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Methods for studying the bacterial urinary microbiome of non-menopausal women

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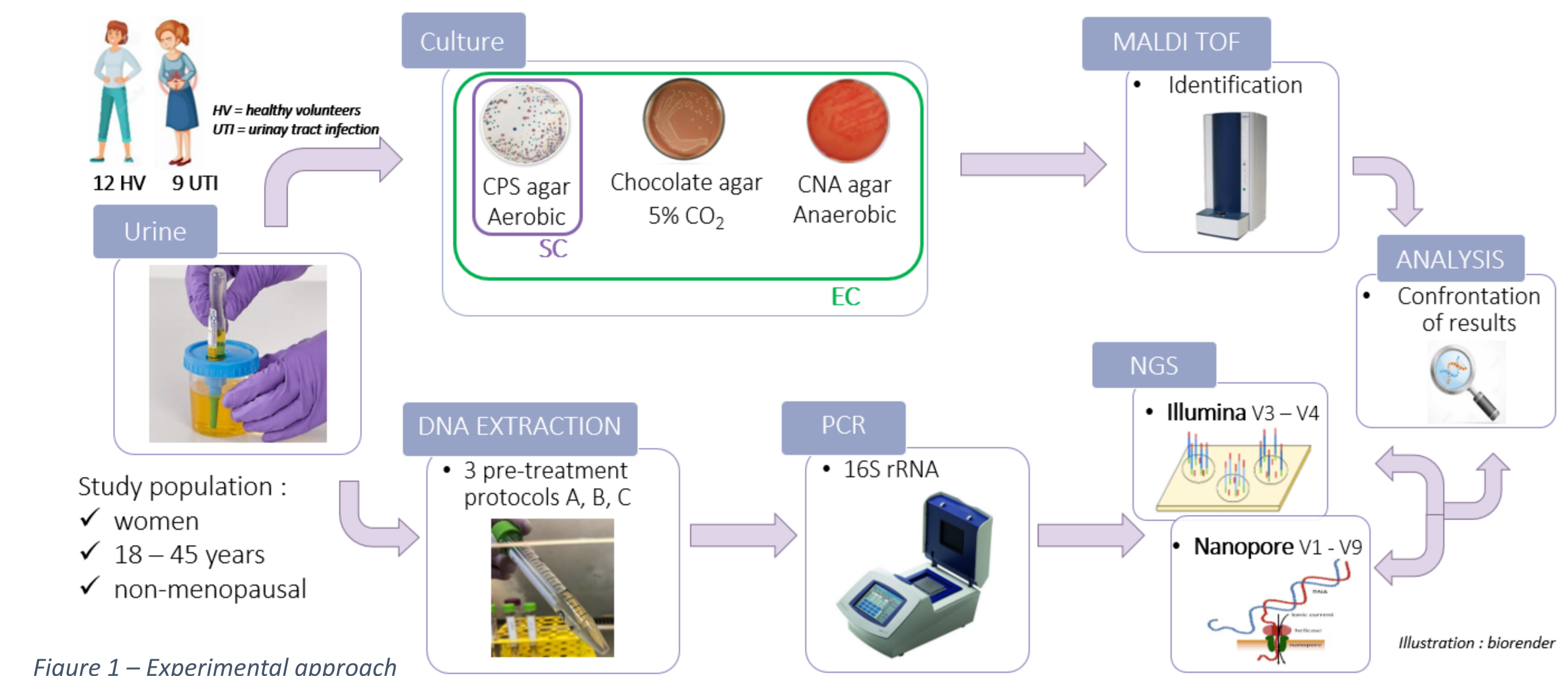
Background

Objectives

Next generation sequencing technologies (NGS) as well as the development of extended cultures (EC) have allowed the recent discovery of an **Urinary Microbiota (UM)**¹. Nevertheless, there are still many unknowns regarding the UM associated with healthy subjects or **urinary tract infections (UTIs)**.

