Evaluation of the VACUETTE® Urine CCM tube for microbial testing of urine samples

Background

The VACUETTE® Urine CCM tube is for the collection, transport and storage of urine samples for bacterial and yeast culture. VACUETTE® Urine CCM tubes are made of PET with a pre-defined vacuum for exact draw volumes. They are fitted with yellow VACUETTE® Safety Caps. The tube interior is sterile. The evacuated tube contains a stabilizer to preserve the urine sample in the range from 4-8°C to 20-25°C for up to 48 hours.

In various studies rapid transportation of urine 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, a delay in processing 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]

According to literature, urine culture results show that microbial counts of $\geq 10^5$ CFU/ml when using midstream urine are indicative of an infection and counts below this value usually imply contamination of the urine sample. In specific patient groups, counts between 10^2 CFU/ml and 10^5 CFU/ml may also be significant, depending on the type of microorganisms detected.[3, 8]

Study objective

A clinical study of the VACUETTE® Urine CCM tube was conducted to evaluate the stabilization of various microorganisms in urine samples. The objective was to demonstrate the stability of microorganisms and microbial counts including bacteria and yeasts for up to 48 hours in comparison to their initial testing when using the VACUETTE® Urine CCM tube stored at room temperature (20°C-25°C) and in the refrigerator (4°C-8°C).

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 bacteria and yeasts.

In a second step, testing of 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 in one log was regarded as clinically significant.[4]

Material and Methods

The following materials were used:

VACUETTE® Tube 10 ml Urine CCM, (item 455052), 16/100 yellow cap-black ring, Round base, non-ridged

VACUETTE® Urine beaker with integrated transfer device, 100 ml (item 724310), yellow lid, single-packed, sterile

Testing of samples from clinically inconspicuous subjects (healthy donors)

A total of 50 urine specimens were obtained from clinically inconspicuous subjects. The received mid-stream urine was pooled and subsequently sterile filtered.

Testing of samples from clinical setting

A total of 35 urine samples from 80 patients with suspected UTI were collected within daily routine procedure in a clinical setting. Urine samples from pathological subjects were tested for leukocytes and nitrite using a test strip (Arkray AUTION Sticks 10EA, AxonLabs AG, Germany) before being included in this study. The urine strip test is based on a reflective photometric measurement of test areas on the strip giving semi-quantitative results expressed in various levels. Only urine samples determined to be positive for leukocytes and/or nitrite were included in this study.

Testing of samples spiked with facultative pathogenic microorganisms

A total of 600 urine specimens were obtained from healthy donors and spiked with 10 different facultative pathogenic microorganisms (*Candida albicans, Candida glabrata, Enterococcus faecalis, Escherichia coli, Staphylococcus saprophyticus, Streptococcus agalactiae, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae*).

The pooled mid-stream urine was sterile filtered by vacuum filtration using 0.45 µm aPES filters followed by 0.2 µm aPES filters (Nalgene Rapid Flow ™, Nalgene Nunc International).

The bacterial strains were cultured in Brain Heart Infusion (BHI, product number 220837, BECTON DICKINSON) for 18-24 hours. *Candida spec.* was cultured in Sabouraud broth (Sabouraud Liquid Broth, product number 221014, Becton Dickinson) for 18-48 hours.

Afterwards, blood agar plates (BD BBL ™ Trypticase ™ Soy Agar with 5% Sheep Blood (TSA II), product number 254098, Becton Dickinson) were inoculated with the microbial suspension to get single colonies. After incubation for 18-24 hours (bacteria) or 48 hours (*Candida spec.*) at 37°C, single colonies of fresh cultures were picked to generate the spiked urine with desired microorganism concentration.
For testing different temperature ranges, microorganisms and concentrations, the spiked urine samples were poured into GBO urine beakers and then immediately transferred to GBO CCM tubes. This resulted in sample tubes for each of the microorganism, concentrations and temperature ranges evaluated. To ensure homogeneous starting conditions, urine was transferred into the GBO CCM Tubes within two hours after inoculation.

For all microorganisms, microbial suspensions were prepared in sterile filtered 0.9% NaCl solution (saline) and adjusted to McFarland nephelometric turbidity values from 0.45 to 0.55 units. McFarland turbidity standards were generated using DensiCHEK plus (product number 24404, lot number 2012243, BioMérieux, France). The correlation of microorganism concentrations (CFU/ml) with the acquired McFarland turbidities was established during internal preliminary tests.
The prepared microbial suspension with a McFarland value of 0.5 was 10-fold diluted in sterile filtered saline and used to spike the sterile filtered urine samples to achieve working concentrations of 1.5×10^2, 1.5×10^3 and 1.5×10^4 colony forming units per ml (CFU/ml).

Determination of colony count for each of the dilution was done by pipetting 100 µl from each dilution to the culture plates in triplicates. For testing to be considered valid, time-zero colony count for each dilution tested had to be between 25 and 250 CFU per plate. If colony counts between 25 and 250 CFU per plate could not be obtained from time-zero inoculum, adjustments to the inoculum volume were prepared. The final count was an average of the triplicates for each dilution yielding 25 to 250 CFU per plate.

