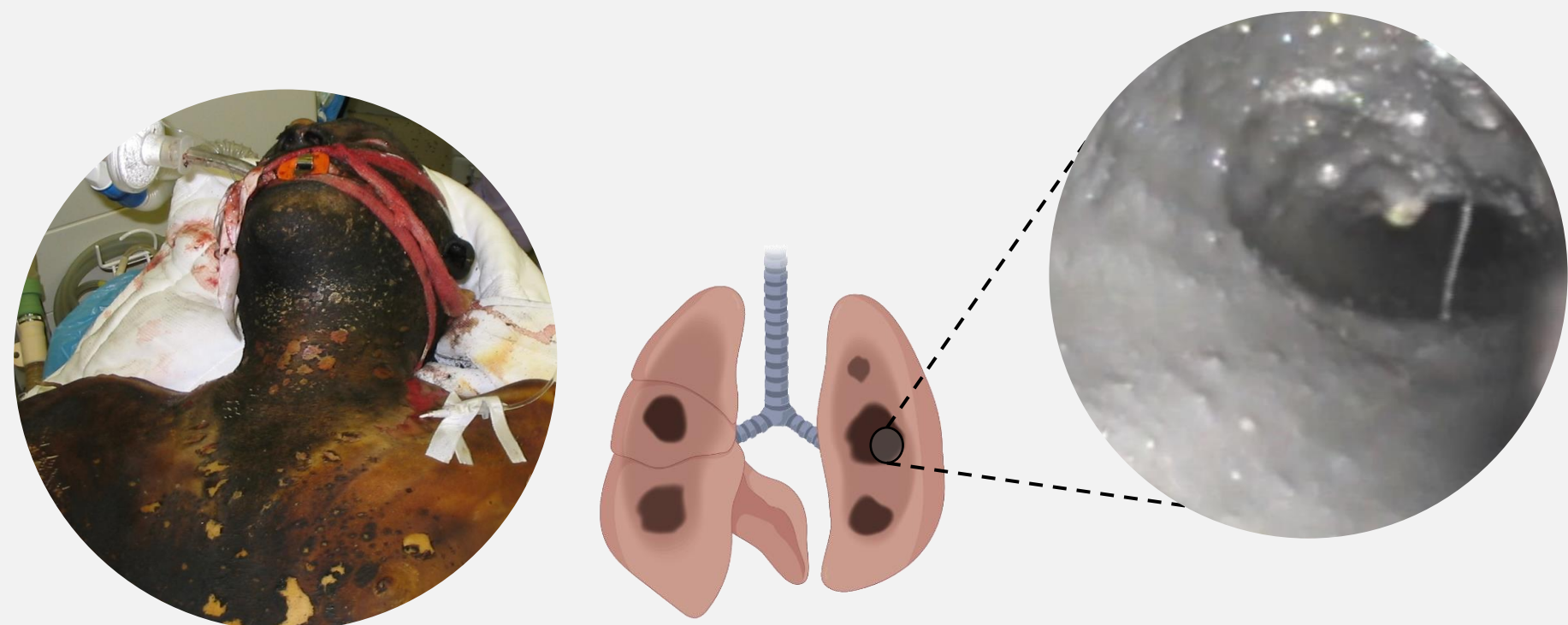


# INHALATION INJURY INFECTIOUS COMPLICATIONS AND MICROBIOME OF AIRWAYS

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## BACKGROUND



- Inhalation injury is caused by inhalation of burning products or steam.
- 2 categories: low-grade and high-grade
- Patients are endangered mainly by secondary complications.
- One of the major complication is microbial infection.<sup>1,2</sup>

## OBJECTIVES OF THE MICROBIAL PART OF THE PROJECT

The main aim of the microbial part of the project, which deals with bacteria, fungi and antibiotic resistance, is to study the dynamic changes in microbiome of the airways in patients with inhalation injury. Results of this study can help an early diagnosis and correctly targeted treatment of infections.

