

A method for analysing a breath sample for screening, diagnosis or monitoring of SARS-CoV-2 carriage or infection (COVID-19) on humans

The invention relates to the SARS-CoV-2 virus carriage or infection (COVID-19) and to tests for screening, diagnosis or monitoring thereof.

The prior art includes the following documents which are referred to in this
5 specification:

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As of September 28th, 2020, about 32 millions of people worldwide had been infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and about 1 million had died from coronavirus disease 2019 (COVID-19).¹ Approximately 5% of patients with COVID-19 will develop acute respiratory distress syndrome
20 (ARDS), septic shock or multiple organ dysfunction ². Around the world, unprecedented research efforts are being focused on the prevention, early detection, diagnosis and management of this lethal disease. To date, only one antiviral drug (remdesivir) has been approved for the treatment of patients hospitalized for COVID-19.³ More recently, a large trial showed that
25 dexamethasone at a daily dose of 6 mg for 10 days substantially reduced the risk of 28 day death (age-adjusted rate ratio [95% confidence interval (CI)]: 0.83 [0.75 to 0.93], particularly in patients with severe disease requiring invasive mechanical ventilation (rate ratio: 0,64 [0.51 to 0.81]).⁴

Although the early immune response may not depend on the severity of the
30 illness, the most severely ill patients show persistent elevations of blood inflammatory markers (such as IL-1 α , IL-1 β , IL-6, IL-10, IL-18 and TNF- α) 10 days or so after SARS-CoV-2 infection, with a very high risk of subsequent organ injury.⁵⁻

⁷ Proteomic and metabolomic studies of serum have described a COVID-19-specific

molecular signature; severe and non-severe forms of COVID-19 differ with regard to amino acid metabolism and the expression of acute phase proteins.⁸

Early detection that a person carries or is infected by SARS-CoV-2 and diagnosis of coronavirus disease 2019 (COVID-19) are of the utmost importance but
5 remain challenging.

An object of the invention is to contribute to the screening, diagnosis, or monitoring of the presence of this virus and associated disease in human.

To this aim, the invention provides a method for analysis, the method comprising:

- 10 - obtaining a sample comprising elements coming from breath exhaled from a person;
- exposing the sample to a spectrometer;
 - determining at least one value of a signal intensity and/or concentration of ions defined by its mass-to-charge ratio (m/z) given by the spectrometer in at
15 least one range,
- the or each range being defined by a median value and limits of the range;
- applying at least one test to the value,
- the range(s) and the test being configured for identifying that:
- a person carries and/or is infected with SARS-CoV-2,
20 - a person does not carry and/or is not infected with SARS-CoV-2,
 - a person suffers from COVID-19, and/or
 - a person does not suffer from COVID-19; and
- communicating a message according to a result of the test or decision rule.

In one embodiment, the step of determining comprises determining only
25 one value of signal intensity and/or concentration of ions defined by its mass-to-charge ratio (m/z) given by the spectrometer in only one range.

In one embodiment, the range comprises $m/z = 99.08$ (99.04-99.14), the method being performed preferably for identifying that a person carries or is infected by SARS-CoV-2 or that a person does not carry or is not infected by SARS-
30 CoV-2.

In another embodiment, the step of determining comprises determining values of signal intensities and/or concentrations of ions defined by their mass-to-charge ratios (m/z) given by the spectrometer in a group of ranges.

In one embodiment, the range comprises one of the following ranges or the group comprises at least one of the following ranges, preferably all of them, and for example consists in all of them:

- 5
- $m/z = 135.09$ (135.03-135.17),
 - $m/z = 143.15$ (143.07-143.22),
 - $m/z = 99.08$ (99.04-99.14), and
 - $m/z = 111.12$ (111.07-111.18).

In another embodiment, the range comprises one of the following ranges or the group comprises at least one of the following ranges, preferably all of them,

10 and for example consists in all of them:

- 15
- $m/z = 135.09$ (135.03-135.17),
 - $m/z = 143.15$ (143.07-143.22),
 - $m/z = 99.08$ (99.04-99.14),
 - $m/z = 111.12$ (111.07-111.18),
 - $m/z = 71.05$ (71.02-71.09),
 - $m/z = 55.05$ (55.03-55.09),
 - $m/z = 83.09$ (83.05-83.13), and
 - $m/z = 93.04$ (93.00-93.09).

20 In another embodiment, the range comprises one of the following ranges or the group comprises at least one of the ranges of the following table, preferably all of them, and for example consists in all of them:

(m/z [$M+H$] ⁺) median	(m/z [$M+H$] ⁺) lower limit	(m/z [$M+H$] ⁺) upper limit
135.09	135.03	135.17
143.15	143.07	143.22
99.08	99.04	99.14
111.12	111.07	111.18
71.05	71.02	71.08
55.05	55.03	55.09
83.09	83.05	83.13
93.04	93.00	93.09
29.01	29.00	29.03
115.11	115.05	115.18
97.07	97.02	97.10
63.01	62.98	63.04
47.05	47.03	47.08
65.06	65.03	65.10
179.15	179.07	179.25

73.07	73.04	73.11
30.98	30.97	31.00
79.06	79.02	79.10
81.07	81.04	81.12
38.03	38.02	38.06
39.02	39.01	39.04
53.04	53.02	53.07
85.10	85.07	85.15
81.02	80.98	81.06
41.00	40.98	41.03
117.09	117.04	117.16
43.99	43.97	44.02
31.00	30.99	31.02
71.09	71.06	71.13
45.03	45.01	45.06
43.97	43.94	43.99
89.06	89.02	89.11
66.96	66.93	67.00
107.09	107.03	107.15
91.06	91.02	91.11
53.00	52.97	53.03
75.05	75.01	75.09
27.00	26.98	27.01
31.02	31.00	31.03
67.06	67.03	67.09
60.05	60.03	60.09
45.00	44.98	45.02
39.03	39.01	39.05
27.01	26.99	27.03
33.03	33.02	33.05
41.04	41.02	41.06
29.00	28.99	29.02
77.06	77.03	77.10
31.05	31.04	31.07
61.04	61.00	61.07
28.00	27.98	28.01
57.03	57.01	57.06
137.13	137.07	137.21
57.07	57.05	57.10
62.03	62.00	62.06
68.06	68.04	68.10
27.02	27.01	27.04

43.06	43.04	43.08
87.08	87.04	87.13
47.01	46.99	47.04
52.96	52.94	52.99
66,05	66.02	66.08
51.04	51.02	51.07
69.07	69.05	69.11
29.04	29.02	29.05

In one embodiment, the method takes into account at least one of the following elements:

- a symptom score,
- whether the person had a corticosteroid therapy before sampling, and
- whether the person had oxygenotherapy before sampling.

The invention relates to the metabolomics of exhaled breath and provides a SARS-CoV-2 and/or COVID-19-specific breath metabolomic signature.

It concerns for example the persons carrying SARS-CoV-2 or those infected with SARS-CoV-2.

It also concerns for example COVID-19 patients, among others critically ill COVID-19 patients, for example those requiring invasive mechanical ventilation.

In instant application, the persons who carry SARS-CoV-2 comprise infected people (COVID-19 disease) and persons who do not suffer from COVID-19 (asymptomatic people).

This signature is formed for example by a set of 65 volatile organic compounds. Ranking aggregation showed that the eight most prominent volatile organic compounds in this signature are $m/z = 135.09$, $m/z = 143.15$, $m/z = 99.08$, $m/z = 111.12$, $m/z = 71.05$, $m/z = 55.05$, $m/z = 83.09$ and, $m/z = 93.04$, by order of importance and as defined by their mass-to-charge ratio ($[M+H]^+$) with PTR-MS detection. ("PTR-MS" stands for proton transfer reaction mass spectrometry.)

Using the whole signature, we can differentiate between COVID-19 and non-COVID-19 patients with an accuracy of 93% (sensitivity: 90%, specificity: 94%, area under the receiver operating characteristic curve: 0.93-0.98).

With the eight most prominent compounds, we can differentiate between COVID-19 and non-COVID-19 patients with an accuracy of 90-93% (sensitivity: 85-90%, specificity: 93-94%, area under the receiver operating characteristic curve: 0.95-0.98).

With the four most important features ($m/z = 99.08$, $m/z = 111.12$, $m/z = 135.09$ and $m/z = 143.15$, as defined by their mass-to-charge ratio ($[M+H]^+$) with PTR-MS detection), we can differentiate between COVID-19 and non-COVID-19 patients with an accuracy of 89-93% (sensitivity: 90-98%, specificity: 88-94%, area under the receiver operating characteristic curve: 0.89-0.95). These four most important features also discriminate the two groups (COVID-19 positive and COVID-19 negative) in the longitudinal modelling of the first 10 days of hospitalization.

