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Application News

Untargeted Metabolomics Analysis of Acute Respiratory Illnesses in Children using LC/QTOF and LabSolutions Insight Profiler

Emily Armitage[1], Parthena Savvidou[2], Olga Begou[2], Alan Barnes[1], Chloe Hutton[1], Elias Iosifidis[2], Helen Gika[2], Neil Loftus[1], Emmanuel Roilides[2], Charalampos Antachopoulos[2]
1 Shimadzu Corporation, Manchester, UK; 2 Aristotle University, Thessaloniki, Greece

User Benefits

- Application of LabSolutions Insight™ Profiler as an innovative software tool for analysis of metabolomics data acquired from the profiling of blood samples from paediatric patients with SARS-CoV-2 or influenza A compared to controls.
- Metabolomics revealed statistically significant phenotypic differences in cases and controls including 75 features significant by ANOVA that could be putatively identified as metabolites or lipids by MS/MS library matching.
- The application delivers an end-to-end solution for untargeted metabolomics, automating complex data processing.

■ Introduction

LabSolutions Insight Profiler is a dedicated software package to streamline workflows for QTOF data analysis, providing an end-to-end solution for metabolomics. A single processing method is set up to perform feature detection and alignment, data filtering, statistical analysis and compound identification. In this application, LabSolutions Insight Profiler was used to analyse metabolomics data acquired to study acute respiratory illnesses (ARIs).

ARIs are a leading cause of morbidity and mortality in paediatrics; however, diagnosis and prognosis can be challenged by children presenting with similar clinical symptoms, regardless of the underlying infection. Being able to distinguish between infections caused by viruses such as SARS-CoV-2 and influenza could lead to a more accurate diagnosis and clinical outcome. In this study, metabolomics analysis of blood samples from children admitted to hospital with an ARI later diagnosed as either SARS-CoV-2 or influenza infection were compared to controls with the aim of phenotyping disease profiles.

The untargeted metabolomics workflow was applied to acquire high-resolution accurate mass data to profile each sample with data independent acquisition (DIA)-MS/MS. Data were processed in LabSolutions Insight Profiler to find potential biomarkers of ARIs. Fig. 1 highlights an example of a boxplot for a lipid identified in the software as being statistically significant in cases versus controls.

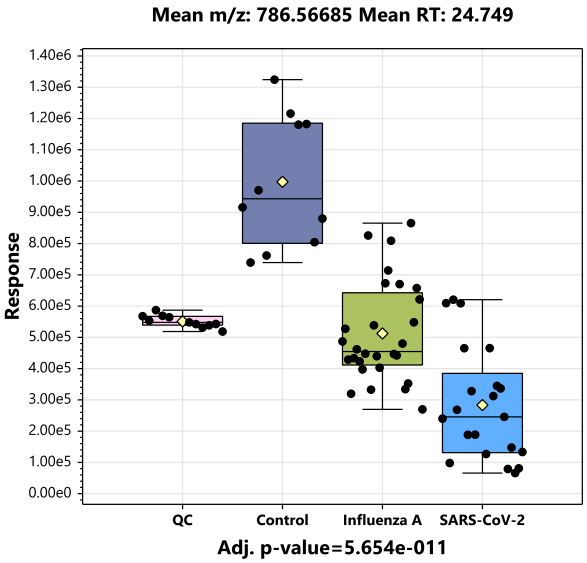

Fig. 1 Boxplot of a statistically significant feature putatively identified as PC O-16:1_18:2 by MS/MS spectral matching to LipidBlast in LabSolutions Insight Profiler. LabSolutions Insight Profiler was applied to untargeted metabolomics analysis of whole blood extracts from paediatric patients with Influenza A infection, SARS-CoV-2 infection or controls to find statistically significant phenotypic differences. The software performs several automated statistical tests including ANOVA, PCA and box plot for a multi-group analysis.