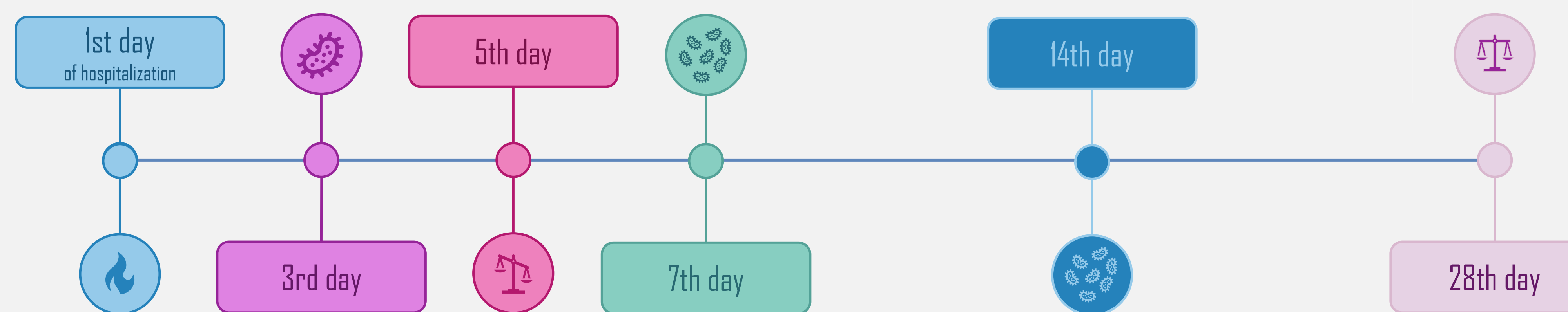
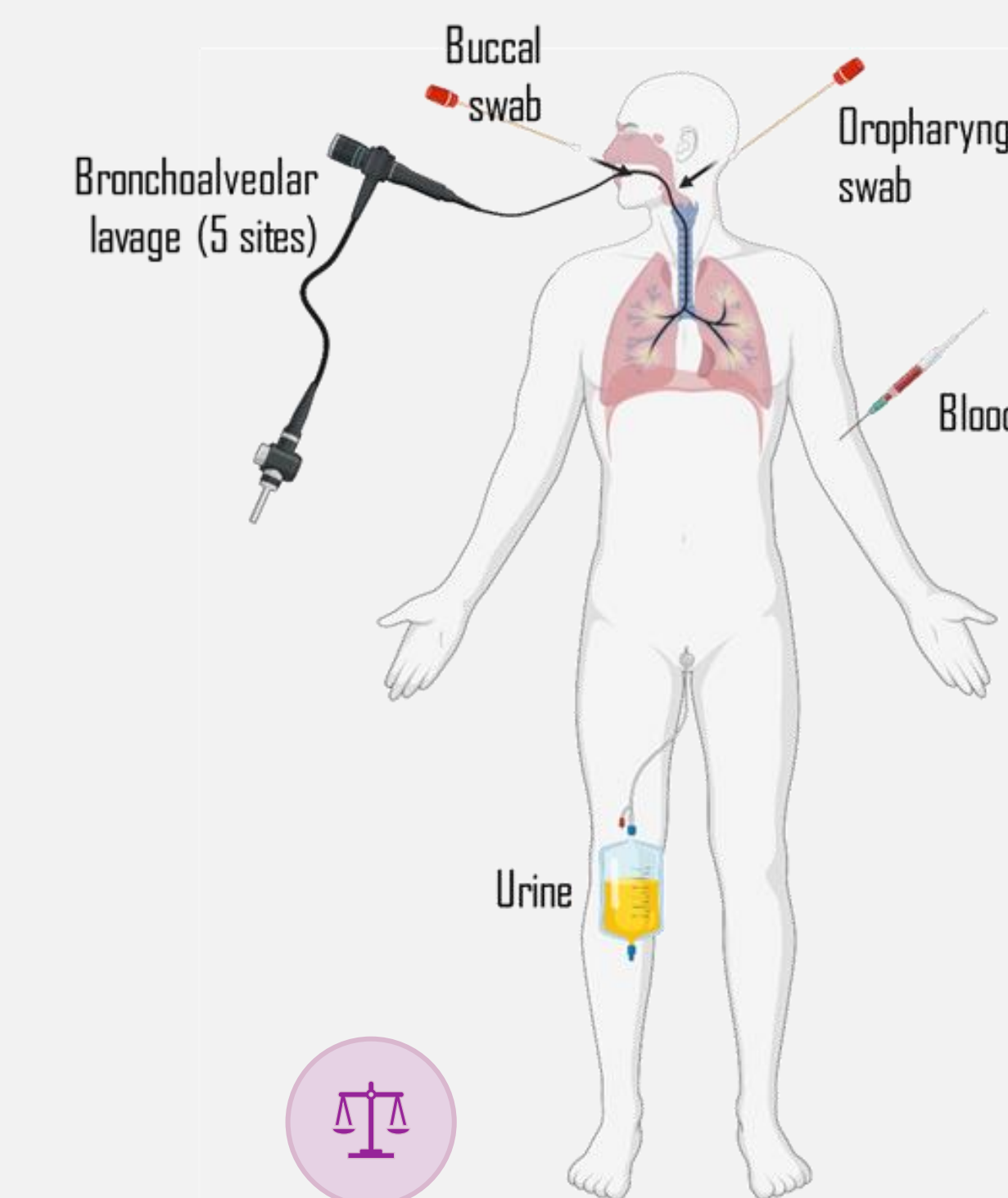
## DESIGN OF THE MICROBIAL PART OF THE PROJECT

### 5 MATRICES FOR ANALYSIS

- It was chosen 5 biological matrices for microbiome analysis – **buccal** and **oropharyngeal swabs**, bronchoalveolar lavage (BAL), **blood**, and **urine**.
- Furthermore, the BAL samples are collected from 5 different parts of the lungs to represent the whole organ microbial composition.

