

# Isolation and culturing of urinary bacteria for VOC-based rapid diagnostics

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## Summary:

Rapid alternatives to traditional 2 - 4 day urine culture are urgently needed to improve patient recovery and combat antimicrobial resistance. Volatile organic compound (VOC) sensing offers a powerful, non-invasive route to bacterial identification, but establishing reproducible metabolic markers requires a workflow that accounts for both pathogen diversity and variable growth dynamics. Using expanded urine culture, we isolated bacteria from 80 patient samples, revealing 35 distinct genera and pronounced inter-individual compositional variability. Growth kinetics of 75 *E. coli* strains were benchmarked under urinary-like conditions, revealing significant lag-time variation that motivates rigorous standardization for reproducible VOC profiling.

**Keywords:** UTI pathogens, method development, UTI rapid diagnostics, expanded urine culture

## Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide. Generalized antibiotic therapy is often administered, contributing significantly to antimicrobial resistance [1]. Volatile organic compound (VOC) profiling of bacterial cultures offers a promising, non-invasive route to rapid pathogen identification: bacteria produce characteristic metabolic volatiles detectable within hours [2]. However, translating VOC signatures into reliable diagnostics requires a well defined, reproducible culture workflow that accounts for the full diversity of urinary pathogens and their variable growth dynamics. This work presents the building of such a workflow, from patient-derived isolate collection to standardized cultivation under urinary-like conditions.

## Methods

Urine samples were collected from 80 patients. Expanded urine culture (EUC) was applied to enhance recovery of slow-growing and low-abundance organisms: samples were plated on multiple media types (BHIS-blood agar, chromogenic agar, MRS) and incubated for up to 5 days under both aerobic and anaerobic conditions. All isolated colonies were identified by MALDI-TOF mass spectrometry. 75 *E. coli* strains were

selected to benchmark growth kinetics. Strains were cultivated in anaerobic artificial urine medium at 37°C, and optical density (OD<sub>600</sub>) was measured every 20 minutes for 22 hours using an automated plate reader. Growth curves were normalized to initial OD ( $\Delta$ OD<sub>600</sub>) and subjected to k-means clustering ( $k = 4$ ) based on lag time and maximum growth rate.

## Results

We isolated 35 different bacterial genera originating from 80 patients. *Escherichia* was the most prevalent genus ( $n = 28$  patients), followed by *Streptococcus* ( $n = 19$ ), *Enterococcus* ( $n = 16$ ), *Klebsiella* and *Staphylococcus* ( $n = 9$  each), as shown in Fig. 1. Beyond the dominant uropathogens, a pronounced “long tail” of low-prevalence genera (e.g., *Veillonella*, *Actinomyces*, *Facklamia*) was observed. These genera were isolated from fewer than 5 patients, highlighting the remarkable inter-individual compositional variability of the urinary microbiome.

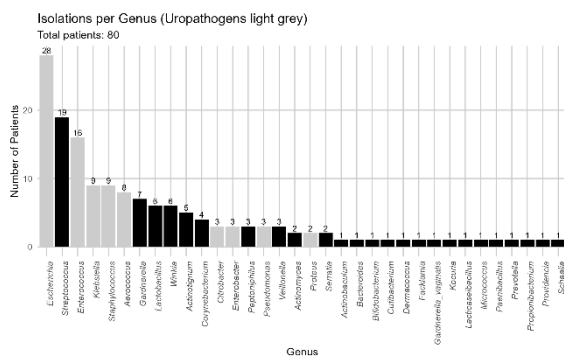


Fig. 1. Isolations per genus from 80 patient urine samples (EUC). Uropathogens shown in light grey. A pronounced long tail highlights low abundance non-uropathogenic genera.

Growth kinetics of 75 *E. coli* strains in artificial urinary media under anaerobic conditions revealed four distinct growth clusters (Fig. 2). The majority of strains (Clusters 1 and 2,  $n = 68$ ) showed moderate growth with  $\Delta OD_{600}$  reaching 0.3–0.4 after 22 hours, while Cluster 0 ( $n = 6$ ) displayed markedly slower kinetics. Notably, one outlier strain (Cluster 3,  $n = 1$ ) exhibited substantially faster and higher-amplitude growth ( $\Delta OD_{600} \approx 0.95$ ), with a steep exponential phase beginning around hour 5. The observed variation in lag times (ranging from under 1 to over 5 hours) underscores why a fixed time-point sampling strategy can lead to inconsistent growth phases, followed by inconsistent VOC profiles. These results motivate the use of growth-phase-aware, standardized cultivation

protocols to ensure reproducibility across strains and growth phases.

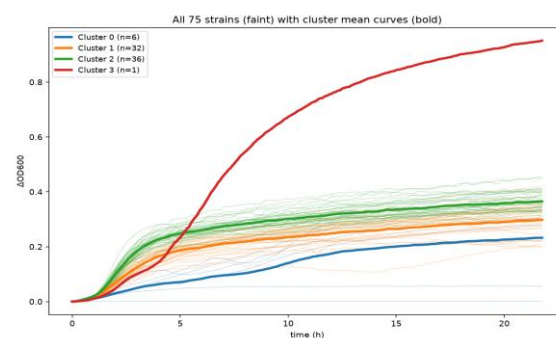


Fig. 2. Growth curves ( $\Delta OD_{600}$ ) of 75 *E. coli* strains in anaerobic artificial urine medium over 22 hours. Individual strain curves (faint) and cluster mean curves (bold) are shown for four clusters.

## References

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