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Background and objectives:

- Exhaled breath analysis** is a promising tool for the early detection of various airway conditions in humans and animals
- Selected Ion Flow Tube Mass Spectrometry (SIFT-MS)** is a highly sensitive analytical technique that quantifies a wide range of volatile organic compounds (VOCs) in matrices such as exhaled breath
- Equine asthma (EA)** and **exercise-induced pulmonary hemorrhage (EIPH)** are among the most prevalent non-septic lower respiratory tract (LRT) inflammatory conditions limiting performance in equine athletes. Their diagnosis is established through endoscopic examination; however, assessing their chronicity, severity, and extent is not straightforward

Short-term aim:

- To assess the feasibility of SIFT-MS for equine breath analysis
- To screen the (untargeted) profile of VOCs in the exhaled breath of horses suffering from non-septic inflammatory airway conditions in comparison to healthy horses

Long-term aim:

- To assess if SIFT-MS could represent a valuable tool, complementary to other ancillary exams, to provide information about the severity, extent and chronicity of airway pathologies such as EA and EIPH by quantification of (more targeted) VOCs

Material and methods:

A total of **7** healthy horses and **31** pathological horses were recruited. Pathological horses were further divided into three groups:

- ✓ **11** horses with EA or other LRT inflammatory conditions
- ✓ **7** horses with EIPH
- ✓ **13** horses with both EIPH and LRT inflammatory conditions

Exhaled air (1 L) was collected in Tedlar® bags via the endoscopic channel or a Bivona® tube prior to tracheal wash (TW) and bronchoalveolar lavage (BAL) procedures (Fig. 1).

Exhaled air was analyzed by SIFT-MS (each sample analyzed in five replicates) using a Syft Tracer i8 with H₃O⁺, NO⁺, and O₂⁺ as precursor ions. Statistical analyses were performed using one-way ANOVA and the non-parametric Kruskal–Wallis test for multiple comparisons.

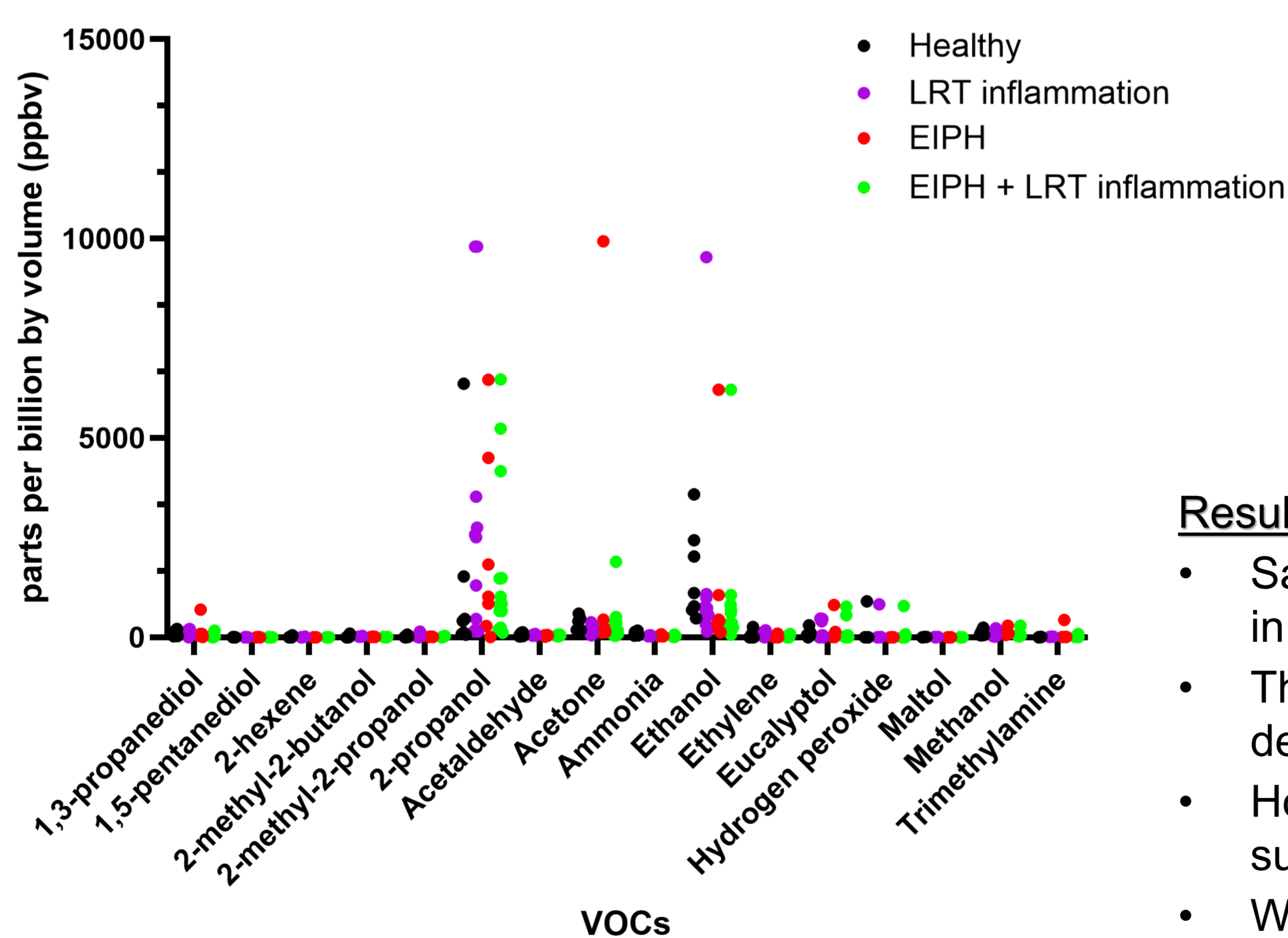


Figure 2. Untargeted screening of VOCs in equine exhaled breath expressed in parts per billion by volume (ppbv) for each group.