■ Sample preparation

Age and gender matched whole blood samples (100 µL) collected from patients with SARS-CoV-2 (n=21), patients with influenza A (n=28) and controls (n=10) were extracted in ice cold methanol (300 µL). Pooled quality control (QC) samples were prepared by mixing equal aliquots from each extract, following the guidelines of the metabolomics Quality Assurance and Quality Control Consortium (mQACC) recommendations[1].

■ Data acquisition

Samples were analysed in a randomised order in positive and negative ESI mode using a reversed-phase metabolomics method for LC/QTOF (Table 1). QCs were used to equilibrate and stabilise the system. Following this, QCs were injected after six samples to monitor system performance throughout the run.

Table 1. Analytical conditions

[UHPLC conditions] (Nexera X2)	
Column	C18 (2.1x100 mm 1.7 µm);
Oven temp.	50 °C
Flow Rate	0.4 mL/min
Mobile Phase	A: Water + 0.1% formic acid B: Acetonitrile + 0.1% formic acid
Rinse (R0/R1/R2)	IPA/MeOH/MeCN/H_2O (1:1:1:1) + 1% formic acid
Time program (%B)	2% B (0-1 min) → 40% B (2 min) → 90% B (25 min, curve -3) →100% B (27.5-31 min) → 2% B (31.01-35 min)
Injection	0.5 µL (+) / 1.0 µL (-), autosampler 4 °C
[MS conditions] (LCMS-9050)	
Interface	ESI:1.5 kV (+) / -3.0 kV (-)
Mode	Data Independent Acquisition (DIA)
Interface temp.	300 °C
Heat block temp.	400 °C
DL temp.	250 °C
Nebulizing gas flow	3 L/min
Heating gas flow	10 L/min
Drying gas flow	15 L/min
CID gas pressure	230 kPa
MS	*m/z* 60-1000 (+) / *m/z* 60-1050 (-)
MS/MS	*m/z* 40-1000 (+) / *m/z* 40-1050 (-), 27 events precursor isolation 35 Da collision energy spread 5-55 V
Total cycle time (MS and MS/MS)	1 second

■ Data Processing using LabSolutions Insight Profiler

LabSolutions Insight Profiler integrates all the steps for untargeted analysis in a single data processing cascade with flexible options for different workflows (Fig. 2). In this application, data processing considered detection, alignment, statistical analysis and compound identification in a single method.

Feature Detection and Alignment
Detects and aligns all ion signals that behave as a peak.
Statistics
Applied to identify significant ion signals and remove highly variable ions according to predefined filtering criteria.
Screening and Identification
Using large scale screening lists and multiple libraries to identify ion signals of interest.
Batch
A single, configurable workflow integrates detection, alignment, and compound identification, enabling automated batch processing for analysis.

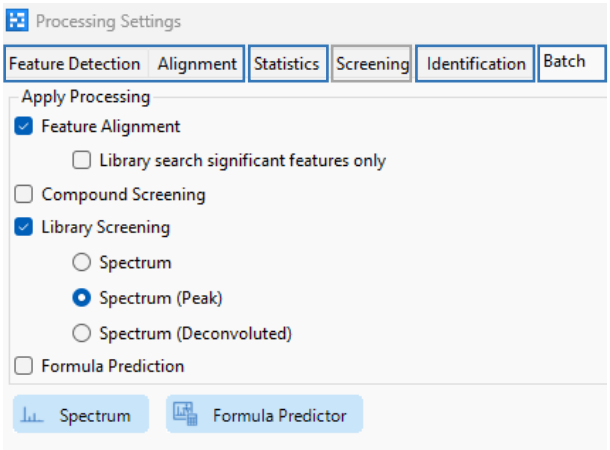

Fig. 2 The LabSolutions Insight Profiler method editor, designed to create a single workflow for the detection of unknowns through to compound identification. The method is applied in batch processing. (The method also supports untargeted screening and profiling workflows for LC/QTOF data, though it was not applied in this application).