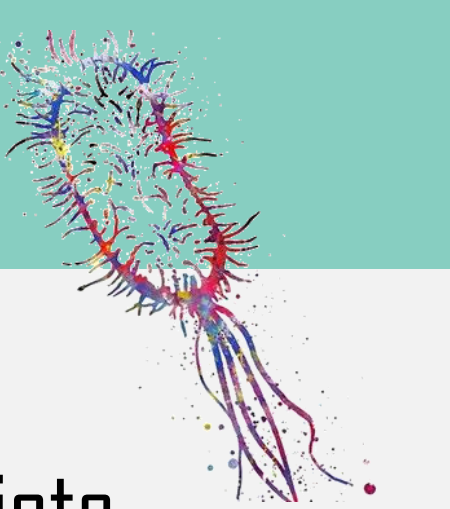
### TIMELINE

- The dynamical changes in microbial composition (especially in the respiratory tract) are studied according to the length of hospitalization.



## HYPOTHESIS

- The patient will be without microbiota immediately after hospitalization.
- The infections developed during the next days. Usually, one or few bacterial/fungal species overgrowth leads to dysbiosis.
- Sum of resistance genes will be related to the length of hospitalization.

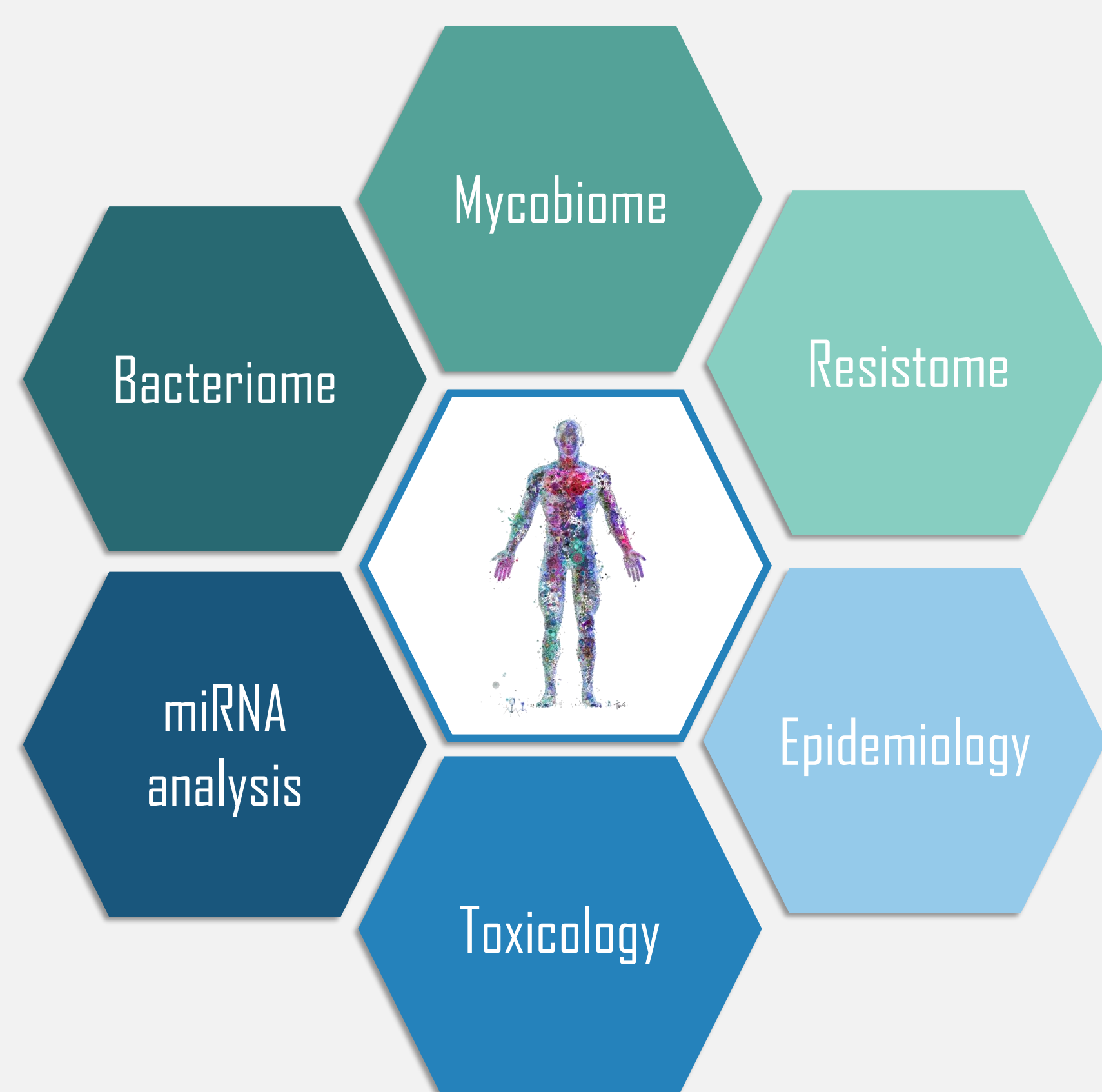


## CONCLUSION

Prediction of infectious complications and early deployment of targeted treatment can positively involve the recovery of the patient.



