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(54) Title: METHODS OF ASSESSING WHETHER A SUBJECT IS AT RISK OF DEVELOPING SEVERE SYMPTOMS OF DISEASE AND/OR BECOMING CONTAGIOUS AFTER EXPOSURE, OR POSSIBLE EXPOSURE, TO A RESPIRATORY VIRUS

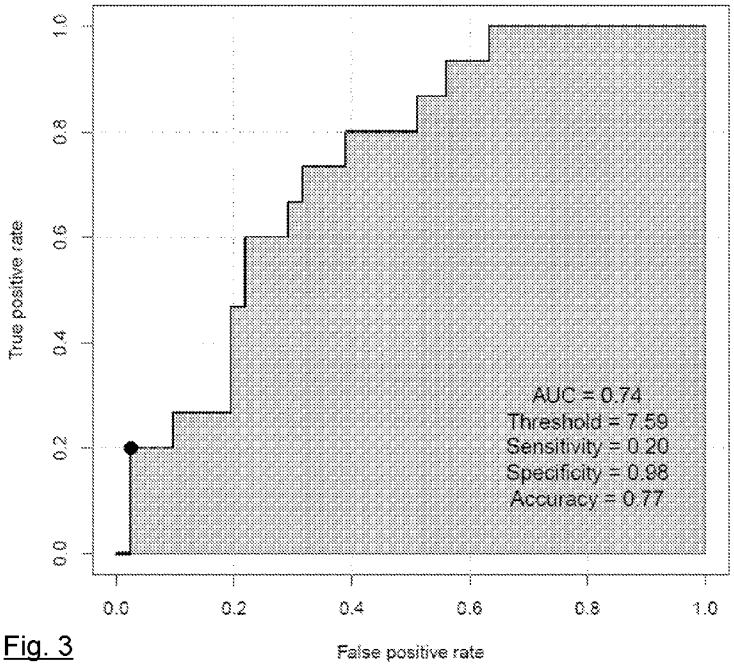

Fig. 3

(57) Abstract: Disclosed is a method of assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, comprising: measuring a level of expression of SPATS2L (spermatogenesis-associated serine-rich 2-like) in a biological sample obtained from the subject; and analysing the measured level of expression of SPATS2L to assess whether the subject is at risk of developing severe symptoms of disease and/or becoming contagious. Also disclosed are related products and methods.

[Continued on next page]

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Methods of assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus

Field of the Disclosure

The present disclosure relates to methods for assessing whether one or more subjects are at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus; especially influenza. The disclosure includes methods of conducting clinical trials or field studies, but more generally, the methods of the disclosure may be used in any healthcare or non-healthcare setting for assessing the risk of an individual patient. For example, the methods of the disclosure may be used to triage patients infected with a respiratory virus to identify those who are at risk of developing severe symptoms of disease and/or becoming contagious and may therefore require early medical intervention. Subjects may have been administered a medicinal product for treatment or prevention of respiratory viral disease, and the methods of the disclosure may therefore be used as a companion analytical product to predict the likely efficacy of the medicinal product. The present disclosure also comprehends related methods, including computer-implemented methods, networks and prognostic kits.

Background of the Disclosure

Acute upper and lower respiratory infections are a major public health problem and a leading cause of morbidity and mortality worldwide. Viruses are the predominant cause of respiratory tract illnesses and include RNA viruses such as respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, metapneumovirus, rhinovirus (HRV) and coronavirus (Hodinka, *Microbiol. Spectr.*, 2016, 4(4)).

The Centre for Disease Control and Prevention (CDC) estimates that in the 2015-2016 period in the US there were 25 million influenza illnesses, 11 million influenza-associated medical visits, 310,000 influenza-related hospitalisations, and 12,000 pneumonia and influenza deaths (Rolfes *et al.*, '*Estimated Influenza Illnesses, Medical Visits, Hospitalizations, and Deaths Averted by Vaccination in the United States*', CDC, 2016). In 2003, the annual economic burden of influenza in the US alone was estimated to be around 87 billion dollars (Molinari *et al., Vaccine*, 2007, 25(27), 5086–5096). The costs of influenza are clearly substantial and any method to treat or diagnose influenza would be of enormous value.

Influenza infects all age groups and causes a range of outcomes from asymptomatic infection and mild respiratory disease to severe respiratory disease and even death. As such,

different subjects exposed to the same influenza virus, which may be a seasonal strain, not necessarily of the highly pathogenic kind, may be asymptomatic, mildly symptomatic, subclinical, exhibit acute symptoms, or require medical attention, or even urgent hospitalisation (Cox *et al., Lancet*, 1999, 354(9186), 1277-1282). Further, the proportion of infections that are asymptomatic or subclinical, and the degree to which these are contagious, as well as the proportion of shedding which occurs prior to onset of symptoms, affect the potential impact of control measures and decisions regarding treatment and the administration of medicinal products (Lau *et al., J Infect Dis*., 2010, 201(10):1509-16).

There are various reasons why it may be advantageous to assess in advance whether a subject is at risk of developing severe disease (such as symptoms or signs requiring hospitalisation) and/or becoming contagious, after exposure (or possible exposure) to a respiratory virus (such as influenza).