To process the data from the metabolomics analysis of whole blood extracts, DIA-MS/MS LC/QTOF data files were loaded, and groups were assigned as 'Control', 'Influenza A', 'SARS-CoV-2' or 'QC'.

The processing method was set to find and align features across the whole dataset and the option for gap filling was selected which performs recursive feature detection for those found in one or more samples but not in others (most likely due to being below the set feature detection threshold).

In this study, the acceptance criteria for statistical analysis included features with a low variance in QCs (< 20% RSD) and present in at least 75% of at least one sample group (not including QCs). Features detected at a near constant value across all samples (<10% RSD) were excluded, on the assumption that these are of low biological relevance.

Remaining missing values after gap filling were set to be replaced by one-fifth minimum value for the feature detected in other samples. The significance level for p-value was set to 0.05 with false discovery rate (FDR) adjustment based on the Benjamini Hochberg algorithm. For a multi-group analysis, the software automatically calculates p-value by one-way analysis of variance (ANOVA) with Tukey HSD post hoc analysis.

Compound identification considered an in-house library developed from authentic standards using the same analytical method (representing metabolomics standards initiative[2] level 1 identification), as well as third-party libraries 'MS-DIAL metabolomics MSP spectral kit'[3] and LipidBlast for level 2 identifications. MSI level 2 identifications are subject to manual review before reporting to avoid mis-identification, as recently described in Theodoridis et al. (2026)[4].

Given the need to provide flexibility in reviewing complex data sets the software application connects tabular results and plots to selected samples and features. It is a highly interactive and intuitive data review platform for the analysis of statistical results (Fig. 3). Selecting any result in the 'Aligned features' table highlights the overlay of chromatograms for samples in which the feature was detected, as well as listing library hits for it. A box plot can be displayed including or excluding the QC group (where applicable) for a selected feature. In a multi-group analysis, results from Tukey HSD post hoc analysis are displayed under the box plot.

