<sup>®</sup> Urine No Additive tube. The samples were gently mixed straight after collection, until the additive was dissolved. The collected urine samples were consecutively cultivated on a dip slide agar<sup>[9]</sup> and incubated at 35°C for 12 hours at the following time points: within 4 hours after urine collection, after 24 hours, after 48 hours and after 72 hours; obtained CFU results were compared.

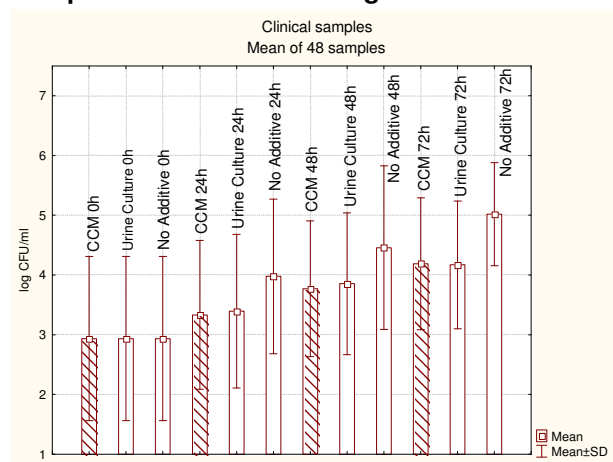
## Testing of samples spiked with facultative pathogenic microorganisms

Urine samples obtained from healthy donors were spiked with a total of 8 different facultative pathogenic microorganisms (*Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*).

A total of five different urine samples were spiked with equally defined concentrations of microorganisms. Aliquots of spiked urine samples were then transferred in to the 3 different urine collection tubes allowing for comparable initial concentrations of each microorganism. Low initial microbial counts ( $10^2$ - $10^5$  CFU/ml) were chosen to observe relevant changes in microbial growth. The determination of the microbial counts was carried out by streaking 10 µl of the urine culture per culture plate and by stating the counted number of microbes per µl. This results in a lower detection limit of 0.1 CFU/µl and of 100 CFU/ml respectively.

## Results

### Samples from clinical setting



**Figure 1: Culture Positive Samples**

Logarithmic data were compared for the time point within 4 hours of urine collection, after 24 hours, after 48 hours and after 72 hours. The microbial count remained stable up to 48 hours in the **VACUETTE**<sup>®</sup> Urine CCM tube and shows comparable results to **VACUETTE**<sup>®</sup> Urine Culture tubes. The **VACUETTE**<sup>®</sup> Urine No Additive tube shows a significant increase of growth at 72 hours (Figure 1).

### Samples spiked with facultative pathogenic microorganisms

Regarding the use of **VACUETTE**<sup>®</sup> Urine CCM tubes for microbial testing of the urine it was demonstrated that *Candida albicans*, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus pyogenes* were not significantly altered in microbial counts in any of the five tested samples at 24 hours, after 48 hours and 72 hours. Similar results were obtained with the **VACUETTE**<sup>®</sup> Urine Culture tubes (Fig. 2-7).