But this signature may be formed in another example by only one volatile organic compound. Indeed, with only one feature such as $m/z = 99.08$ in combination with the following elements:

- a symptom score,
- whether the person had a corticosteroid therapy before sampling, and
- whether the person had oxygenotherapy before sampling,

we can differentiate between persons who carry/are infected with the virus and those who are not (area under the receiver operating characteristic curve: 0.85).

Thus, non-invasive detection of volatile organic compounds in exhaled breath may identify patients with COVID-19 and provide a diagnosis of COVID-19. It also permits to identify the persons who carry or are infected by the virus, with or without clinical symptoms. It also permits to identify the persons who do not carry or are not infected by the virus, and therefore allows to exclude the diagnosis of COVID-19. The knowledge of this specific breathprint enables the development of rapid, non-invasive, point-of-care tests for large-scale COVID-19 screening or monitoring disease severity and control.

Thus, in one embodiment, the method is a method for the diagnosis of COVID-19.

It cannot be excluded that the invention contributes not only to the diagnosis, but also to the prognosis/evaluation of the severity and specific impairment of certain organs as well as to the evaluation of the effect of the treatments administered.

Different kinds of tests or decision rules may be used such as the algorithms known as elastic net, random forest or support vector machine, as explained below.

In one embodiment, the determining step comprises determining whether one or at least two, for example three, four, five, six or seven of the components are present in the sample.

In one embodiment, the spectrometer is a mass spectrometer, for example
5 a proton transfer reaction mass spectrometer.

Using a proton transfer reaction mass spectrometer enables to have an immediate analysis of the sample and an immediate result. But other types of mass spectrometers (other than PTR-MS) would allow the detection of the same compounds.

10 Similarly, the method may be performed according to different modalities, for example not by directly analyzing the breath exhaled by the person or the patient (either by direct connection to a ventilator or by using a device for breath collection for breath analysis such as the Buffered End-Tidal Breath Sampling Inlet (BET-med) device (Ionicon, Innsbruck, Austria) but by adding a storage step of the
15 sample: the exhaled breath is taken from the patient's bed and stored in bags (Tedlar bags type) or on desorption tubes (Tenax type) which are then analyzed in another place (e.g. laboratory)) with identical (PTR-MS) or similar technologies (GC-MS, GC-IMS...).

The method of the invention may also have at least one of the following
20 features:

- the person is undergoing invasive mechanical ventilation;
- the sample is a sample of the exhaled breath;
- the step of obtaining a sample comprises obtaining the sample via a transfer line in direct communication with the person, for example the transfer
25 line being connected to an end of an endotracheal tube installed on the person.
- it comprises heating the transfer line; and
- the person is inspiring air, a mixture of nitrogen and oxygen or pure oxygen;

The invention also provides a device for the diagnosis, screening or
30 monitoring of SARS-CoV-2 and/or COVID-19, the device comprising:

- means for determining at least one value of a signal intensity and/or concentration of ions defined by its mass-to-charge ratio (m/z) given by the spectrometer in at least one range,
the or each range being defined by a median value and limits of the range;

- means for applying at least one test to the value,
the range(s) and the test being configured for identifying that:
 - a person carries and/or is infected with SARS-CoV-2,
 - a person does not carry and/or is not infected with SARS-CoV-2,
 - 5 - a person suffers from COVID-19, and/or
 - a person does not suffer from COVID-19.

The device of the invention may also comprise at least one of the following features:

- means for receiving the breath sample;
- 10 - a transfer line having a connector configured for connecting to an end of an endotracheal tube;
- means for heating the transfer line; and
- a spectrometer, such as a mass spectrometer, for example a proton-transfer-reaction mass spectrometer;

- 15 The invention also provides a program comprising code instructions configured for controlling an execution of the method of the invention or for controlling a device according to the invention, as well as a process for providing this program on a communication network in view of downloading the program.

20 Presentation of the figures

We will now present two non-limiting examples embodying the invention with the help of the following figures on which:

- figures 1 to 6 show graphs illustrating the results of analysis of components in the breath exhaled from patients suffering from Covid-19 in a first example
25 embodying the invention;
- figure 7 shows an embodiment of a device according to the invention;
- figure 8 shows graphs obtained when performing the method of the invention with this device;
- figure 9 shows graphs obtained for the analysis of the four most prominent
30 features ($m/z = 99.08$, $m/z = 111.12$, $m/z = 135.09$ and $m/z = 143.15$, as defined by their mass-to-charge ratio ($[M+H]^+$)) with proton transfer reaction-mass spectrometry;
- figure 10 illustrates one of the tests or decision rules used in the embodiment of the method of the invention;

- figure 11 is a graph representing the level of expression of the m/z 99.08 signal according to the groups of persons in a second example embodying the invention;

5 - figure 12 is a graph giving more details about the “No infection group” of figure 11;

- figure 13 is a graph confronting the m/z 99.08 signal intensity with the Ct (cycle threshold) in the second example;

- figure 14 is a graph confronting the m/z 99.08 signal intensity with the time elapsed between the PCR being performed in the second example;

10 - figures 15 and 16 are a graph and a matrix showing diagnostic performances in this example; and

- figures 17 and 18 are similar graph and matrix about a complementary analysis in this example.

15 Detailed description

First example

Breath analysis is an innovative, non-invasive, technique for detecting volatile organic compounds (VOCs) with potential for use in diagnosis and large-scale screening.^{9,10} Thousands of VOCs have been identified in human breath following infectious, inflammatory or pathological events.^{11,12} The analysis of exhaled breath can be used to diagnose tuberculosis, invasive fungal infections, and bacterial colonization of the respiratory tract¹³⁻¹⁶, together with ARDS and ventilator-associated pneumonia in patients in an intensive care unit (ICU).¹⁷⁻²²

20 Likewise, VOC analysis is of value in the diagnosis of viral infections in patients with chronic obstructive pulmonary disease and of influenza infections in a swine model.^{23,24}

We used real-time, online, proton transfer reaction time-of-flight mass spectrometry to perform a metabolomic analysis of exhaled breath from adults undergoing invasive mechanical ventilation in an intensive care unit due to severe COVID-19 or non-COVID-19 acute respiratory distress syndrome (ARDS).

30

Between March 25th and June 25th, 2020, we included 40 patients with ARDS, of whom 28 had proven COVID-19. In a multivariate analysis, we identified a characteristic breathprint for COVID-19. This signature is formed by a set of 65

volatile organic compounds. Ranking aggregation showed that the eight most prominent volatile organic compounds in this signature are $m/z = 135.09$, $m/z = 143.15$, $m/z = 99.08$, $m/z = 111.12$, $m/z = 71.05$, $m/z = 55.05$, $m/z = 83.09$ and, $m/z = 93.04$, by order of importance and as defined by their mass-to-charge ratio ($[M+H]^+$) with PTR-MS detection. Using the whole signature, we can differentiate between COVID-19 and non-COVID-19 patients with an accuracy of 93% (sensitivity: 90%, specificity: 94%, area under the receiver operating characteristic curve: 0.93-0.98). With the eight most prominent compounds, we can differentiate between COVID-19 and non-COVID-19 patients with an accuracy of 90-93% (sensitivity: 85-90%, specificity: 93-94%, area under the receiver operating characteristic curve: 0.95-0.98). With the four most important features ($m/z = 99.08$, $m/z = 111.12$, $m/z = 135.09$ and $m/z = 143.15$, as defined by their mass-to-charge ratio ($[M+H]^+$) with PTR-MS detection), we can differentiate between COVID-19 and non-COVID-19 patients with an accuracy of 89-93% (sensitivity: 90-98%, specificity: 88-94%, area under the receiver operating characteristic curve: 0.89-0.95).

The real-time, non-invasive detection of volatile organic compounds in exhaled breath permits to identify patients with COVID-19.

Study design, oversight and participants

This study was conducted at the ICU of Raymond Poincaré Hospital (Garches, France). Adult patients (aged 18 or over) in ICUs were eligible for inclusion if they had ARDS and required invasive mechanical ventilation. ARDS was defined as all of the following: (i) acute onset, i.e. within one week of an apparent clinical insult, followed by progression of the respiratory syndrome, (ii) bilateral opacities on chest imaging not explained by another lung disease (e.g. pleural effusion, atelectasis, nodules etc.), (iii) no evidence of heart failure or volume overload, and (iv) $PaO_2/FiO_2 \leq 300$ mm Hg, and positive end expiratory pressure (PEEP) ≥ 5 cm H_2O .²⁶ The main exclusion criteria were pregnancy, an expectation of death within 48 hours, and the withholding or withdrawal of treatment.