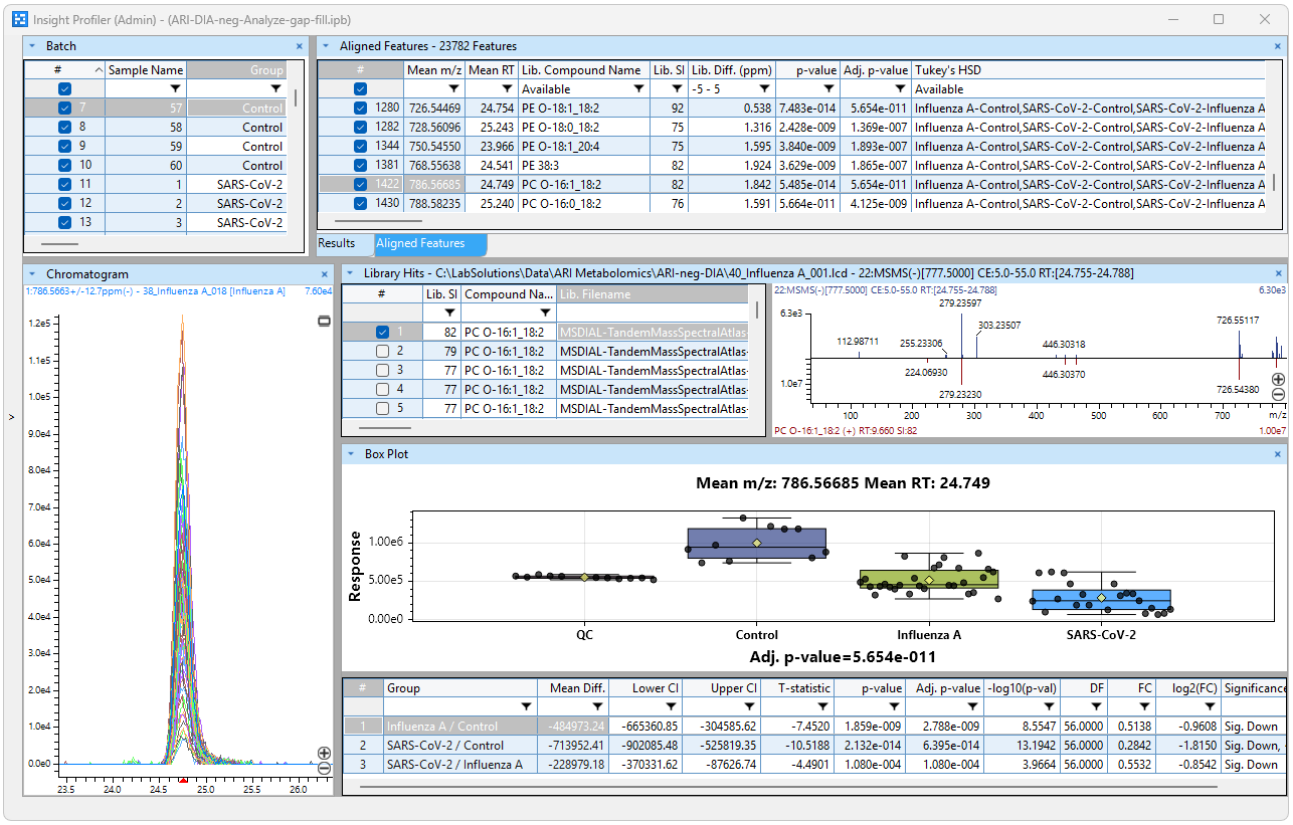

Fig. 3 LabSolutions Insight Profiler analysis of whole blood extracts from children diagnosed with influenza A, SARS-CoV-2 or controls following untargeted metabolomics analysis (LC/QTOF). Interactive and intuitive data review enables analysis of statistically significant features. Chromatogram, spectrum and library identification are linked with statistical analysis plots, helping data interpretation to find potential biomarkers. The example highlights the putative identification of PC O-16:1_18:2 in negative ion mode data which was significant in both negative and positive ion mode datasets.

■ Results

Current diagnostic strategies for ARIs in children can suffer from a lack of sensitivity and specificity required to make an accurate and timely diagnosis enabling an optimal care plan. Metabolomics may be a useful approach to improve our understanding of different ARI phenotypes and potentially reveal specific biomarkers that could enhance treatment pathways. In this study, blood samples from patients aged between 1 month and 16 years who presented at hospital with ARI symptoms and tested positive for SARS-CoV-2 or influenza A were compared to age matched controls who tested negative for both illnesses. Children with immunosuppression or comorbidities were excluded from the study.

Untargeted metabolomics analysis of whole blood extracts revealed statistically significant phenotypic differences in cases and controls. Fig. 4 highlights the six most significant features by ANOVA, putatively identified as PC O-16:1_18:2, PC 18:2_18:2, PC 18:2_20:4, PE O-16:1_18:2, PC O-16:0_18:2, and PC 17:0_18:2. Tukey HSD post hoc analysis revealed that all were significantly diminished in both infections versus the control group, while PC O-16:1_18:2 and PC O-16:0_18:2 were also significantly lower in SARS-CoV-2 relative to influenza A. MSI level 2 identifications were made by spectral matching of DIA-MS/MS data to LipidBlast in LabSolutions Insight Profiler.