- Locally implement **EC** and **NGS** (Illumina and Nanopore) to describe **female bacterial UM**
- Identify **urotype** (dominant bacterial taxon) for each urine specimen
- Compare bacterial UM of patients with **UTIs** with that of **healthy volunteers (HV)**

Methods



EC vs Standard Culture (SC)

- 2 agar plates in addition to the chromogenic UTI agar :
 - **CHOC** (chocolate) : 5% CO₂
 - **CNA** (colimycin nalidixic agar) : anaerobic
- 2 volumes of inoculation : **10 and 100 µL**
- Incubation **48h at 37°C**

NGS : Illumina vs Nanopore

- Illumina :**
 - PCR 16S rRNA **V3-V4** (550 pb)
 - Metagenomics Analysis Module
 - Database derived from **GreenGenes (13_5)**
- Nanopore :**
 - PCR 16S rRNA **V1 - V9** (1550 pb)
 - EPI2ME 16S Workflow
 - Database derived from **NCBI**

Figure 1 – Experimental approach

Results

Analysis of UM by culture

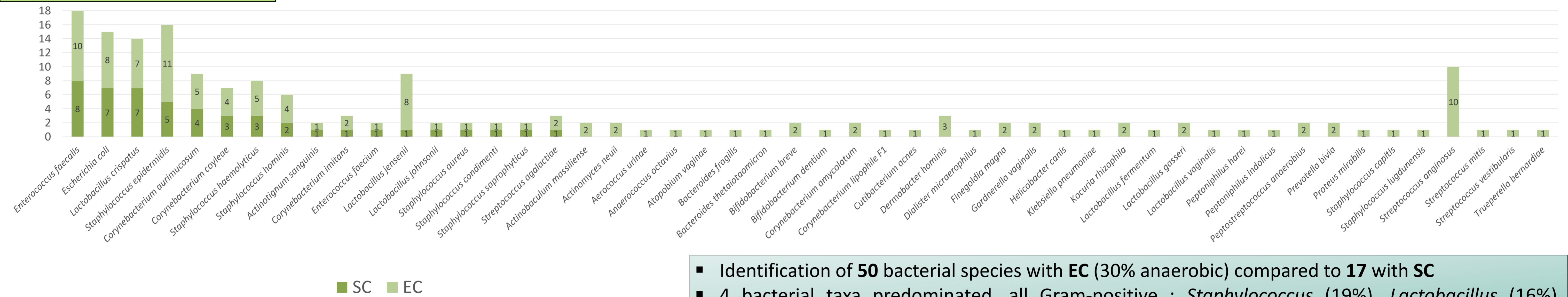


Figure 2 – Prevalence of species identified in standard culture (SC) and in extended culture (EC)

- Identification of **50 bacterial species** with EC (30 anaerobic) compared to **17** with SC
- 4 bacterial taxa** predominated, all Gram-positive : *Staphylococcus* (19%), *Lactobacillus* (16%), *Streptococcus* (11%) and *Corynebacterium* (11%)