Study measurements and procedures

Variables recorded at baseline were patient demographics and anthropometrics, the source of infection and the severity of illness (according to the Simplified Acute Physiology Score (SAPS) II and the Sequential Organ Failure

Assessment (SOFA)).^{27,28} The following variables were recorded at baseline and daily during the hospital stay: core body temperature, vital signs, central hemodynamic data, standard laboratory data, microbiological and virologic data. Samples of blood and nasopharyngeal, bronchial or bronchoalveolar lavage fluids were assayed for SARS-CoV-2 and other respiratory viruses, using a PCR test. Were also recorded life-supportive therapies including mechanical ventilation, renal replacement therapy, intravenous fluids bolus and the administration of vasopressors, and adjunct therapies including corticosteroids, thiamine, vitamin C, other vitamins, nutritional supplements, blood products, anticoagulants, sedatives, stress ulcer prophylaxis and anti-infective drugs.

Breath analysis

Each patient's exhaled breath was analyzed daily in the morning until discharge. Measurements were made with a proton-transfer-reaction quadrupole time-of-flight mass spectrometer (PTR-Qi-TOF MS) (Ionicon Analytik GmbH, Innsbruck, Austria) placed outside the patient room. Samples were obtained via a heated transfer line (length: 1,6 m) connected directly to the end of the endotracheal tube (i.e. without disconnection from the mechanical ventilator) and with an air flow of 50 mL/min. To eliminate the dependency on the oxygen concentration in the sample matrix, recordings were performed in patients with a fraction of inspired oxygen of 100% for at least 3 mins.²⁹ The acquisition took 2 mins. H_3O^+ was used as the primary ion and the instrument settings were as follows: source voltage, 120 V; drift tube pressure, 3.8 mbar; drift tube temperature, 60°C; and drift tube voltage, 959 V. The mass spectrum was acquired up to $m/z = 392$, with a time resolution of 0.1 s.

We recall that, when presenting data of a spectrometer, it is common to use the (officially) dimensionless m/z . This quantity, although it is informally called the mass-to-charge ratio, more accurately speaking represents the ratio of the mass number m and the charge number, z . A spectrometer can measure this mass-to-charge ratio of ionized compounds. For example for a molecule M , the form $[M+\text{H}]^+$ may be detected with PTR-MS when using H_3O^+ as the primary ion.

Data and statistical analysis

Patient characteristics were expressed as the median [interquartile range (IQR)] for continuous variables and the frequency (percentage) for categorical variables. Patients with and without COVID-19 were compared using Fisher's exact test for categorical variables, and a t-test or the Mann-Whitney test for normally and non-normally distributed continuous variables (as evaluated with the d'Agostino-Pearson test), respectively.

Mass spectrometry data were processed with the *ptairMS* software developed by CEA under the CeCILL licence (<https://github.com/camilleroquencourt/ptairMS>) and included mass calibration, expiratory phase detection, peak detection and quantification, normalization, alignment, isotope identification and the imputation of missing values. After aligning each individual peak, ions detected in more than 70% of at least one group (COVID vs. non-COVID-19) were kept; this resulted in 81 features. Missing values (corresponding to ions in exhaled breath that were not detected by the preprocessing algorithm) were imputed with the *ptairMS* package, which returns to the raw data and integrates the noise at the exact missing *m/z*. Data were then log2-transformed and standardized. Outliers (patients with a z-score >3 for at least five features) were deleted. In the remaining patients, saturated ions (acetone, H₃O⁺, H₂O-H₃O⁺, oxygen) and isotopes were deleted to leave a final table of 65 features.

For the univariate analysis, a Wilcoxon test was performed and *p*-values were adjusted to control for the false discovery rate.³⁰ For multivariate analysis, data were first analyzed with unsupervised learning principal component analysis and then with supervised machine learning algorithms (orthogonal partial least-squares discriminant analysis, linear support vector machine, elastic net, and random forest) with the R packages *ropls*, *e1071*, *glmnet*, and *randomForest*.³¹⁻³⁴ A 10-fold cross-validation was repeated four times, in order to avoid overfitting the small number of data points. The model's parameters were tuned to optimize the cross-validation accuracy.

To rank the features in order of importance, we used rank aggregation (with *RankAggreg* R package³⁵) of the ordered *p*-values from the Wilcoxon test, the rank in decreasing order of absolute value for the loadings in the principal component analysis, the variable importance in projection from the orthogonal partial least-squares discriminant analysis, the coefficient values from the elastic net and the

support vector machine, and the feature importance from the random forest. The effects of tidal volume, PEEP, respiratory rate, serum C-reactive protein (CRP) level, body temperature, and renal replacement were also investigated in a correlation test; the secondary principal component in the principal component analysis was used to determine whether or not these covariates were confounding factors (using Pearson's test for continuous variables and a chi-squared test for categorical variables).

Longitudinal analysis of the most important features was performed with a mixed effects model, using a sum of 4 spline functions uniformly distributed over time for the fixed effect. Intergroup differences in trends and mean concentrations were assessed with an F-test (p -value <0.05) adjusted to correct for the false discovery rate. Correlations between VOC concentrations, the SAPS II, the SOFA score and the viral load were analyzed using Pearson's correlation test, with correction for the false discovery rate.

Results

Patients

Between March 25th and June 25th, 2020, 40 patients (of whom 28 had confirmed COVID-19-related ARDS) were included in the study and a total of 303 measurements were made.

Compared with the patients with non-COVID-19 ARDS, the patients with COVID-19 ARDS had (i) a higher incidence of treatment with glucocorticoids prior to admission, (ii) a higher respiratory rate, FiO₂, PEEP and CRP on admission, (iii) a higher incidence of treatment with hydroxychloroquine and fludrocortisone after admission, and (iv) a greater likelihood of renal replacement therapy (Table 1).

Table 1: Patient characteristics and treatments

	COVID-19 ARDS	Non-COVID-19 ARDS	p value
Number of patients (n)	28	12	-
Males/females (n)	20/8	6/6	0.28
Age (years)	61 [55-72]	72 [54-79]	0.75
Body weight (kg)	80.0 [66.6-87.6]	86.5 [65.3-94.1]	0.71
Height (cm)	170 [164-175]	173 [169-175]	0.55

Body mass index (kg/m ²)	26.3 [23.7-32.4]	28.9 [23.0-30.9]	0.79
SAPS II score in the first 24 hours	62 [49-68]	46 [40-57]	0.051
SOFA score in the first 24 hours	11 [7-12]	8 [5-12]	0.37
Comorbidities: (<i>n</i> (%))			
- high blood pressure	11 (39)	6 (50)	0.73
- chronic obstructive pulmonary disease	2 (7)	1 (8)	>0.99
- ischemic cardiac disease	5 (18)	3 (25)	0.68
- cancer	2 (7)	3 (25)	0.15
Treatments before admission: (<i>n</i> (%))			
- glucocorticoids	0 (0)	3 (25)	0.022
- conversion enzyme inhibitors	5 (18)	1 (8)	0.54
- angiotensin antagonists	2 (7)	2 (16)	0.57
Interventions after admission: (<i>n</i> (%))			
- catecholamines	17 (61)	4 (33)	0.17
- renal replacement therapy	9 (32)	0 (0)	0.038
Treatments after admission: (<i>n</i> (%))			
- hydroxychloroquine	27 (96)	1 (8)	<0.0001
- remdesivir	2 (7)	0 (0)	>0.99
- lopinavir/ritonavir	7 (25)	0 (0)	0.081
- glucocorticoids	11 (39)	6 (50)	0.73
- fludrocortisone	1 (4)	4 (33)	0.022
- eculizumab	12 (43)	4 (33)	0.73
Body temperature at first sample (°C)	37.4 [36.5-38.3]	37.3 [36.8-37.8]	0.84
Respiratory rate at first sample (breaths per min)	26 [25-28]	20 [18-23]	<0.0001
Tidal volume at first sample (mL)	420 [400-475]	438 [400-490]	0.99
Fraction of inspired oxygen at first sample (%)	80 [50-100]	48 [31-68]	0.007
Positive end-expiratory pressure at first sample (cm H ₂ O)	10 [8-13]	5.5 [5-8]	0.0002
Serum creatinine at first sample (μM)	74 [56-137]	67 [44-86]	0.30
Serum C-reactive protein at first sample (mg/L)	195 [175-268]	76 [23-119]	0.002

Continuous data are presented as the median [IQR].