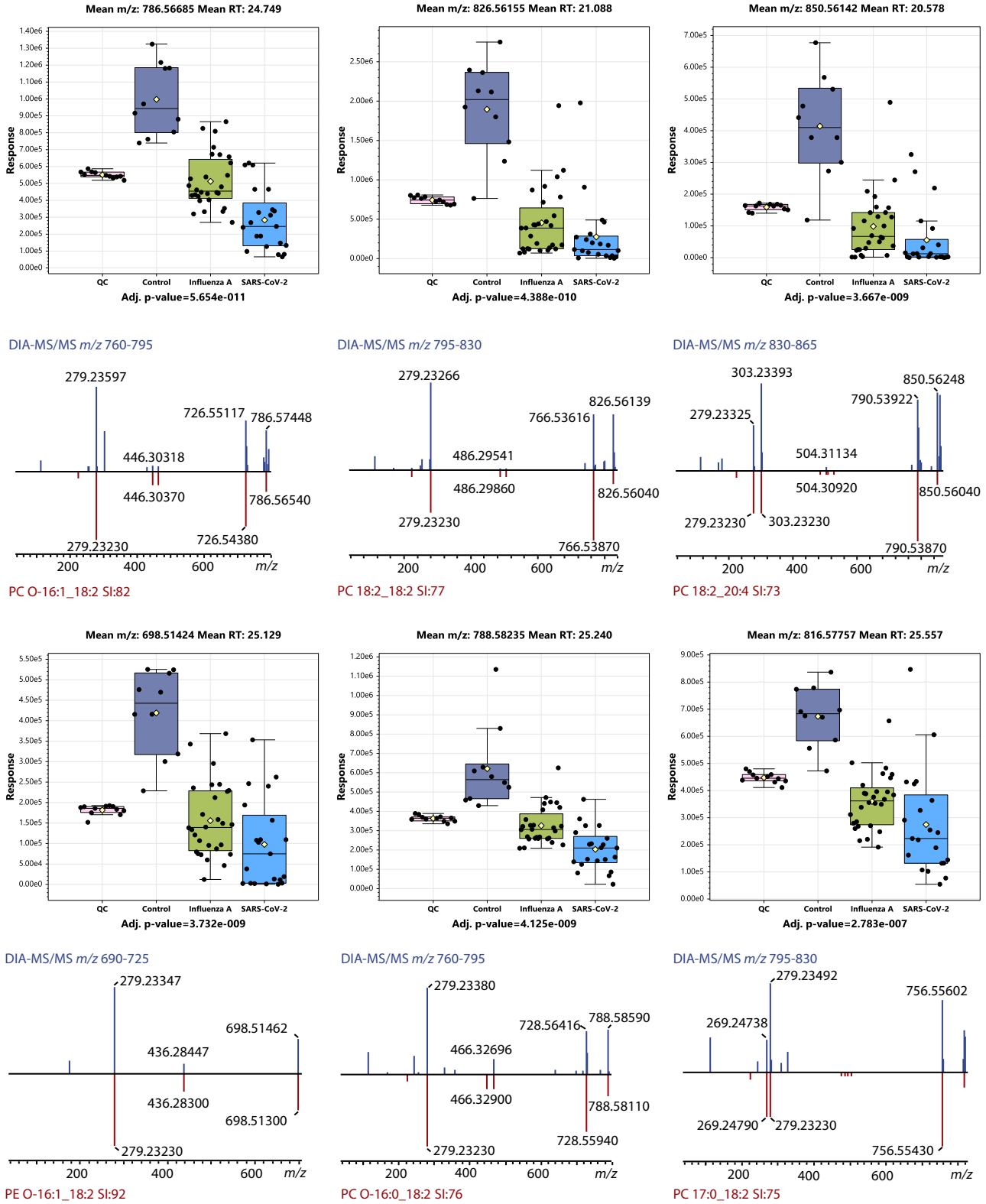

Fig. 4 LabSolutions Insight Profiler revealed 75 annotated metabolites and lipids that were significantly different between groups (ANOVA, FDR adjusted p<0.05). PCs were significant in both positive and negative ion mode datasets. Box plots and MS/MS library identifications for the top 6 most significant annotated features are displayed here (the upper spectra show the acquired DIA-MS/MS spectra and the lower spectra are LipidBlast library MS/MS spectra).