All microbial specimens for colony counts were generated and sampled on the same day within two hours (T0), after 24 hours (T1) and after 48 hours (T2) after sample collection (clinically inconspicuous and conspicuous) or spiking of urine and sample tube filling. Between the sampling time points the specimens were stored at room temperature (20-25°C) and refrigeration (4-8°C), respectively.

The identification of microorganisms in urine from pathological and clinically inconspicuous subjects as well as of strains used for spiking purposes was done by MALDI-TOF (matrix-

assisted laser desorption ionization – time of flight) mass spectrometry. The MALDI-TOF analysis was generated on MALDI Biotyper microflex LT (Bruker Daltonics, Germany).

Results:

Samples from clinically inconspicuous subjects

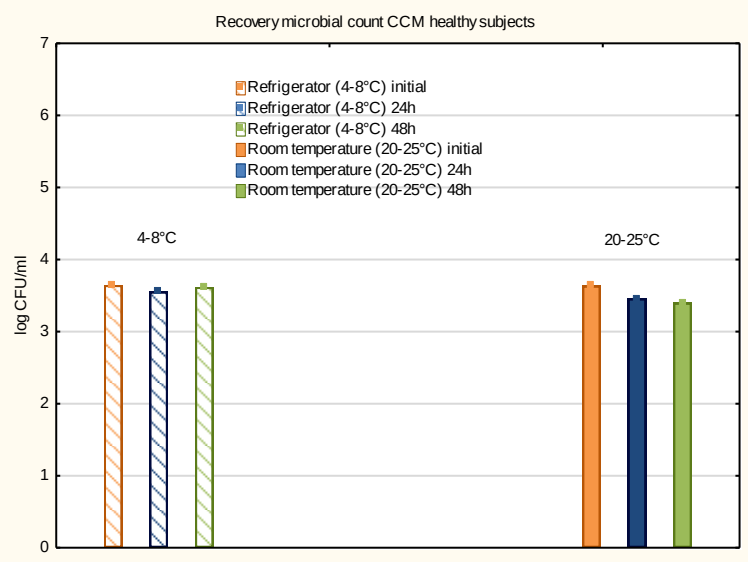

Figure 1: Recovery for VACUETTE® CCM tube

The colony counts at time point 48h remained stable in both temperature ranges examined in relation to the colony counts at time point T0. The results above indicate no significant deviation.

Samples from clinical setting

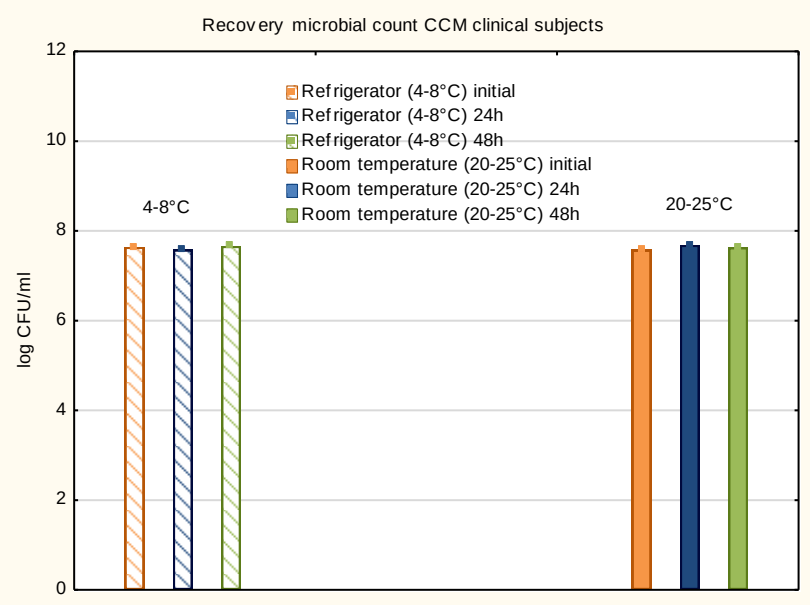

Figure 2: Recovery for VACUETTE® CCM tube: Culture Positive Samples

Logarithmic data were compared for the time point within 2 hours of urine collection (initial value), after 24 hours, and after 48 hours. The colony counts at time point 48h remained stable in both temperature ranges examined in relation to the colony counts at time point T0.

Considering the colony count over time (initial to 48h), the results above meet the acceptance criteria defined in CLSI M40-A2 (to be considered acceptable, colony counts changes in CFU shall be no more than one log step between initial time point and the CFU count after incubation for a specific period) and indicate no significant deviation.