Metabolomic analysis of exhaled breath

We first used an untargeted metabolomic strategy to identify biomarkers associated with COVID-19 ARDS. To this end, we used the first breath sample collected after admission. Twelve of the 40 participants had been hospitalized for more than 10 days at the start of the sampling period and so were excluded from this first part of the study. Hence, we analyzed 18 patients with COVID-19 ARDS and 10 with non-COVID-19 ARDS.

A principal component analysis and an orthogonal partial least-squares discriminant analysis showed that COVID-19 was associated with a specific signature in the exhaled breath, i.e. the breathprint could discriminate between COVID-19 and non-COVID-19 patients. Figure 1 shows this unsupervised analysis. A primary component analysis (left) and an orthogonal primary least-squares discriminant analysis (right) of the breath signature in intubated, mechanically ventilated ICU patients with a positive (red) or negative (blue) PCR test for SARS-CoV-2.

The use of three machine learning classification algorithms (elastic net, support vector machine and random forest) yielded an accuracy of up to 93%, with a 10-fold cross validation repeated 4 times, using 19 (elastic net), 16 (random forest) or 65 (support vector machine) features from the 65 features of the whole signature. The corresponding receiver operating characteristic curves are shown in Fig. 2a. These curves are for models classifying patients with COVID-19 vs. non-COVID-19 ARDS. After internal cross-validation, the sensitivity was 90% and the specificity was 94%.

In order to identify possible confounding effects, we first tested the relationship between the COVID-19 status and all the available covariables for patient demographics (sex, age, body weight, height, and body mass index), clinical and biological data (body temperature, SAPS II and SOFA scores, serum CRP and serum creatinine), comorbidities (high blood pressure, chronic obstructive pulmonary disease, ischemic cardiac disease and cancer), ventilation parameters (respiratory rate, positive end-expiratory pressure and tidal volume) and treatments (catecholamines, renal replacement, hydroxychloroquine, remdesivir, lopinavir/ritonavir, glucocorticoids, fludrocortisone and eculizumab). We applied

a Wilcoxon test to quantitative variables and a chi-squared test to qualitative variables, with false discovery rate correction in both cases.

The variables significantly related to COVID-19 status (p -value < 0.05) were the positive end-expiratory pressure and the respiratory rate (Fig. 3 - A). Figure 3 shows the effect of the positive end-expiratory pressure and respiratory rate as follows:

A. The positive end-expiratory pressure (PEEP) and respiratory rate, according to COVID-19 ARDS status. The p -value from the Wilcoxon test is indicated.

10 B. A principal component analysis of the subsets corresponding to patients with PEEP < 10 (left) or a respiratory rate > 20 (right).

The “+” and “-” symbols refer to the patient’s COVID-19 status. The p -value from the Pearson correlation test comparing the factor intensities (displayed as a color gradient) and the scores in the secondary component of the principal component analysis (i.e. the component that best discriminated between COVID-19 ARDs and non-COVID-19 ARDS) is shown.

To determine whether these variables had a confounding effect, we therefore restricted the sample to two rebalanced groups by keeping only patients with a positive end-expiratory pressure < 10 on one hand or a respiratory rate > 20 on the other. We then applied Pearson’s correlation test to the secondary component from the principal component analysis of this restricted dataset. We observed that in both cases the secondary component still distinguished between cases of COVID-19 ARDS and non-COVID-19 ARDS disease and that there was no correlation with the positive end-expiratory pressure or the respiratory rate (Fig. 3 - B).

We used the same procedure for the other covariables with the complete dataset. None of them was significantly correlated with the secondary component of the principal component analysis (Fig. 4). Figure 4 shows the effect of tidal volume, CRP and body temperature in a principal component analysis and a correlation analysis. COVID-19 status is represented by the “+” and “-”, whereas the color scale represents the factor’s intensity. The p -value of the Pearson correlation test comparing the secondary component and the corresponding factor is shown on each graph.

To determine which VOCs were most discriminant for COVID-19 status, we performed rank aggregation using the various metrics from the previously mentioned models and the hypothesis tests.

The eight most prominent volatile organic compounds in this signature were
5 $m/z = 135.09$, $m/z = 143.15$, $m/z = 99.08$, $m/z = 111.12$, $m/z = 71.05$, $m/z = 55.05$,
 $m/z = 83.09$ and, $m/z = 93.04$, by order of importance and as defined by their
mass-to-charge ratio ($[M+H]^+$) with PTR-MS detection. With these eight most
prominent compounds, we can differentiate between COVID-19 and non-COVID-19
patients with an accuracy of 90-93% (sensitivity: 85-90%, specificity: 93-94%, area
10 under the receiver operating characteristic curve: 0.95-0.98).

With the four most important features ($m/z = 99.08$, $m/z = 111.12$, $m/z =$
135.09 and $m/z = 143.15$, as defined by their mass-to-charge ratio ($[M+H]^+$) with
PTR-MS detection), we can differentiate between COVID-19 and non-COVID-19
patients with an accuracy of 89-93% (sensitivity: 90-98%, specificity: 88-94%, area
15 under the receiver operating characteristic curve: 0.89-0.95).

The corresponding receiver operating characteristic curves are shown in Fig.
2b for the eight most prominent compounds ($m/z = 135.09$, $m/z = 143.15$, $m/z =$
99.08, $m/z = 111.12$, $m/z = 71.05$, $m/z = 55.05$, $m/z = 83.09$ and, $m/z = 93.04$)
and Fig. 2c for the four most prominent compounds ($m/z = 135.09$, $m/z = 143.15$,
20 $m/z = 99.08$ and $m/z = 111.12$).

Figure 9 shows graphs obtained for the analysis of the four most prominent
features ($m/z = 99.08$, $m/z = 111.12$, $m/z = 135.09$ and $m/z = 143.15$, as defined
by their mass-to-charge ratio ($[M+H]^+$)) with proton transfer reaction - mass
spectrometry. Each curve represents a patient of the study. And the curves of the
25 patients having a COVID+ status and a COVID- status are visually differentiated.
The range associated with each median value is illustrated.

We investigated the expression of the four most prominent VOCs in the
whole study population throughout the period of mechanical ventilation (Fig. 5b).
Figure 5 shows the longitudinal analysis of VOCs in exhaled breath. The four
30 features (m/z 99.08, 135.09, 143.15 and 111.12) contributing the most to the
models were assessed in the first sample available for each patient (a) and over
time (b) during the ICU stay of intubated, mechanically ventilated patients with
COVID-19 (in red) or non- COVID-19 ARDS (in blue). All the points for a given patient

are connected, and the bold lines correspond to the fixed effect of the mixed model for each group.

We observed that the VOC concentrations (i) were significantly higher in the breath of patients with COVID-19 ARDS than in the breath of patients with non-
 5 COVID-19 ARDS, and (ii) tended to decrease over the first 10 days of hospitalization. The putative annotations for the four compounds at m/z 135.09, 143.15, 99.08 and 111.12 were respectively 1-chloroheptane, nonanal, methylpent-2-enal, and 2,4-octadiene.

10 Correlation with viral load and severity scores

The viral load in bronchoalveolar fluid was measured for 18 patients. The median [IQR] value in the first sample was 7.2 [6.2-8.4] log eq. copies/mL. The VOC concentrations in the first sample were not significantly correlated with the bronchoalveolar fluid viral load or with the severity of illness (i.e. the SAPS II and
 15 SOFA score^{27,28}) measured during the first 24 hours in the ICU (Table 2).

Table 2: Absence of significant correlations between VOC concentrations and the SAPS II, SOFA score and viral load.

VOC (m/z)	SAPS II score		SOFA score		Viral load	
	r	p -value	r	p -value	r	p -value
143.15	0.12	0.62	0.27	0.25	-0.23	0.24
135.09	0.05	0.85	0.35	0.14	-0.0004	1.00
99.08	0.04	0.88	0.36	0.13	0.08	0.70
111.12	0.02	0.93	0.28	0.25	-0.14	0.48

r : Pearson's correlation coefficient.

20

This study provided proof of concept for the measurement of VOCs and the determination of a specific VOC breathprint in the exhaled breath from patients with COVID-19-related ARDS requiring invasive mechanical ventilation in the ICU. This breathprint was independent of the severity of illness and the viral load. Four

features (m/z 99.08, 111.12, 135.09 and 143.15) discriminate between COVID-19 and non-COVID-19 ARDS in the longitudinal analysis.