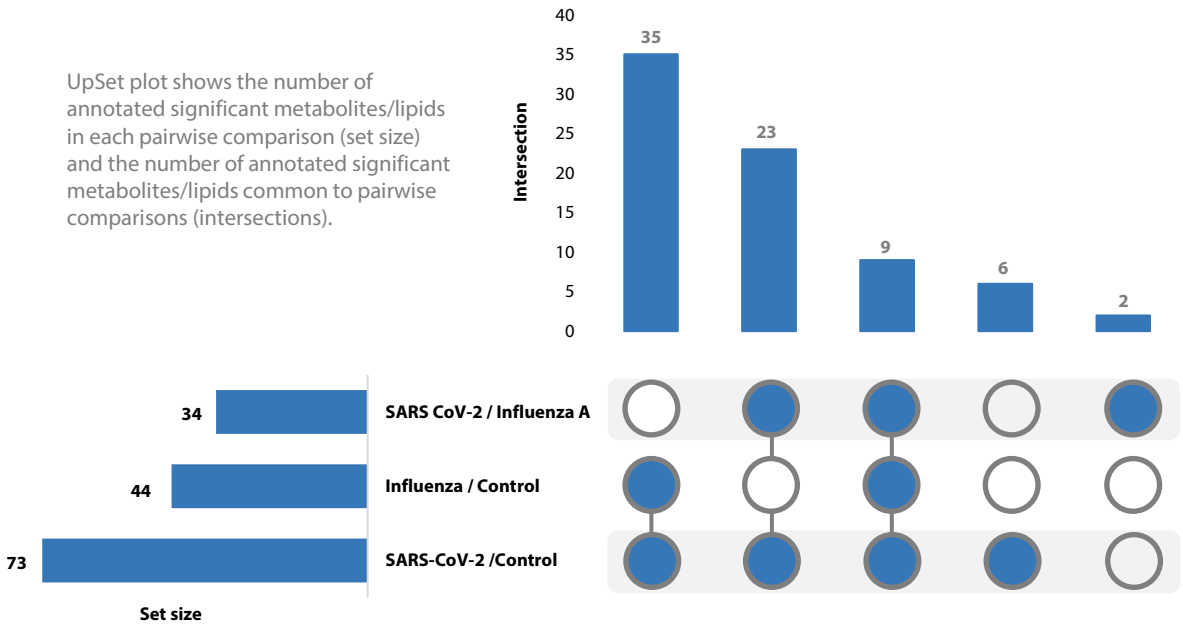

Fig. 5 UpSet plot highlighting interactions in annotated metabolites and lipids found to be statistically significantly different according to Tukey HSD post hoc analysis between each pair of groups: SARS-CoV-2 versus control, Influenza A versus control and SARS-CoV-2 versus Influenza A.

Consistent with the clinical presentation of each illness, metabolic alterations were similar for children infected with either SARS-CoV-2 or influenza A. LabSolutions Insight Profiler revealed 75 annotated metabolites and lipids that were significantly different between groups (ANOVA, FDR adjusted $p<0.05$). As depicted in the UpSet plot in Fig. 5, Tukey HSD post hoc analysis revealed that 73 of these were significantly different between SARS-CoV-2 and controls, 35 of which were also significant between influenza A and controls and 9 of these were also significantly different between the infections.

■ Conclusion

LabSolutions Insight Profiler is a single software application developed to automate feature detection and alignment, statistical analysis and filtering with spectral annotation. To highlight a workflow for untargeted metabolomics, the application has been applied to a biomarker discovery study of acute respiratory illnesses.

The analysis of UHPLC-DIA-MS/MS data acquired from whole blood extracts from paediatric patients with SARS-CoV-2 or influenza A infection and healthy controls enabled the detection of statistically significant metabolites and lipids distinguishing the phenotypes. Consistent with the clinical presentation of each illness, metabolic alterations were similar for children infected with either SARS-CoV-2 or influenza A. LabSolutions Insight Profiler software enabled a streamlined automated approach to revealing metabolic signatures of each infection, highlighting statistically significant features (PCA and ANOVA) putatively identified with integrated MS/MS library searching. Using a single processing method cascade to automate metabolomics data analysis helps to simplify complex data processing workflows without compromising data quality.

■ References

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