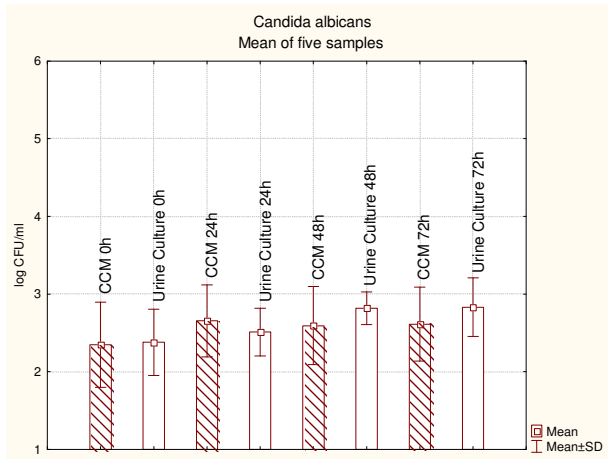


Figure 2: Stability of *Candida albicans*

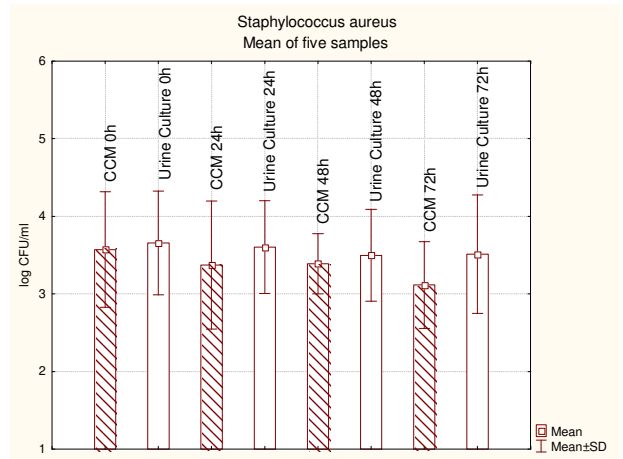


Figure 5: Stability of *Staphylococcus aureus*

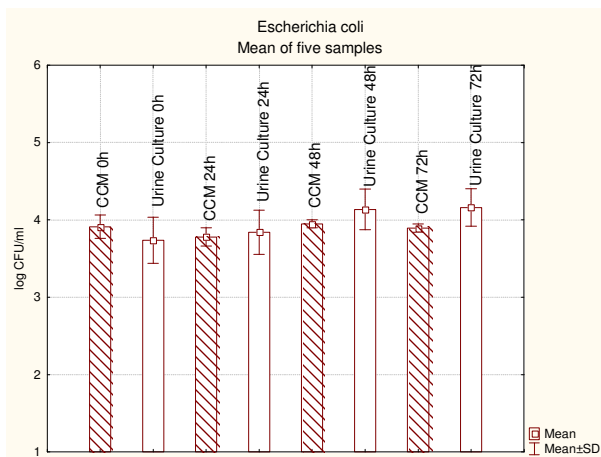


Figure 3: Stability of *Escherichia coli*

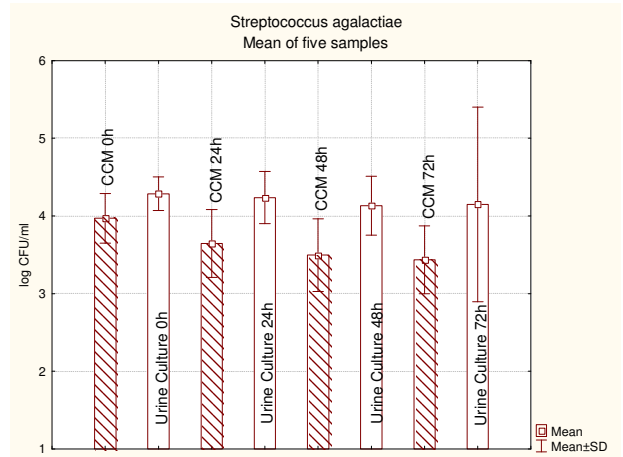


Figure 6: Stability of *Streptococcus agalactiae*

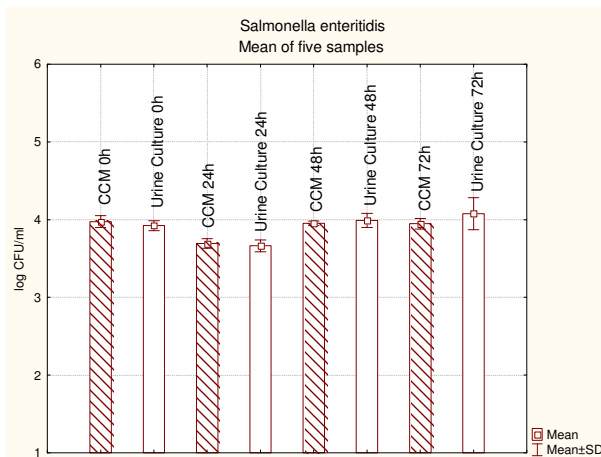


Figure 4: Stability of *Salmonella enteritidis*

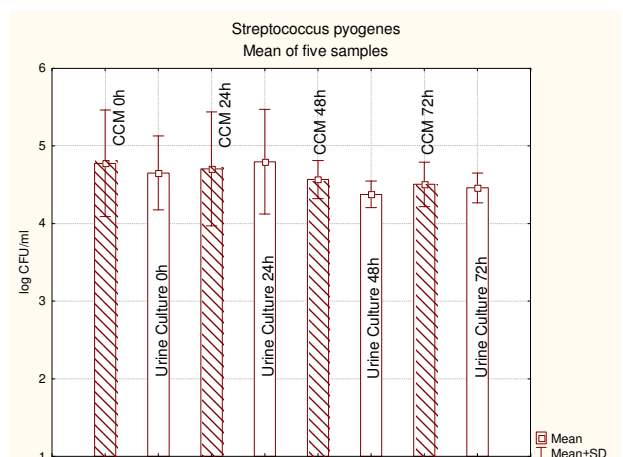
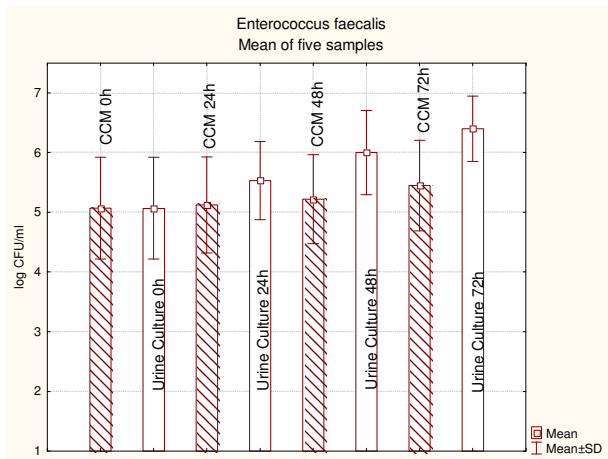
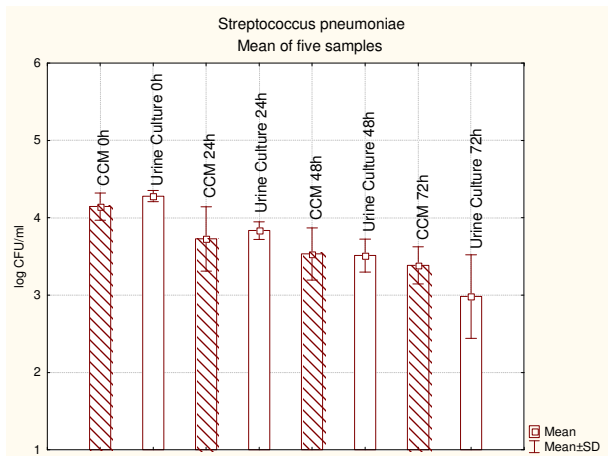


Figure 7: Stability of *Streptococcus pyogenes*



**Figure 8:** Stability of *Enterococcus faecalis*

For *Enterococcus faecalis* the microbial count in the **VACUETTE**<sup>®</sup> Urine CCM tube remained stable up to 48 hours, however, there was a slight increase of counts observed with the **VACUETTE**<sup>®</sup> Urine Culture tube at 72 hours (Figure 8).



**Figure 9:** Stability of *Streptococcus pneumoniae*

For *Streptococcus pneumoniae* the microbial count remained stable for both collection tubes containing preservatives up to 48 hours. At 72 hours a significant decrease was observed with both tubes (Fig. 9).

## Discussion:

The results obtained when samples from the clinical setting were investigated showed comparable results up to 48 hours of both, the **VACUETTE**<sup>®</sup> Urine CCM tube and the **VACUETTE**<sup>®</sup> Urine Culture tube. The microbial count significantly increased when the **VACUETTE**<sup>®</sup> Urine No Additive tube was used at 72 hours, which leads to a falsification of microbial count compared to stabilized samples.