Analysis of UM by NGS

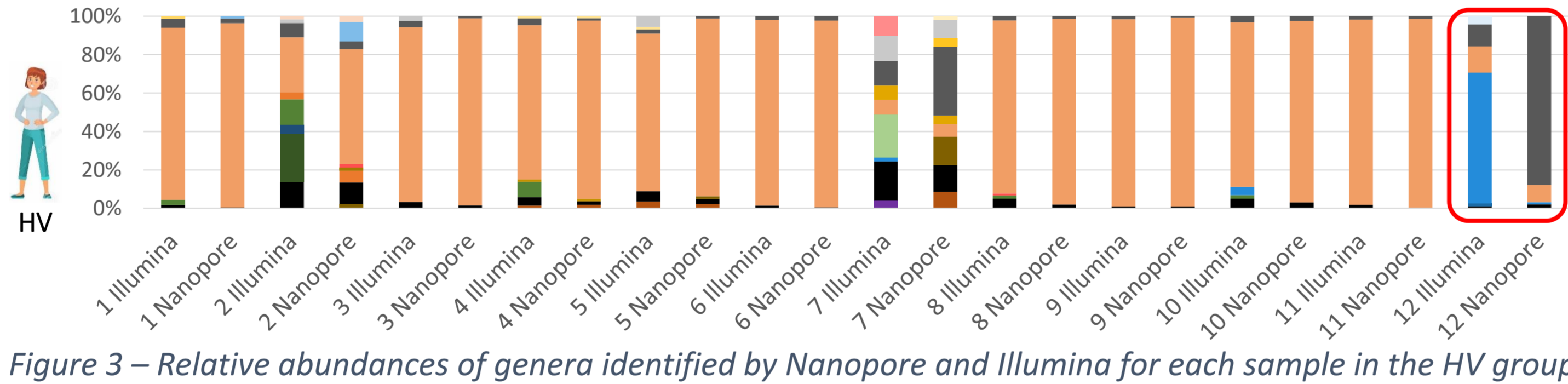
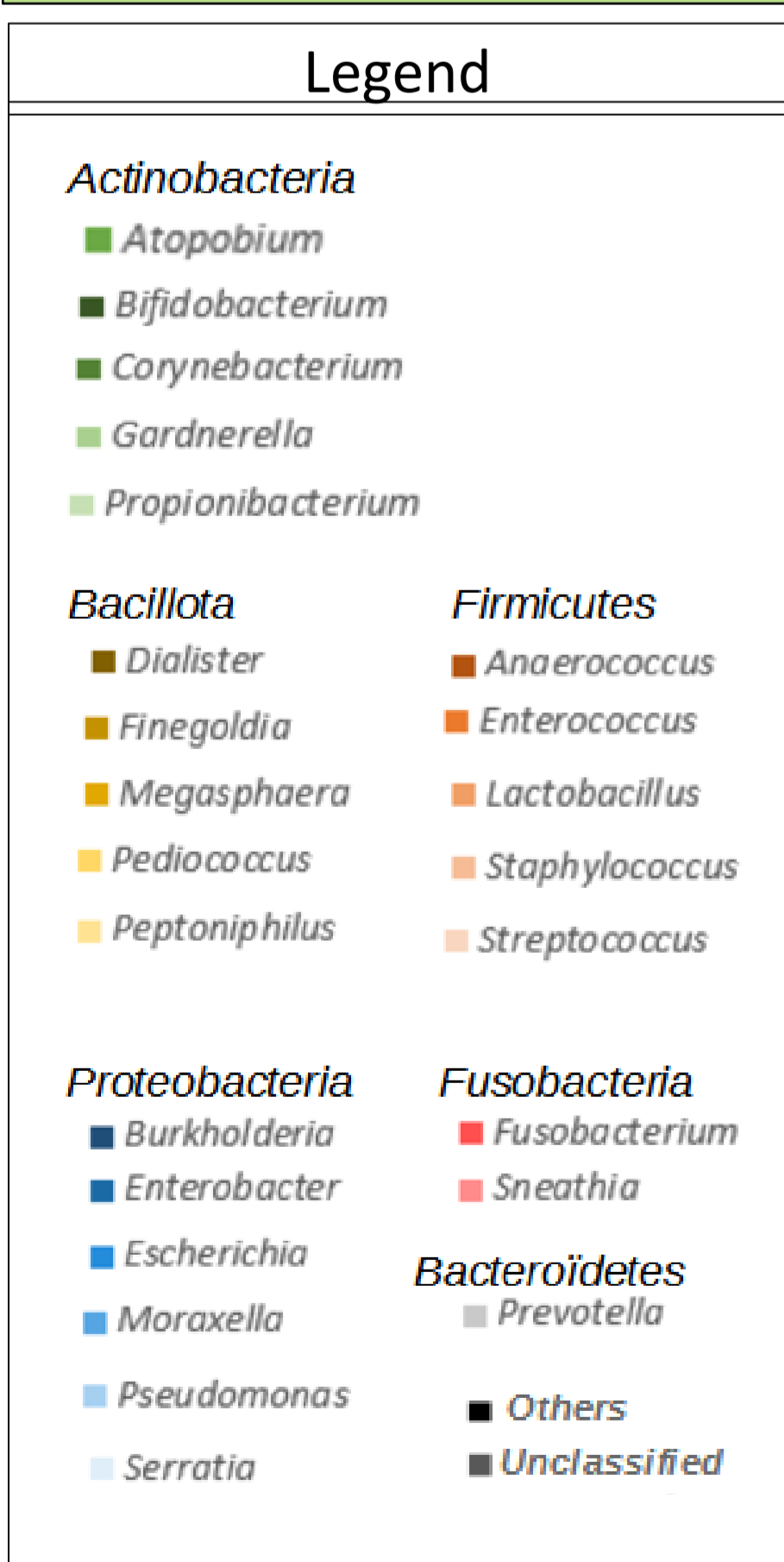