Two of these four prominent VOCs (m/z = 143.15 and m/z = 99.08, which may correspond to methylpent-2-enal and nonanal) are aldehydes, while 2,4-octadiene (m/z = 111.12) is an alkadiene; all three compounds are known to be expressed in breath.^{38,39} Nonanal (m/z = 99.08) is a sub-product of the destruction of the cell membrane as a result of oxidative stress; reactive oxygen species may be generated by various type of inflammatory, immune and structural cell in the airways.⁴⁰ In studies of exhaled breath from patients with ARDS, Schubert *et al.* found abnormally low isoprene concentrations and Bos *et al.* reported abnormally high concentrations of octane, acetaldehyde and 3-methylheptane.^{21,22} Differences in study populations (non-COVID-19 vs. COVID-19 ARDS) and analytical methods (offline vs. online) might explain the differences between the VOCs identified in the present study and those identified in previous studies of ARDS.^{21,22}

In line with previous reports, the VOC concentrations measured here were not correlated with the severity of illness (as judged by the SAPS II and the SOFA score).²¹ This finding suggests that the exhaled breath signature is a marker of COVID-19 *per se*, rather than of the severity of illness. Likewise, the VOC concentrations were not correlated with viral load, suggesting that this signature may be a marker of the disease related to SARS-CoV-2 rather than of virus carriage.

Our interpretation of the present data may have been limited by differences between the COVID-19 and non- COVID-19 ARDS subgroups. Patients with COVID-19 ARDS cohort had higher respiratory rate, FiO_2 , PEEP and CRP values on admission. The respiratory rate, PEEP and CRP were not found to be confounding factors, and all the patients were sampled when breathing 100% FiO_2 (to avoid mass spectrometry interference by oxygen).²⁹

The FiO_2 represents the percentage of oxygen in the air with which patients are ventilated, the values range from 21% (ambient air) to 100% (pure oxygen, for the most severe patients). This percentage of oxygen causes interference for analysis with a PTR-MS. For this reason, for all patients, we first performed a first analysis with their basal FiO_2 level (between 21 and 100%), then all patients were ventilated with 100% FiO_2 for 5 mins to have a standardized value and a second analysis was performed. Only the results of this second analysis are detailed here.

The graphs of figure 6 represent, for each of the four compounds of interest, the values measured in the two analyses. The first four graphs show the values obtained for all patients as a function of the initial % FiO₂ ("raw" values (cps, upper line), or normalized (ncps, lower line, normalization process not detailed here).

- 5 The four other graphs of the figure represent the variations observed for each patient between the 1st measurement (value on the left, "base") and the 2nd measurement in 100% FiO₂ (value on the right). The statistical analyses performed do not show any significant difference, which suggests that the percentage of oxygen does not disturb the analysis of the 4 compounds of interest.
- 10 Similarly, patients with COVID-19 ARDS were more likely to have been treated with hydroxychloroquine and fludrocortisone. However, these drugs were administered to the patients after their first sample had been analyzed. Although the VOC concentrations decreased over time, the treatments did not change. Lastly, there is no correspondence between VOCs described in the present study
- 15 and the molecular masses of the known metabolites of hydroxychloroquine or fludrocortisone.

The *ptairMS* R package used for data analysis is fully available at <https://github.com/camilleroquencourt/ptairMS>.

- 20 Due to these findings, we can apply a highly sensitive, rapid, non-invasive, real-time mass spectrometry breath analysis.^{36,37} This contrasts with offline technologies, which require a sampling step and remote, time-consuming analytical steps.^{21,22} The subsequent diagnostic validation and clinical implementation can be based on less cumbersome technologies, such as mass spectrometers dedicated to targeted analyses or portable "electronic noses" with
- 25 a set of sensors that are relatively selective for different families of VOCs (as previously used in patients with ARDS).²⁰

Embodiment

- We will now present a first embodiment of the device for screening,
- 30 diagnosis or monitoring of COVID-19, with reference to figures 7 and 8.

The device 102 comprises a body 104.

The device comprises means for receiving a breath sample. These means comprise an elongated transfer line 106. One end of the transfer line has a connector 108 configured for connecting to an end of an endotracheal tube 110.

This tube is configured for installation on the face of a person 112 for invasive mechanical ventilation. Another end 114 of the transfer line 106 extends inside body 104.

5 The device also comprises means 116 for heating the transfer line, which helps to preserve the components or compounds from the moment they exit the patient to the moment the breath sample is analyzed inside device 102.

The device comprises inside body 104 means configured for determining whether $m/z = 135.09$, $m/z = 143.15$, $m/z = 99.08$ and $m/z = 111.12$ are present in a breath sample. These means comprise in this example a proton-transfer-
10 reaction mass spectrometer 118 configured for analyzing a breath sample transferred through line 106.

The device has computer and/or electronic control means 120. These means comprise a program comprising code instructions configured for controlling device 102 for performing the method presented hereafter. This program can be provided
15 on a communication network in view of downloading the program into the device when it is connected to this network with classical means in this view.

The method of the invention is performed as follows with this device 102.

We assume that the person 112 is undergoing invasive mechanical ventilation. An endotracheal tube 110 is installed on the person. The connector
20 108 of transfer line 106 is connected to the endotracheal tube 110. The person is breathing for example air of 100% FiO_2 , which means that this person inspires pure oxygen.

The method comprises the step of heating the transfer line with the heating means 116.

25 It then comprises the step of obtaining a sample of exhaled breath from the person 112 via the heated transfer line. Thus, the sample of exhaled breath enters body 104 for being exposed to the spectrometer and analyzed.

The method then comprises the step of determining whether $m/z = 135.09$, $m/z = 143.15$, $m/z = 99.08$ and $m/z = 111.12$ are present in the sample.

30 To this end, the spectrometer 118 and the control means 120 perform the steps of determining values of signal intensities and/or concentrations of ions defined by their mass-to-charge ratios (m/z) given by the spectrometer in a group of ranges, each range being defined by a median value and limits of the range. In this example, the group consists of the following ranges:

- $m/z = 135.09$ (135.03-135.17),
- $m/z = 143.15$ (143.07-143.22),
- $m/z = 99.08$ (99.04-99.14),
- $m/z = 111.12$ (111.07-111.18).

5 Figure 8 shows on the second graph the amount of certain components into the breath received inside the device as a function of time. The sample is received inside the device roughly between 1 min 10 s and 1 min 20 s. At that moment, the amount of some components rises and then decreases.

The first graph shows the intensity or concentration of different
10 components as a function of their mass (m/z). Each peak thus designates a different component. The higher the peak, the higher the intensity or concentration of the component in the exhaled breath. The device can directly and in real time evaluate whether one, two, three or all of the eight components are present in the exhaled breath.

15 For this purpose, the device determines the value m/z of each of the components ($[M+H]^+$) in the sample.

Then we use these signal intensities or concentrations to predict the probability of COVID infection thanks to a machine learning model trained with the data. For that, the method comprises the step of applying at least one test or
20 decision rule to the values, the group and the test or decision rule being configured for identifying a patient with COVID-19.

In this embodiment, the tests or decision rules of elastic net, random forest and support vector machine (SVM) can be used for example. This testing step is performed as follows for example.

25 We log-transform the measured concentration in ppb of k VOCs of interest. We note $\mathbf{x} = (x_1, \dots, x_k)$ those values.

Elastic net³²

We generally define the probability that this individual is in the COVID-19
30 positive group as follows:

$$p = \text{logit}^{-1}(\alpha_0 + \sum_{i=1}^k \alpha_i \times x_i)$$

where $(\alpha_0, \dots, \alpha_k)$ are known coefficient estimated from our data, and

$$\text{logit}^{-1}(x) = \frac{\exp(x)}{1 + \exp(x)}$$

If p is greater than a certain threshold (e.g. 0.5), the individual is considered to be in the COVID-19 positive group.

In our example with the four VOC of interest, we note
 5 $x_{99}, x_{111}, x_{135}, x_{143}$ the logarithm of ions 99.08, 111.12, 135.09 and 143.15 concentrations.

After training and tuning the model, we obtain the decision rules:

$$p = \text{logit}^{-1}(4.32692 + 0.0717 \times x_{99} - 0.88062 \times x_{111} + 1.51188 \times x_{135} + 1.82797 \times x_{143})$$

10 If p is greater than 0.5, the individual is considered to be in the COVID-19 positive group.

Random forest

The Random Forest approach is based on the aggregation of the predictions
 15 from many decision trees³³. Here, five hundred decision trees as the one illustrated on figure 10 have been built from our data. Each tree is built from a random subset of our variables. For the illustrated tree, if the logarithm of the ions 135.09 is less than -1.416, the patient is considered to belong to the positive group. Else, we look at the log-concentration of the ion 99.09. If this is less than -0.7923, the
 20 patient is in the negative group. We note the result for the five hundred trees, which are negative (0) or positive (1). If there is more positive outputs than negative outputs, the individual is considered to be in the COVID-19 positive group.