Regarding the samples spiked with facultative pathogenic microorganisms, the results showed that the **VACUETTE**<sup>®</sup> Urine CCM tubes reliably stabilize the causative pathogens of UTI at room temperature for up to 48 hours. It therefore allows for accurate determination of microbial counts to diagnose UTI infections. Compared to the **VACUETTE**<sup>®</sup> Urine Culture tube, the **VACUETTE**<sup>®</sup> Urine CCM tube seems to be superior in suppressing *Enterococaceae faecalis*, a contaminant frequently found in urine, and to better stabilize sensitive microorganisms such as *Streptococcaceae* spp.

## Conclusion

The **VACUETTE**<sup>®</sup> Urine CCM tube is suitable for the clinical use of microbial urine testing for up to 48 hours.

## References:

- [1] Wilson, M. (1996) General Principles of Specimen Collection and Transport. *Clinical Infectious Diseases*. 22:766-77.
- [2] Porter, I. (1969) Boric Acid Preservation of Urine Samples. *British Medical Journal*. 2, 353-355.
- [3] NHS. Health Protection Agency. Investigation of urine. BSP41.
- [4] CLSI. Quality Control of Microbiological Transport Systems; Approved Standard. M40-A. Vol. 23 No 34.
- [5] CLSI. Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline – Third Edition. GP16-A3. Vol 21 No 19.
- [6] European Urinalysis Guidelines. *Scand J Clin Lab Invest* 2000; 60: 1-96.
- [7] Kouri, T. (2008) Limits of preservation of samples for urine strip tests and particle counting. *Clin Chem Med*. 46 (5):703-713.
- [8] Schmiermann, G. (2010) The Diagnosis of Urinary Tract Infection: A systematic review. *Deutsches Ärzteblatt Int*. 107 (21):361-7.
- [9] UrinAX Dip slides. Product Manual from Axon Lab A