Samples spiked with facultative pathogenic microorganisms

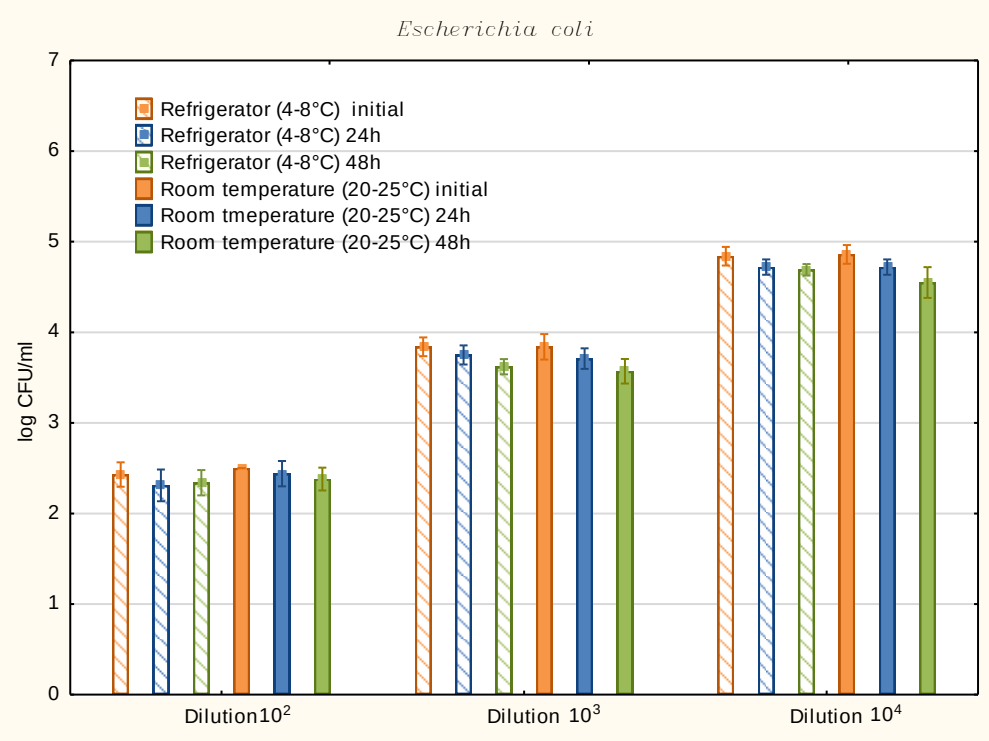

Figure 3: Stability of *Escherichia coli*

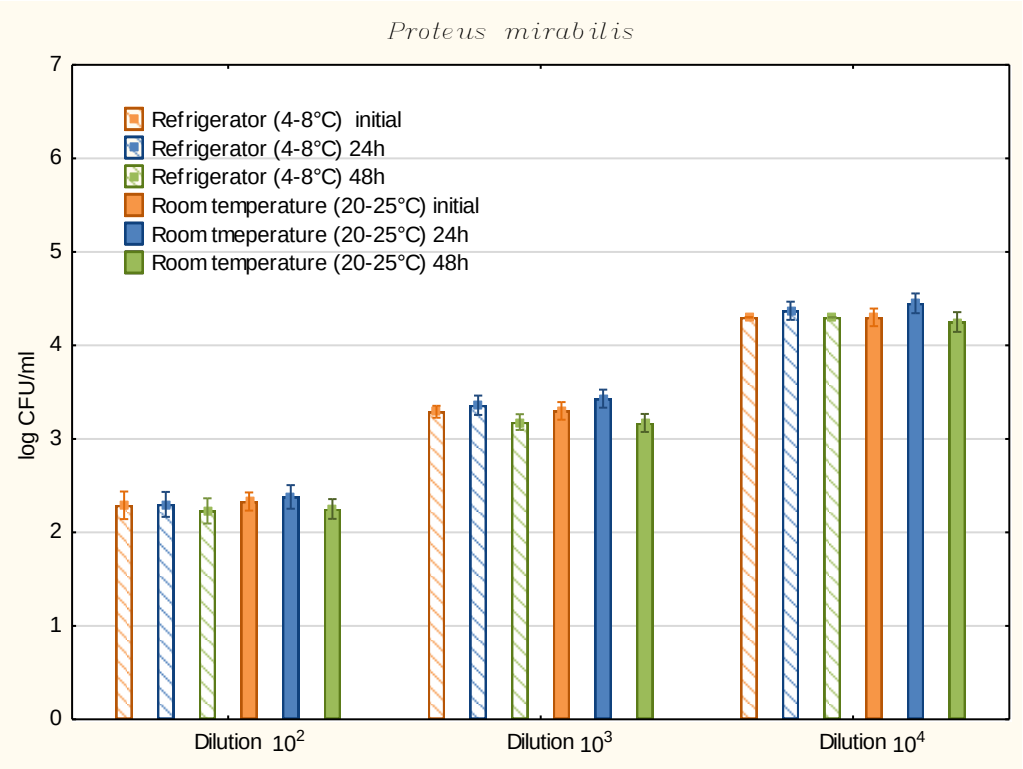

Figure 4: *Stability of Proteus mirabilis*

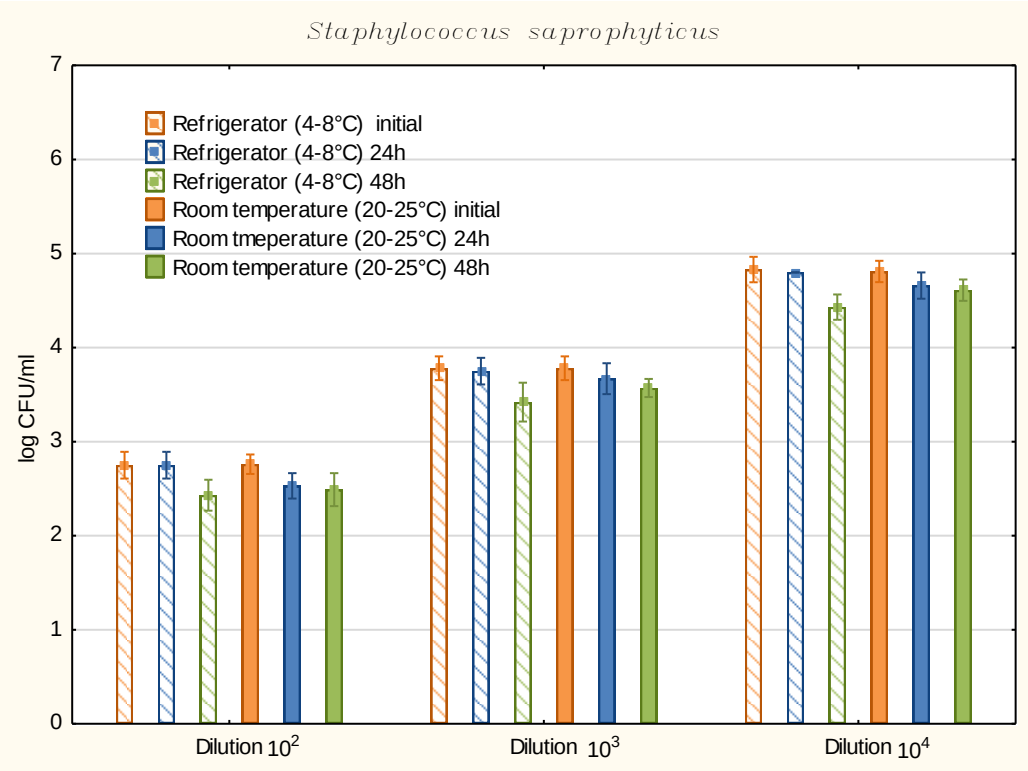

Figure 5: Stability of *Staphylococcus saprophyticus*

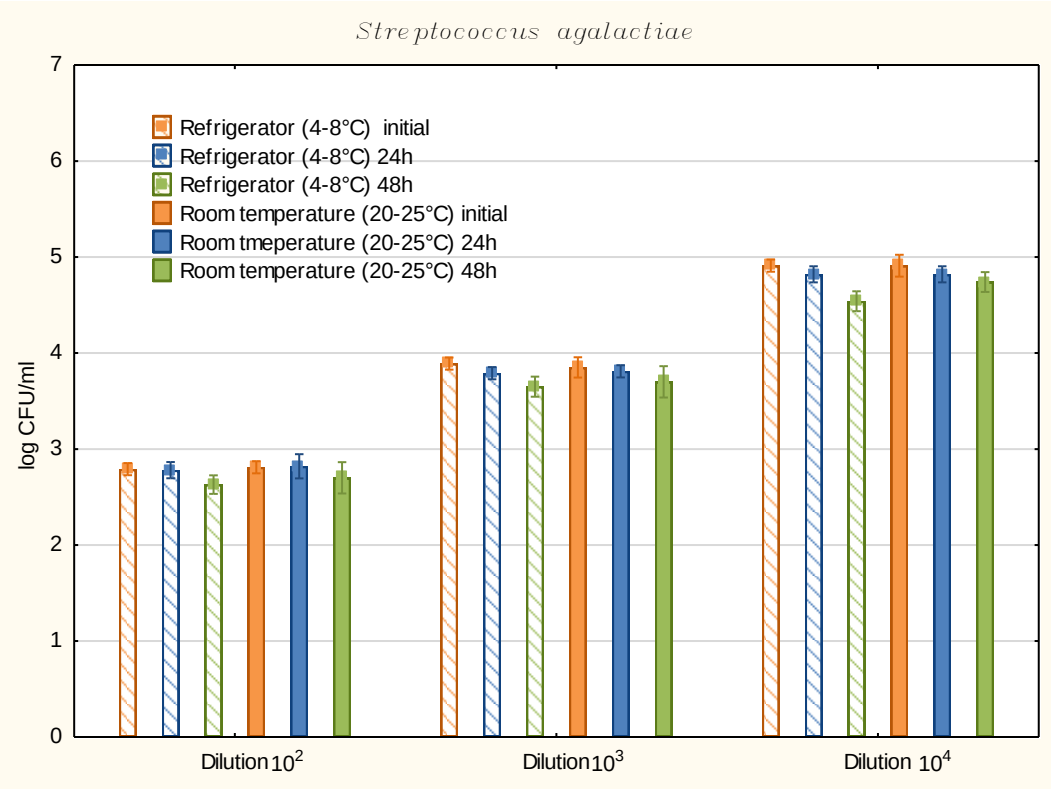

Figure 6: Stability of *Streptococcus agalactiae*

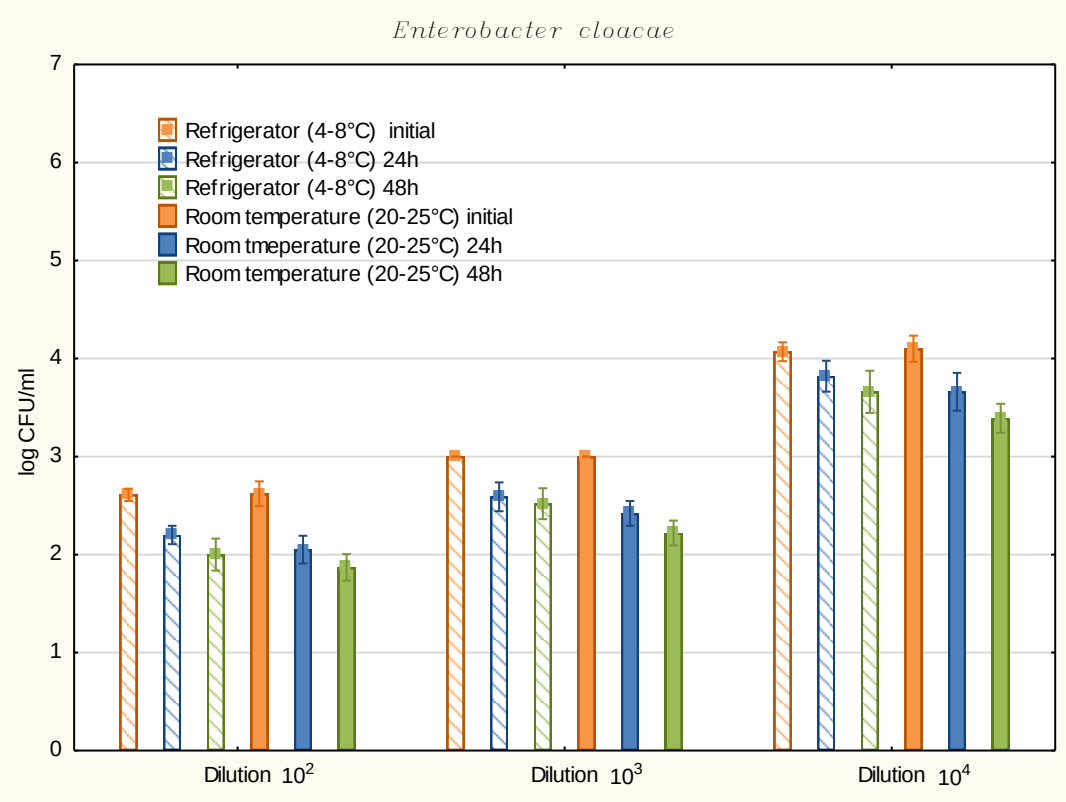

Figure 7: Stability of *Enterobacter cloacae*