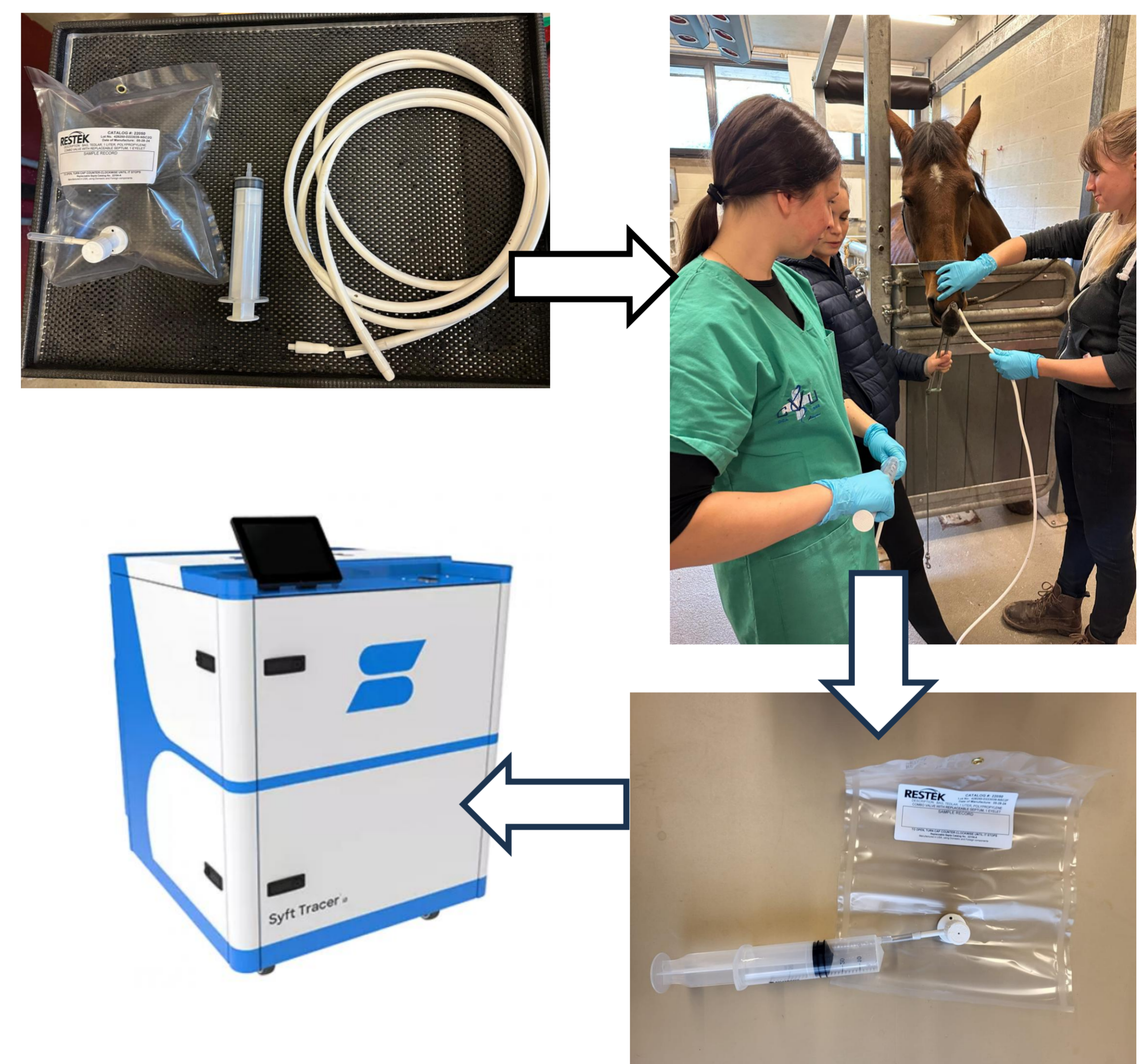


Figure 1. Schematic illustration of the material required for exhaled breath sampling using a Bivona® tube and a Tedlar® bag.

Results:

- Sampling of equine exhaled breath is **easy and fast**. It can be performed in conjunction with endoscopic examination prior to BAL/TW procedures
- The limited number of horses, particularly healthy ones, was **too small** to detect significant differences between groups
- However, some VOCs stood out in the general untargeted screening, such as **2-propanol and ethanol** (both alcohols)
- We are confident that **alcohol-related VOCs** warrant further targeted investigation in inflammatory airway conditions in horses.

Discussion and conclusions:

- Collection and analysis of VOCs is a promising technique that may, in the future, enable the identification of biomarkers for different airway pathologies
- Despite the lack of statistical significance, **alcohols have attracted our attention in this preliminary study** — they have also been reported in the limited equine research published on this topic, as well as in studies on human **COPD** and **cystic fibrosis**
- We aim to continue profiling biomarkers in non-septic inflammatory airway conditions in horses to better quantify the extent and severity of these disorders, particularly of **multifocal diseases** such as EIPH