Figure 3 – Relative abundances of genera identified by Nanopore and Illumina for each sample in the HV group

- Similar profiles at the gender level for **10/12 samples** with both NGS technologies
- Discordance for *Escherichia*** identification between Nanopore and Illumina for 1 sample (boxed in red)

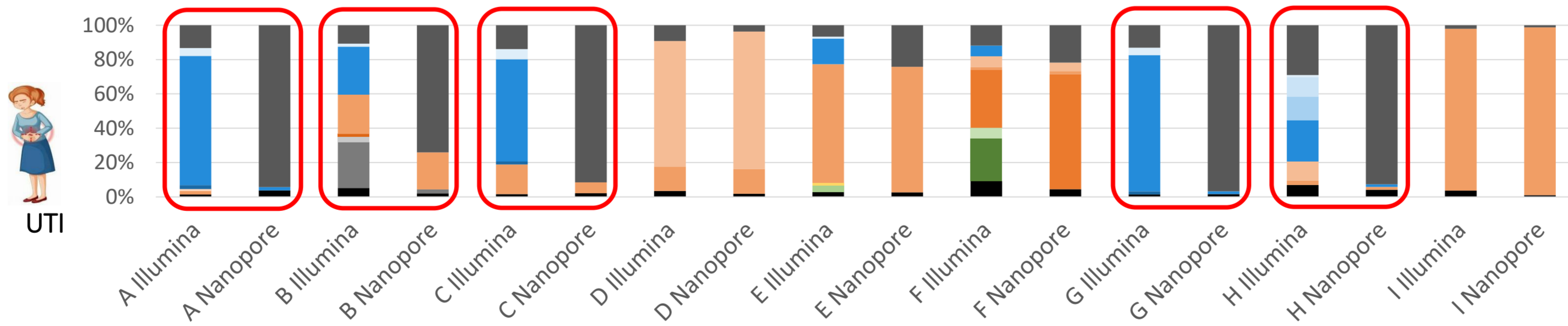


Figure 4 – Relative abundances of genera identified by Nanopore and Illumina for each sample in the UTI group

- For 5/9 samples : majority of **unclassified** reads at genus level by Nanopore while ***Escherichia*** identified by Illumina (boxed in red). However, predominance of Enterobacteriaceae for four of them (A, B, C and G) at the family level.