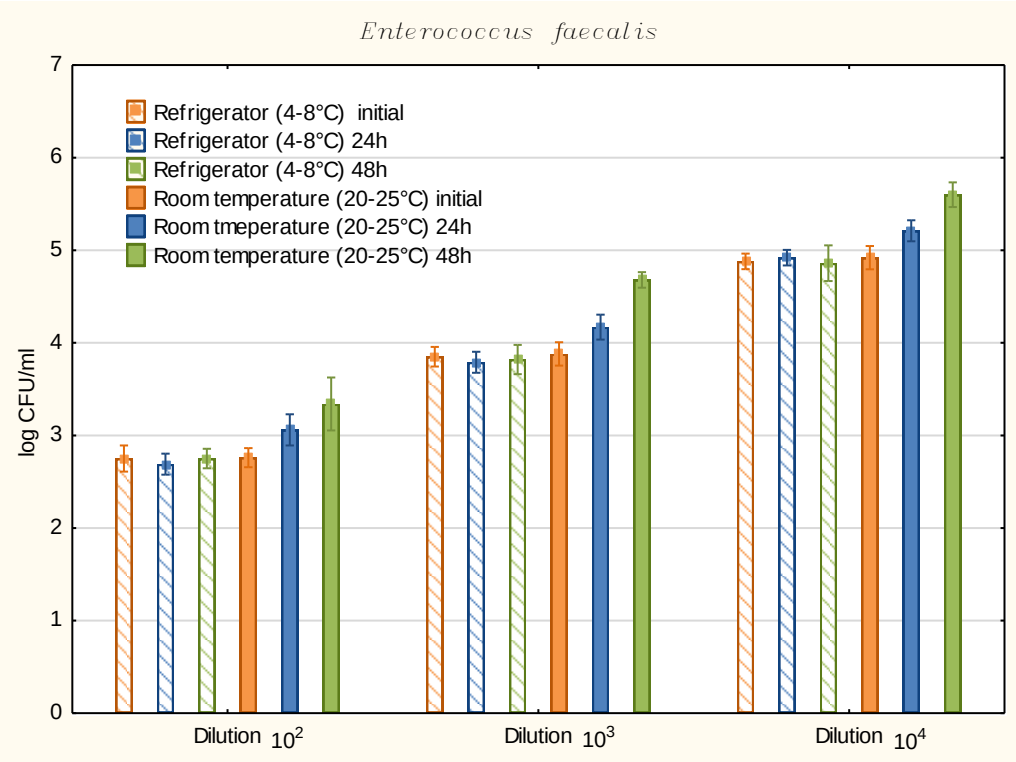

Figure 8: Stability of *Enterococcus faecalis*

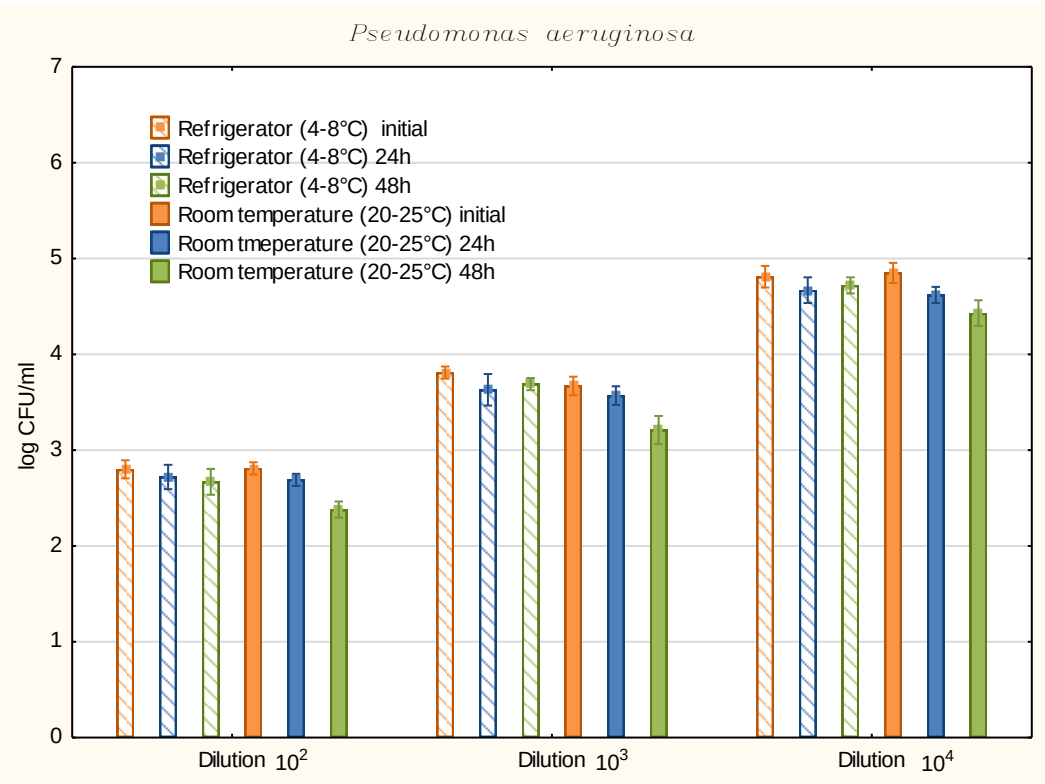

Figure 9: Stability of *Pseudomonas aeruginosa*

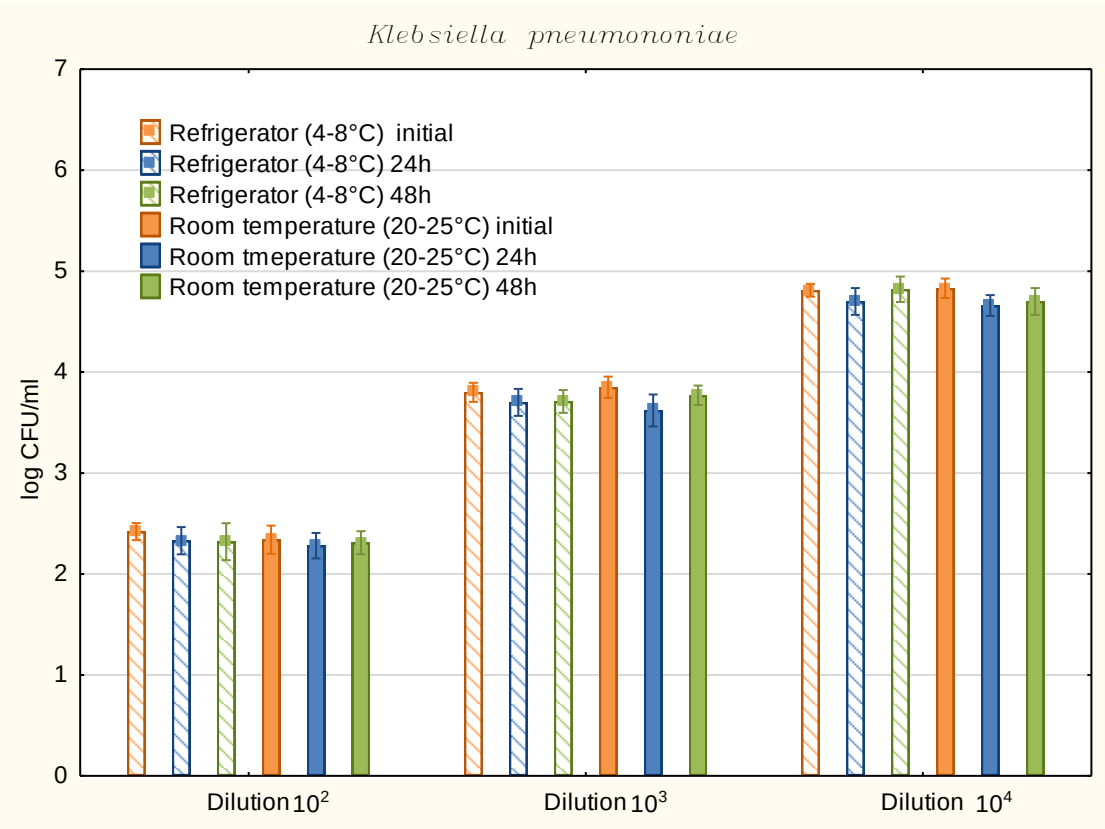

Figure 10: Stability of *Klebsiella pneumononiae*

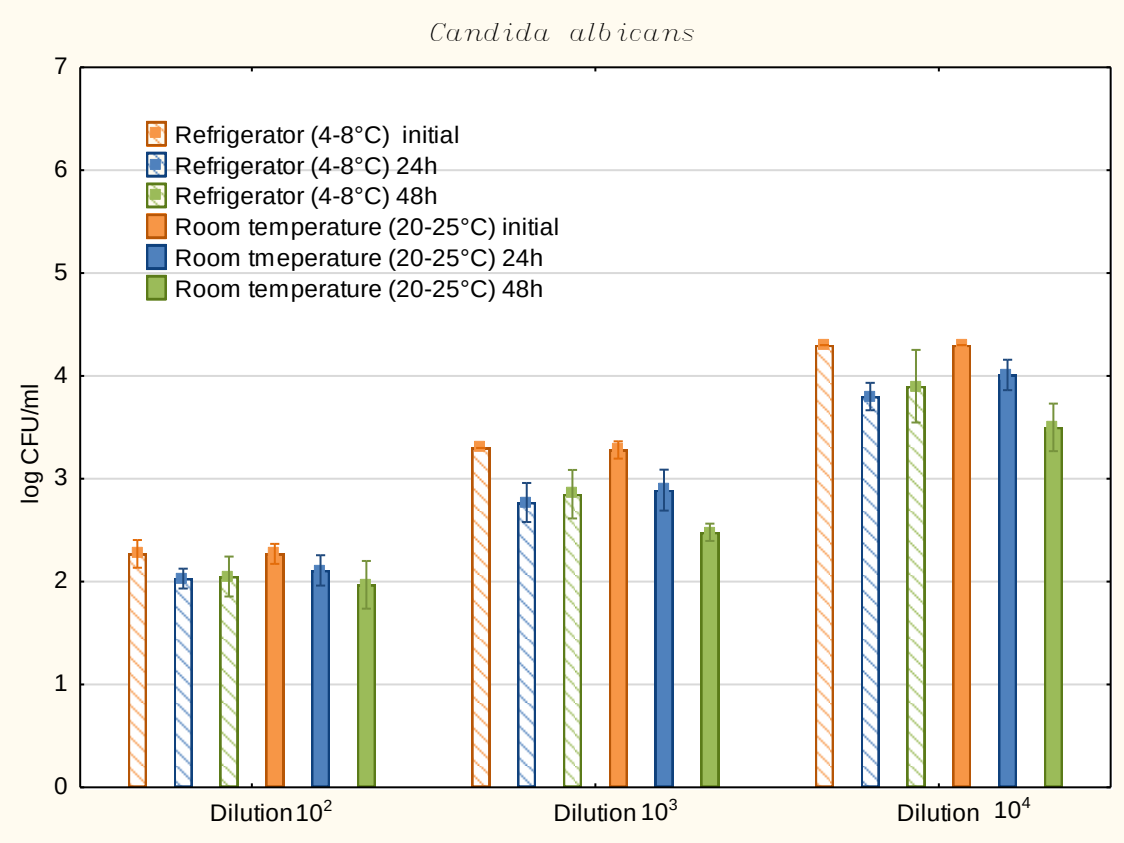

Figure 11: Stability of *Candida albicans*

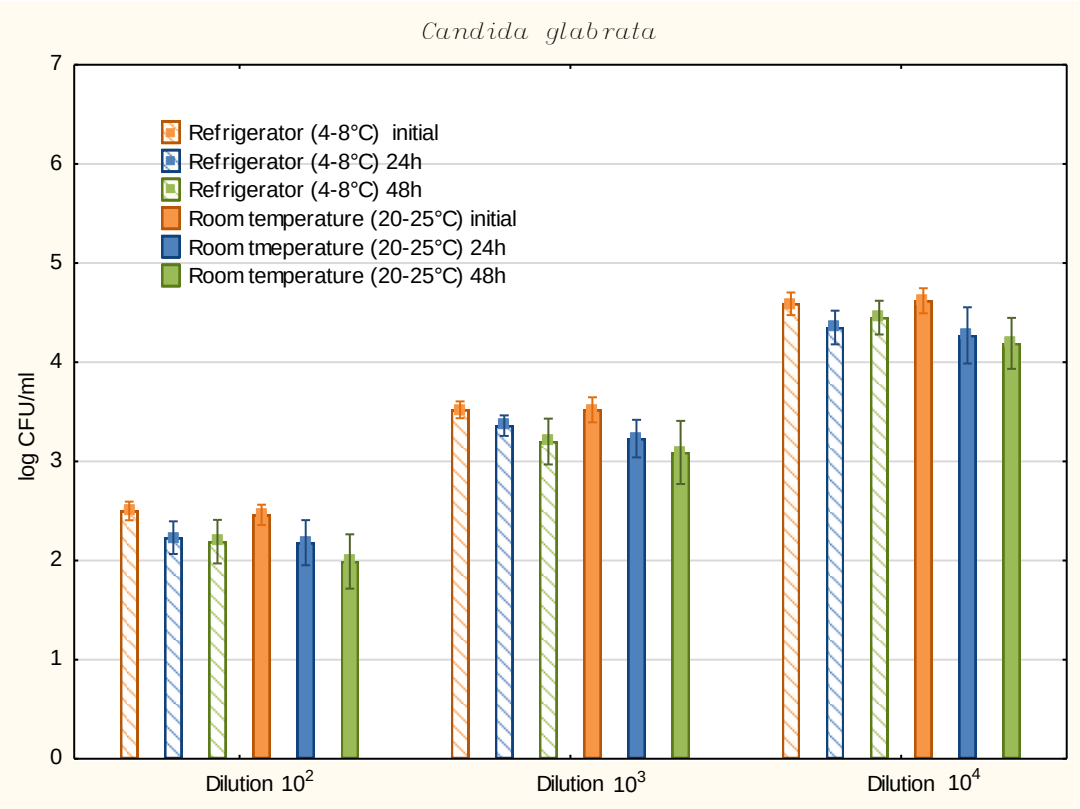

Figure 12: Stability of *Candida glabrata*