SVM³⁴

25 An equation of a hyperplane $h(\mathbf{x})=0$ separating the two classes has been estimated from our data, for example in the linear case:

$$h(\mathbf{x}) = \sum_i^k \alpha_k x_k + \alpha_0$$

or in general case:

$$h(\mathbf{x}) = \sum_j^p \alpha_j K(\mathbf{x}^j, \mathbf{x}) + \alpha_0$$

where $(x^1, \dots, x^p)$ are the features value for patient used to build the hyperplane (called support vector), K a kernel and (α_i) the known estimated coefficients.

If $h(x) > 0$ (respectively $h(x) \leq 0$), the patient is in the COVID-19 positive (respectively negative) group.

In our examples with the four VOC of interest, we note $x_{99}, x_{111}, x_{135}, x_{143}$ the logarithm of ions $m/z = 99.08, 111.12, 135.09, 143.15$ concentration, whose mean and variance have been scaled according to the values from the training data.

After training and tuning the model, we obtain the decision rule:

$$h(x_{99}, x_{111}, x_{135}, x_{143}) = 0.8776471 + 1.1364761 x_{135} + 0.8867700 x_{143} + 0.3212751 x_{99} - 0.1639539 x_{111}$$

If $h(x_{99}, x_{111}, x_{135}, x_{143}) > 0$, the patient is in the COVID-19 positive group.

One or at least two of these tests or decision rules are performed.

Then a message is communicated according to the result of the test(s) or decision rule(s). This comprises for example displaying the result of the test or decision rule on a screen of the device, sending an e-mail to a physicist, etc.

The above-examples for the elastic net and SVM models have been given for the four VOC of interest. Here are the values of coefficients for using the elastic net test with the 65 VOC of interest.

Table 3: Coefficients of the elastic net test for 65 VOC of interest

m/z	coef
26.9994	0
27.0105	0
27.0218	0
27.9951	0
29.0026	0
29.014	-0.17099834952128
29.0375	0
30.9838	-0.19272254930386
31.0035	0
31.018	0
31.0545	-0.0868809541462363
33.0338	0
38.0341	-0.373544816475641
39.0231	0
39.033	0

41.0035	0
41.0392	0
43.0553	0
43.9676	0
43.9907	0
44.9981	0
45.0342	0
47.0135	0
47.05	0
51.0446	0
52.9618	0
53.002	0
53.0393	0
55.0549	-0.204060119119194
57.0346	0
57.0706	0
60.0531	0
61.0356	0
62.0299	-0.172910672454371
63.0079	-0.133793047603517
65.0606	0
66.0475	0
66.9591	0
67.055	-0.0185542639450894
68.0625	0
69.0706	0
71.0501	-0.29614511174346
71.0861	0
73.0656	0
75.045	0
77.0604	0
79.0553	0.1565639655472
81.019	0
81.0708	0
83.0866	-0.264806002067097
85.1024	0
87.0815	0
89.061	0
91.0563	0
93.0378	0.0134534777254841
97.0667	0.142381904312499
99.0818	0.116216917765426

107.0865	0.0435199756862549
111.1192	0.139762358652873
115.1135	0
117.0928	0
135.0885	0.34647275629029
137.1346	0.0746880721623011
143.1451	0.548099225194985
179.1459	0

Here are the values of coefficients for using the SVM test with the 65 VOC of interest.

Table 4: Coefficients of the SVM test for 65 VOC of interest

features	coef	
intercept	1.78457393935063	
26.9994	0.0203164775583294	
27.0105	-0.00646293739846489	
27.0218	0.0131373528739178	
27.9951	0.00396558638862555	
29.0026	0.0105578163957263	
29.014	-0.0490358531227796	
29.0375	0.0122541721514019	
30.9838	-0.0794211818615996	
31.0035	-0.0371246281462103	
31.018	-0.00301229548201719	
31.0545	-0.099084453254533	
33.0338	-0.00907141264166196	
38.0341	-0.029855704498521	
39.0231	-0.0397990392900494	
39.033	0.00637568953724734	
41.0035	-0.0230482514619407	
41.0392	0.011438473256849	
43.0553	0.0526010770568651	
43.9676	-0.00876987827383436	
43.9907	0.0229029819998173	
44.9981	0.00858726062702962	
45.0342	-0.00751150864277307	
47.0135	0.0140843908608015	
47.05	-0.0152800023536971	
51.0446	-0.0276965017027041	
52.9618	-0.0294181148271273	
53.002	-0.0114321026066049	

53.0393	-0.0329220742342297
55.0549	-0.0796086805043102
57.0346	-0.0156023981925246
57.0706	0.0165741125645432
60.0531	0.0169707074015356
61.0356	0.0476857892192711
62.0299	0.00128519033348025
63.0079	-0.0347890132637384
65.0606	-0.00565899268637548
66.0475	-0.0159928201806251
66.9591	-0.00725756349009642
67.055	-0.0442809159240086
68.0625	-0.0224115129980686
69.0706	-0.0183969983383
71.0501	-0.074340950426599
71.0861	0.0085464014404322
73.0656	-0.0358032773974026
75.045	0.00282342778864358
77.0604	-0.0216485052925101
79.0553	0.0323336124905708
81.019	-0.0225294351687507
81.0708	0.0121522697786908
83.0866	-0.0769266668049326
85.1024	0.012202175586782
87.0815	-0.00812314515499904
89.061	0.0196373308932
91.0563	0.00999750102871999
93.0378	0.040514992832867
97.0667	0.0308882660672048
99.0818	0.080845663301971
107.0865	0.0264354320284494
111.1192	0.0274064508385647
115.1135	0.0518974143614893
117.0928	0.0379433650316464
135.0885	0.0901668878172821
137.1346	0.028631519713326
143.1451	0.0981198699268599
179.1459	-0.0135128151592857

Thus, this method uses real-time, online, proton transfer reaction time-of-flight mass spectrometry to perform a metabolomic analysis of exhaled breath from adults undergoing invasive mechanical ventilation in an intensive care unit

due to severe COVID-19 or non-COVID-19 acute respiratory distress syndrome (ARDS). It is a real-time, non-invasive detection in exhaled breath for identifying patients with COVID-19.

5 Second example

We now present PTR-MS breath analysis results for SARS-CoV-2 in and spontaneously breathing persons (non-intubated) who may carry or be infected with SARS-CoV-2, made with the invention.

These results were obtained thanks to the VOC-COVID-DIAG (NCT04614883) and VOC-SARSCOV-DEP (NCT04817371) clinical studies promoted by the Foch Hospital and for which the scientific coordinators and principal investigators are Pr Philippe Devillier and Dr Hélène Salvator.

The aim of these studies was to validate the value of volatile organic compounds (VOCs) analysed by mass spectrometry, electronic noses and/or olfaction for the diagnosis of SARS-CoV-2 infection in patients admitted to hospital with suspected SARS-CoV-2 (COVID-19) or other respiratory-tropic viruses, or hospitalised with systematic screening for SARS-CoV-2 infection, as well as in vaccinated volunteers. For each patient, a breath sample was taken for analysis and a sweat sample for VOC analysis by canine olfaction or other non-detailed mass spectrometry techniques. A symptom score was also established by the investigator, on a scale of 0 to 4, according to the presence of certain symptoms (fever, fatigue, cough, diarrhoea, anosmia, agueusia, headache, dyspnoea, polypnoea, hypoxia...).

The results concerning the invention relate to PTR-MS analysis and are an analysis of the data available for a total of 136 patients that can be used with this technology.

While the results of the first example involved intubated and mechanically ventilated patients in an intensive care unit, the patients in these studies were spontaneously breathing and therefore the experimental design had some differences. Although we will designate them collectively for simplicity as “patients” in the following description of this example, some of them were not actually suffering from COVID-19 and were merely persons possibly carrying or infected by SARS-CoV-2, with or without clinical symptoms.

Here, the patients' exhaled breath was collected on the ward in a special exhaled breath collection bag (Tedlar bag®) by exhaling the patient into the bag using the appropriate device. The bag, once closed, was then transported to the analysis room. A flexible tube was then connected to the Tedlar bag and allowed
5 the contents of the bag to be aspirated directly into the mass spectrometer for real-time analysis at a flow rate of 50 ml/min. Other analytical and data processing procedures were similar to those described above.