UM of UTI vs HV

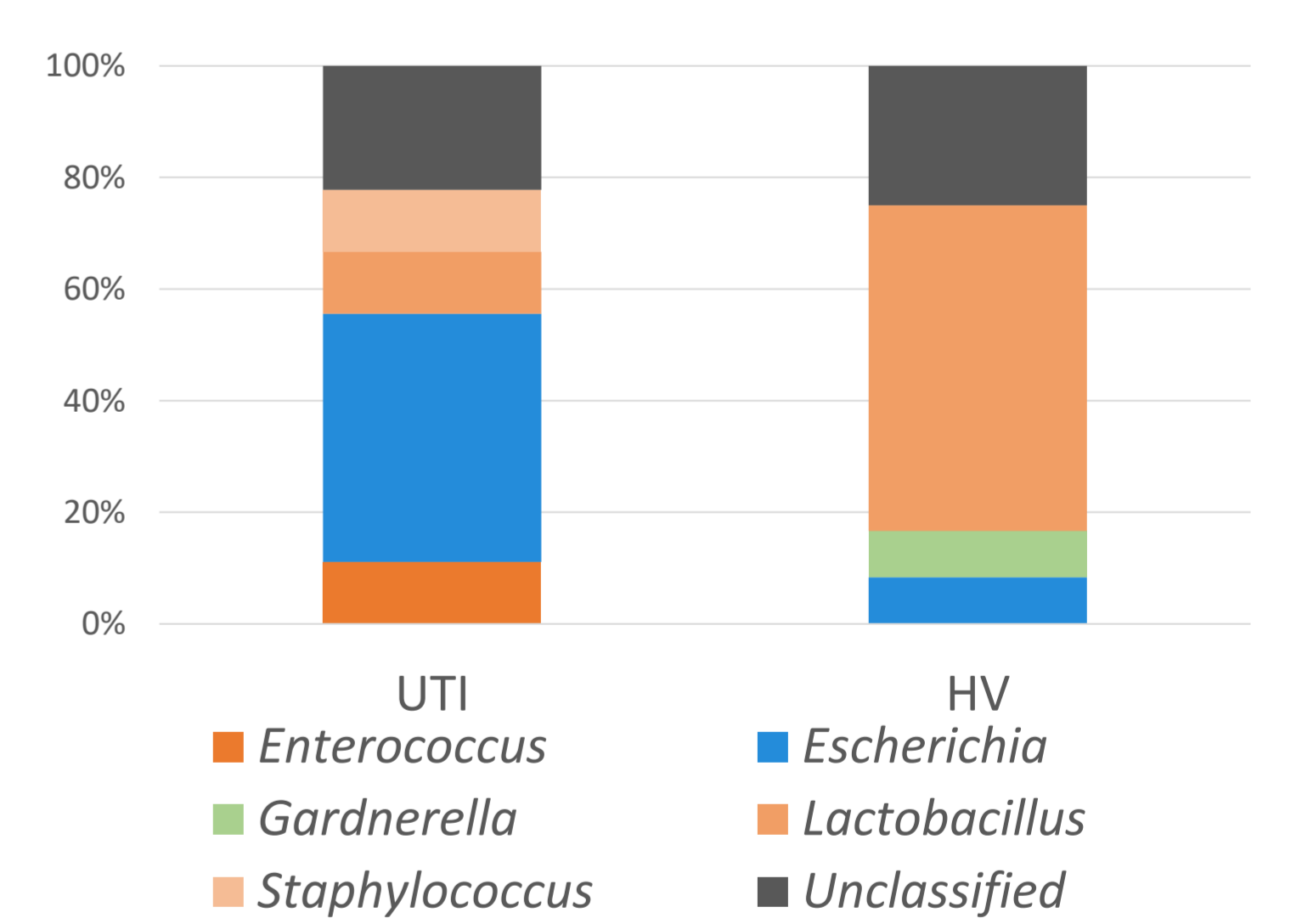


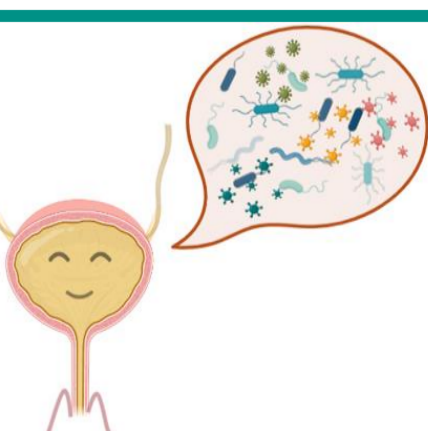
Figure 5 – Comparison of urotypes identified concordantly by Illumina and EC in UTI cases and HV. Unclassified = discordant urotypes between Illumina and EC

Major Urotypes

- UTI (4/9) : *Escherichia***
- HV (7/12) : *Lactobacillus***

Conclusions

- EC** improves detection of uropathogens compared to SC procedure¹. Both **EC and NGS** results showed a **difference in the composition of the UM** between **HV** and **UTI** patients. This study highlights a high occurrence of ***Lactobacillus*** in the female UM, as already reported².
- Nevertheless, these results must to be confirmed with a larger sample collection, and the analyses of the NGS data need to be further investigated, particularly by using other databases than those suggested by the manufacturers.



1. Price, Travis K., Tanaka Dune, Evann E. Hilt, Krystal J. Thomas-White, Stephanie Kliethermes, Cynthia Brincat, Linda Brubaker, Alan J. Wolfe, Elizabeth R. Mueller, and Paul C. Schreckenberger. "The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms." *Journal of Clinical Microbiology* 54, no. 5 (May 2016): 1216–22.
 2. Ugarcina Perovic, Svetlana, Magdalena Ksiezarek, Joana Rocha, Elisabete Alves Cappelli, Márcia Sousa, Teresa Gonçalves Ribeiro, Filipa Grosso, and Luísa Peixe. "Urinary Microbiome of Reproductive-Age Asymptomatic European Women." *Theis. Microbiology Spectrum* 10, no. 6 (December 21, 2022): e01308-22.