The three concentrations tested of colony counts of *Escherichia coli* (ATCC® 25922), *Enterococcus faecalis* (ATCC® 29212), *Enterococcus faecalis* (ATCC® 29212), *Proteus mirabilis* (ATCC® 7002), *Pseudomonas aeruginosa* (ATCC® BAA-427), *Staphylococcus saprophyticus* (ATCC® 15305), *Enterobacter cloacae* (ATCC® 13047), *Klebsiella pneumoniae* (ATCC® 13883), *Streptococcus agalactiae* (ATCC® 13813), *Candida albicans* (ATCC® 24433)*, and Candida glabrata* (ATCC® 24433) remained stable at time point 48h at both temperatures in relation to the colony counts at the initial time point.

Conclusion:

None of the 50 examined urine specimens obtained from clinically inconspicuous subjects or 35 examined urine specimens obtained from clinically conspicuous subjects showed a significant deviation of colony counts (CFU/ml) at time point 48h when compared to the colony counts at initial time point (CFU/ml) of the same tube.

Furthermore, none of the 600 examined urine specimens of sterile filtered urine obtained from clinically inconspicuous subjects, spiked with ten different microorganisms showed a significant deviation of colony counts (CFU/ml) at time point 48h when compared to the colony counts (CFU/ml) at the initial time point of the same tube. The colony counts remained stable in correctly filled sample tubes in all examined temperature ranges and microorganism concentrations.

The **VACUETTE®** Urine CCM Tubes stabilize the tested microorganisms in urine specimens from clinically inconspicuous and conspicuous subjects as well as in spiked urine specimens for 48 hours of storage at room temperature (20-25°C) and refrigeration temperature (4-8°C), respectively.

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-A2. Vol. 34 No 9.

[5] CLSI. Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline – Third Edition. GP16-A3. Vol 29 No 4.

[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] Schmiemann, G. (2010) The Diagnosis of Urinary Tract Infection: A systematic review. Deutsches Ärzteblatt Int. 107 (21):361-7.