The patients were divided into three groups:

- no infection: SARS-CoV-2 PCR negative: 69 patients (50 vaccinated and 19
10 unvaccinated);
- old infection: SARS-CoV-2 PCR negative but history of COVID-19 infection in the previous weeks or months: 12 patients; and
- recent infection: SARS-CoV-2 PCR positive: 55 patients.

The data were analysed with the ptairMS software developed by Camille
15 Roquencourt and Etienne Thévenot (CEA).

1 - Univariate analysis

Univariate analysis showed a significant difference between groups for the m/z 99.08 signal (p-value <0.001 Wilcoxon test), which was one of the 4 significant
20 signals in the study in intensive care patients. The graph representing the level of expression of the m/z 99.08 signal according to the groups of patients is presented on figure 11.

The following points should be noted.

In the "Recent infection" group, illustrated on the right, the 12 patients with
25 the lowest signal intensity had specificities, in particular 5 of them had received prior treatment with dexamethasone (anti-inflammatory glucocorticoid drug) between 1 and 9 days before sampling; 2 were organ transplant patients (kidney or lung) and under immunosuppressive treatment, 1 patient was asthmatic with inhaled glucocorticoid treatment.

30 The "Old infection" group is illustrated at the center.

In the "No infection" group, illustrated on the left, the two patients with the highest intensities were patients with other infections, namely clostridial colitis and septicaemia (Escherichia coli positive blood cultures).

In the "No infection" group, 50 patients were vaccinated and 19 non-vaccinated. No significant difference in m/z 99.08 signal intensity was observed between vaccinated and non-vaccinated patients (see figure 12), suggesting that vaccination status does not interfere with the proposed screening method.

5 We then assessed whether the m/z 99.08 signal intensity correlated with the Ct (cycle threshold) value measured by the PCR assays on nasopharyngeal swabs when this value was available in the 'Recent Infection' group. This is obtained for SARS-CoV-2 detection with reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) on nasopharyngeal swabs. Ct is a
10 reflection of the viral load present in each sample: the higher the number of copies of the SARS-CoV-2 gene mRNA detected by the test, the lower the Ct. The results are presented on figure 13 and show the lack of correlation between the two parameters, suggesting that breath testing is likely to detect patients with high and low viral loads in the same way.

15 In the same group, we also looked to see if the m/z 99.08 signal intensity correlated with the time elapsed between the PCR being performed. The results are presented on figure 14 and show that there was no correlation and suggest that breath testing can detect very recent infections in the same way as less recent infections (maximum delay of 15 days).

20 This example shows that we may perform a method for identifying that a person carries or is infected by SARS-CoV-2 or that a person does not carry or is not infected by SARS-CoV-2, the method comprising:

- obtaining a sample comprising elements coming from breath exhaled from a person;
- 25 - exposing the sample to a spectrometer;
- determining only one value of signal intensity and/or concentration of ions defined by its mass-to-charge ratio (m/z) given by the spectrometer in only one range comprising $m/z = 99.08$ (99.04-99.14),
- applying at least one test to the value, and
- 30 - communicating a message according to a result of the test.

The test comprises for example the step of determining whether the intensity or concentration is above a predetermined threshold, for example 1.67 ppb (hence the person carries or is infected with SARS-CoV-2) or not (hence the person does not carry or is not infected with SARS-CoV-2). With a logistic regression

based on the concentration of the m/z 99.08, a threshold estimated at 1.67 ppb allows the detection of the carriage or infection with SARS-CoV-2 with an accuracy of 74% (40% precision, 97% recall and AUC 0.853).

5

2 - Multivariate analysis

A - Untargeted analysis

After the steps of alignment, imputation, isotope suppression, saturated ions (m/z 37 and m/z 59) and oxygen ions, and outlier suppression, we obtain a
10 matrix of 82 VOCs and 134 patients. For this analysis, only two groups were considered: patients with negative PCR test for SARS-CoV-2 (corresponding to the two “No infection” and “Old infection” groups of the univariate analysis) and patients with positive PCR test for SARS-CoV-2, resulting in 55 positive patients and 79 negative patients.

15 Five data were added to build the model:

- previous corticosteroid therapy before sampling (0 no, 1 yes),
- vaccinal status for COVID-19 (0 no, 1 yes),
- symptom score (0 to 4),
- oxygenotherapy during the sampling (0 no, 1 yes), and
- 20 - time since PCR in days.

We then used the Elastic Net model to predict the COVID-19 status. To select the best penalization parameters, we performed a 10-fold cross validation, repeated 4 times, with a proportion of 40% infected and 60% not infected patients in each fold. Parameters giving the best accuracy were selected.

25 The selected model, which includes the signal intensity of feature m/z 99.08, the symptom score, previous corticosteroid therapy before sampling and oxygenotherapy, allows the detection of COVID-19 with an accuracy of 91% in cross validation. The diagnostic performances are presented in the graph of figure 16, with an area under the Receiver operating characteristic curve (AUC) of 0.961,
30 which is close to 1. Figure 15 shows the confusion matrix with a precision of 85.5% and recall 93.4%.

Thus, this example also shows that we may perform a method for identifying that a person carries or is infected by SARS-CoV-2 or that a person does not carry or is not infected by SARS-CoV-2, the method comprising:

- obtaining a sample comprising elements coming from breath exhaled from a person;
- exposing the sample to a spectrometer;
- determining only one value of signal intensity and/or concentration of ions
- 5 defined by its mass-to-charge ratio (m/z) given by the spectrometer in only one range comprising $m/z = 99.08$ (99.04-99.14),
- determine the symptom score, whether the person had a corticosteroid and/or oxygen therapy before sampling, and
- applying at least one test to the value to predict the probability of the patient
- 10 carrying or being infected by SARS-CoV-2 (as described for the model elastic Net)

B - Targeted analysis

In this second example, a complementary analysis was made using the same

15 model as the model used for intubated, mechanically ventilated patients, using only the concentration of the four signals m/z 99.08, m/z 111.12, m/z 135.10 and m/z 143.14. It shows that we predict the COVID-19 status with an accuracy of 78% in 10-fold cross validation, with a logistic regression. The confusion matrix of the model and the Receiver operating characteristic curve are presented on figures 17

20 and 18.

In this case, the example shows that we may perform a method for identifying that a person carries or is infected by SARS-CoV-2 or not, the method comprising:

- 25 - obtaining a sample comprising elements coming from breath exhaled from a person;
- exposing the sample to a spectrometer;
- determining values of signal intensities and/or concentrations of ions defined by their mass-to-charge ratios (m/z) given by the spectrometer in a group comprising the following ranges:
- 30 - $m/z = 135.09$ (135.03-135.17),
- $m/z = 143.15$ (143.07-143.22),
- $m/z = 99.08$ (99.04-99.14), and
- $m/z = 111.12$ (111.07-111.18).
- applying at least one test to the values, and
- 35 - communicating a message according to a result of the test.

Apart from that, the method is performed in the same way as in the first example.

Conclusion

5 The invention is not limited to the above-described embodiments and many modifications of these embodiments could be made without departing from the invention.

Other types of spectrometer may be used. But this kind of spectrometer can directly receive exhaled breath and provide immediately the result of the analysis.

10 Other kinds of spectrometer may need more operations and deliver the result after a certain period of time.

For a given M molecule, the m/z values indicated above are characteristic of the mass spectrometer we used (PTR-TOF-MS). With this type of instrument, the molecule M whose mass would be equal to 200 is detected in its form $[M+H]^+$ (the spectrometer "grafts" a proton to the molecule in order to give it an electric charge making detection possible), i.e. $m/z = 201$. It would also be possible to detect the same molecule M with other types of mass spectrometers based on different technologies, for example GC-MS, and in this case for this same molecule M, we would obtain masses corresponding to 4 or 5 fragments, for example 72, 125, 140 and 172. Still other types of mass spectrometers could also give values strictly identical to ours (SESI-MS...). Accordingly, the same range may be defined by different median values and limits of the range.

Examples include gas chromatography mass spectrometry (GCMS), liquid chromatography mass spectrometry (LCMS) and proton transfer reaction mass spectrometry (PTR-MS)), ion mobility spectrometry, field asymmetric ion mobility spectrometry, differential mobility spectrometry (DMS); infrared spectroscopy (IR spectroscopy) such as near-infrared (NIR), selected ion flow tube mass spectrometry (SIFT), secondary electrospray ionization (SESI) mass spectrometry, Fourier Transform-Infrared (FOR) spectroscopy and ring-down, cavity spectroscopy or light absorption spectroscopy.