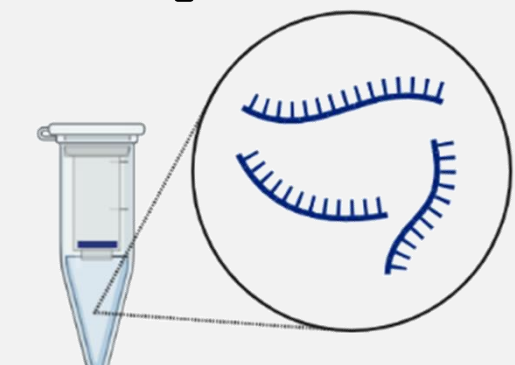
## PROJECT multidisciplinary research



## METHODOLOGY OF THE MICROBIAL PART OF THE PROJECT

### Isolation of DNA

- Isolation kit: **QIAamp DNA Blood Mini Kit (QIAGEN)**.<sup>5,6</sup>
- Isolation protocol is optimized.
- Isolation kit suitable for all 5 biological matrices.



### Amplification

#### Bacteriome

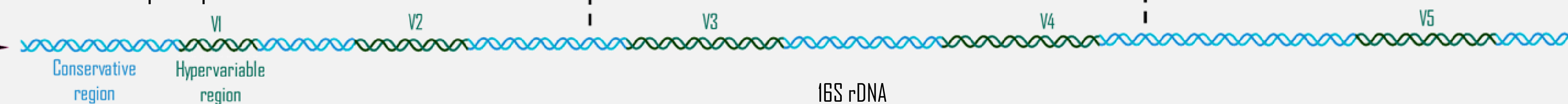
- PCR for bacteriome: microbial **16S rDNA**.
- Primers: **IL primers** targeted V3 and V4 regions.
- Note: the standard bacterial MOCK community is added into each amplification.
- qPCR of total bacterial DNA.
- Multiplex qPCR of selected bacteria.

#### Mycobiome

- PCR for mycobiome: microbial **ITS rDNA**.
- Primers: **ITS primers** targeted ITS2 regions.

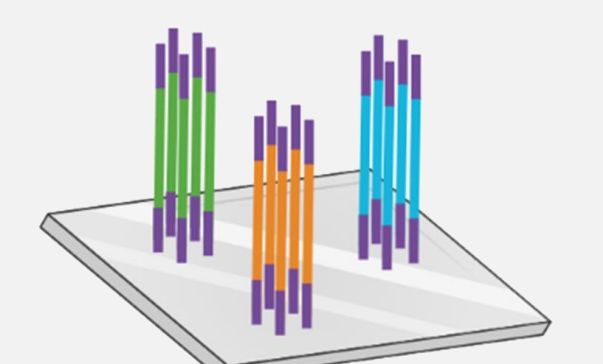
#### Resistome

- Resistomap.
- Multiplex qPCR for resistome: quantification of selected resistance genes (*cmIA*, *ermB*, *tetM*, *blaTEM*, *sull*, *ampC*).<sup>3,4</sup>



### Sequencing

- **NOVAseq instrument** by Illumina which sequence 300 bp long amplicons.



## REFERENCES

1. Walsh, D. M. *et al.* Alterations in airway microbiota in patients with PaO2/FiO2 ratio  $\leq$  300 after burn and inhalation injury. 2017. 2. Corcione, S. *et al.* Microbiome in the setting of burn patients: implications for infections and clinical outcomes. 2020. 3. Mac Aogin, M. *et al.* Metagenomics Reveals a Core Macrolide Resistome Related to Microbiota in Chronic Respiratory Disease. 2020. 4. Taylor, S. L. *et al.* Understanding the impact of antibiotic therapies on the respiratory tract resistome: a novel pooled-template metagenomic sequencing strategy. 2018. 5. Bali, L. *et al.* Comparative Study of Seven Commercial Kits for Human DNA Extraction from Urine Samples Suitable for DNA Biomarker-Based Public Health Studies. 2014. 6. Gosiewski, T. *et al.* Comparison of Methods for Isolation of Bacterial and Fungal DNA from Human Blood. 2014

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