Mass spectrometry can be used in tandem with chromatographic separation techniques. For example. GCMS is an analytical method that combines the features of gas chromatography and mass spectrometry to identify compounds. Similarly, liquid chromatography mass spectrometry (LCMS) is an analytical method that

combines the features of liquid chromatography and mass spectrometry to identify compounds.

Ion-mobility spectrometry (IMS) is an analytical technique used to separate and identify ionized molecules in the gas phase based on their mobility in a carrier
5 buffer gas. This technique can be coupled with mass spectrometry and/or chromatographic separation techniques. Having the person inspire pure oxygen is not compulsory. Other percentages of FiO₂ may be used.

This device may be used on a person undergoing invasive mechanical ventilation, but it may also be used on a person who is not undergoing that. It
10 could be a person who is sick or suspected to be sick and/or who has been diagnosed as affected by the COVID-19. It could be a person having ARDS or no ARDS. It could be a person having symptoms or no symptoms. For persons not undergoing mechanical ventilation, sampling devices other than the transfer line directly connected to the end of the endotracheal tube may be used, such as the
15 Buffered End-Tidal Breath Sampling Inlet (BET-med device, Ionicon, Innsbruck).

The number of VOC (or ranges of m/z) of interest has been illustrated with examples of 1, 4, 8 and 65. But many other numbers are possible, for example 10, 12, 20, 30, etc., provided a test or decision rule is properly designed to be used with this number of VOC.

20 As shown, this number of VOC may also be only one. In this case, the VOC could correspond to any of the following ranges already mentioned in the group of 8:

- $m/z = 135.09$ (135.03-135.17),
- $m/z = 143.15$ (143.07-143.22),
- 25 - $m/z = 99.08$ (99.04-99.14),
- $m/z = 111.12$ (111.07-111.18),
- $m/z = 71.05$ (71.02-71.09),
- $m/z = 55.05$ (55.03-55.09),
- $m/z = 83.09$ (83.05-83.13), and
- 30 - $m/z = 93.04$ (93.00-93.09).

Besides, when using a number of VOC less than 65, the chosen VOC (or ranges of m/z) of interest need not be among the most prominent ones in the complete signature of 65. A good signature may be obtained with a number of VOC (and an associated test or decision rule) not among the most prominent ones.

Claims

1. A method for analysis, the method comprising:
 - obtaining a sample comprising elements coming from breath exhaled from
 - 5 a person (112);
 - exposing the sample to a spectrometer;
 - determining at least one value of a signal intensity and/or concentration of ions defined by its mass-to-charge ratio (m/z) given by the spectrometer in at least one range,
 - 10 the or each range being defined by a median value and limits of the range;
 - applying at least one test to the value,
 - the range(s) and the test being configured for identifying that:
 - a person carries or is infected by SARS-CoV-2, with or without clinical symptoms,
 - 15 - a person does not carry or is not infected by SARS-CoV-2,
 - a person suffers from COVID-19, and/or
 - a person does not suffer from COVID-19; and
 - communicating a message according to a result of the test.
 - 2. A method according to the preceding claim wherein the spectrometer is
 - 20 a mass spectrometer, for example a proton transfer reaction mass spectrometer.
 - 3. A method according to any of the preceding claims wherein the step of determining comprises determining only one value of signal intensity and/or concentration of ions defined by its mass-to-charge ratio (m/z) given by the spectrometer in only one range.
 - 25 4. A method according to the preceding claim wherein the range comprises $m/z = 99.08$ (99.04-99.14), the method being performed for example for identifying that a person is carrying or is infected by SARS-CoV-2 or that a person is not carrying or is not infected by SARS-CoV-2.
 - 5. A method according to any of claims 1 to 2 wherein the step of
 - 30 determining comprises determining values of signal intensities and/or concentrations of ions defined by their mass-to-charge ratios (m/z) given by the spectrometer in a group of ranges.

6. A method according to any of the preceding claims, wherein the range comprises one of the following ranges or the group comprises at least one of the following ranges, preferably all of them, and for example consists in all of them:

- 5
- $m/z = 135.09$ (135.03-135.17),
 - $m/z = 143.15$ (143.07-143.22),
 - $m/z = 99.08$ (99.04-99.14), and
 - $m/z = 111.12$ (111.07-111.18).

7. A method according to any of the preceding claims, wherein the range comprises one of the following ranges or the group comprises at least one of the following ranges, preferably all of them, and for example consists in all of them:

- 10
- $m/z = 135.09$ (135.03-135.17),
 - $m/z = 143.15$ (143.07-143.22),
 - $m/z = 99.08$ (99.04-99.14),
 - $m/z = 111.12$ (111.07-111.18),
 - 15 - $m/z = 71.05$ (71.02-71.09),
 - $m/z = 55.05$ (55.03-55.09),
 - $m/z = 83.09$ (83.05-83.13), and
 - $m/z = 93.04$ (93.00-93.09).

8. A method according to any of the preceding claims, wherein the range comprises one of the following ranges or the group comprises at least one of the ranges of the following table, preferably all of them, and for example consists in all of them:

(m/z [$M+H$] ⁺) median	(m/z [$M+H$] ⁺) lower limit	(m/z [$M+H$] ⁺) upper limit
135.09	135.03	135.17
143.15	143.07	143.22
99.08	99.04	99.14
111.12	111.07	111.18
71.05	71.02	71.08
55.05	55.03	55.09
83.09	83.05	83.13
93.04	93.00	93.09
29.01	29.00	29.03
115.11	115.05	115.18
97.07	97.02	97.10
63.01	62.98	63.04
47.05	47.03	47.08
65.06	65.03	65.10

179.15	179.07	179.25
73.07	73.04	73.11
30.98	30.97	31.00
79.06	79.02	79.10
81.07	81.04	81.12
38.03	38.02	38.06
39.02	39.01	39.04
53.04	53.02	53.07
85.10	85.07	85.15
81.02	80.98	81.06
41.00	40.98	41.03
117.09	117.04	117.16
43.99	43.97	44.02
31.00	30.99	31.02
71.09	71.06	71.13
45.03	45.01	45.06
43.97	43.94	43.99
89.06	89.02	89.11
66.96	66.93	67.00
107.09	107.03	107.15
91.06	91.02	91.11
53.00	52.97	53.03
75.05	75.01	75.09
27.00	26.98	27.01
31.02	31.00	31.03
67.06	67.03	67.09
60.05	60.03	60.09
45.00	44.98	45.02
39.03	39.01	39.05
27.01	26.99	27.03
33.03	33.02	33.05
41.04	41.02	41.06
29.00	28.99	29.02
77.06	77.03	77.10
31.05	31.04	31.07
61.04	61.00	61.07
28.00	27.98	28.01
57.03	57.01	57.06
137.13	137.07	137.21
57.07	57.05	57.10
62.03	62.00	62.06
68.06	68.04	68.10

27.02	27.01	27.04
43.06	43.04	43.08
87.08	87.04	87.13
47.01	46.99	47.04
52.96	52.94	52.99
66.05	66.02	66.08
51.04	51.02	51.07
69.07	69.05	69.11
29.04	29.02	29.05

9. A method according to any of the preceding claims, taking into account at least one of the following elements:

- a symptom score,
- whether the person had a corticosteroid therapy before sampling, and
- whether the person had oxygenotherapy.

10. A method according to any of the preceding claims wherein the person (112) is undergoing invasive mechanical ventilation.

11. A method according to any of the preceding claims wherein the sample is a sample of exhaled breath.

12. A method according to any of the preceding claims wherein the step of obtaining a sample comprises obtaining the sample via a transfer line (106) in communication with the person (112), for example the transfer line (106) being connected to an end of an endotracheal tube (110) installed on the person (112), the method for example comprising heating the transfer line (106).

13. A device (120) for the diagnosis, screening or monitoring of SARS-CoV-2 and/or COVID-19,

the device comprising:

- means for determining at least one value of a signal intensity and/or concentration of ions defined by its mass-to-charge ratio (m/z) given by the spectrometer in at least one range,

the or each range being defined by a median value and limits of the range;

- means for applying at least one test to the value,

the range(s) and the test being configured for identifying that:

- a person carries and/or is infected with SARS-CoV-2,
- a person does not carry and/or is not infected with SARS-CoV-2,
- a person suffers from COVID-19, and/or
- a person does not suffer from COVID-19.

14. The device of the preceding claim, comprising a spectrometer, such as a mass spectrometer, for example a proton-transfer-reaction mass spectrometer (118).

15. A program comprising code instructions configured for controlling an
5 execution of a method according to claims 1 to 11 or for controlling a device (120; 220) according to claims 13 to 14.

16. A process for providing a program according to the preceding claim on a communication network in view of downloading the program.