By way of example, this may enable informed treatment decisions, leading a physician to administer suitable care. In some circumstances, a proportion of the population may have mild (even asymptomatic) infections, especially in response to seasonal strains. Treating everyone having an infection, including those subjects assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious, may mean exposing subjects unnecessarily to drugs with side effects. This may be especially undesirable for subject groups that are more susceptible to side effects, such as may be the case amongst patients who are resident in hospital (particularly patients who may be resident in the hospital for reasons other than viral infection) and/or infants (i.e. younger than one year old), children (i.e. younger than ten years old), elderly subjects (i.e. 65 years old or more) and pregnant women.

By way of a further example, it may help to improve the trial design for investigational medicaments. Current trial designs within human challenge models for assessing investigational treatment drugs and medicinal products for influenza, RSV, coronaviruses, or HRV rely on either:

a. "*Universal dosing*" - universally treating all subjects inoculated with virus on a given day post inoculation (e.g. 24 hrs or 48 hrs post inoculation) irrespective of whether the subjects become infected or not;
b. "*Triggered dosing*" - treating only those subjects having either one or both of the following:
    i. their first (or confirmed) PCR positive respiratory sample (i.e. treating only those who are expected to be infected post-inoculation); and
    ii. initial respiratory symptoms that are indicative of onset of viral infection; or

c. "*Triggered dosing + universal dosing*" (de Vincenzo *et al., N. Engl. J. Med.,* 2014, 371(8):711-22; de Vincenzo *et al., N. Engl. J. Med.,* 2015, 373(21):2048-58) - this uses the principles of triggered dosing for the primary endpoint. However, at a certain day post-inoculation (e.g. Day 5) subjects who still do not have a positive viral sample (or symptoms) are subsequently given the drug regardless. Subjects who are universally given the drug in this scenario may be included for analysis in two sub-analysis approaches:

   i. On their own as a sub-group;

   ii. Combined with the triggered sub-group.

In research models such as human challenge models, knowing which subjects will develop significant symptoms in advance would allow dosing of an investigational medicinal product to be triggered only in subjects who would otherwise go on to develop severe symptoms of disease and/or become contagious. A method capable of assessing who is at risk of developing severe symptoms of disease and/or become contagious would allow the identification and selection of subjects for administration of the medicinal product. Benefits of this volunteer selection method for dosing may include:

- An improved ability to detect a clinically relevant reduction in disease, by only evaluating the effects of a medicinal product (or products) in subjects who would have gone on to present with severe symptoms of disease and/or become contagious. This contrasts with trial designs where triggering of treatment might be based on the presence of symptoms, or universal administration of the medicinal product (or products) to all inoculated people. Selecting appropriate subjects for a trial in advance may avoid problems associated with assessing the efficacy of the medicinal product (or products) in populations where the ability to detect a difference is more difficult (i.e. uninfected, asymptomatic infected or people who only have a mild infection with minimal viral loads).
- Fewer people will be exposed to the medicinal product (or products) unnecessarily, thereby:
   i. reducing medicinal product requirements, leading to manufacturing and cost benefits;
   ii. providing a treatment regime with an improved benefit/risk profile by selecting to provide treatment only to subjects who are assessed to be at risk of developing severe symptoms and/or become contagious;
   iii. providing an improved benefit/risk profile for both the medicinal product and the study, by requiring fewer people to be exposed to an investigational medicinal product.

Woods *et al.*, *'A Host Transcriptional Signature for Presymptomatic Detection of Infection in Humans Exposed to Influenza H1N1 or H3N2'* (*PLOS ONE*, 2013, 8(1): e52198) describes the generation of a viral gene signature (or factor) for symptomatic influenza that is reported to be capable of detecting 94% of infected cases. The authors report that the gene signature is detectable as early as 29 hours post-exposure and achieves maximal accuracy on average 43 hours ($p = 0.003$, H1N1) and 38 hours (p-value equals 0.005, H3N2) before peak clinical symptoms.

Muller *et al.*, *'Development of an objective gene expression panel as an alternative to self-reported symptom scores in human influenza challenge trials'* (*J. Transl. Med.* (2017) 15:134) discloses methods for detecting and differentiating between different levels of symptoms of disease in an influenza challenge trial, using a panel of biomarkers to provide a more accurate assessment of symptoms than may be attained by means of (subjective) self-reported symptom scores.

However, there remains a need for methods of assessing as early as possible whether a subject is at risk of developing severe disease (such as symptoms or signs requiring hospitalisation; and/or symptoms or signs comprising, by way of example, one or more of: tachypnoea, hypoxemia, an arterial oxygen saturation of $\leq 92\%$ on room air by a transcutaneous method and radiological pulmonary infiltrates) and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, to enable informed treatment decisions, to administer the correct level of care, and/or to improve the trial design for investigative medicaments.

Summary of the Disclosure

In a first aspect of the present disclosure, therefore, there is provided a method of assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, comprising:

(a) measuring a level of expression of SPATS2L (spermatogenesis-associated serine-rich 2-like) in a biological sample obtained from the subject; and

(b) analysing the measured level of expression of SPATS2L to assess whether the subject is at risk of developing severe symptoms of disease and/or becoming contagious.

By "measuring the level of expression of SPATS2L" herein may be meant quantifying mRNA transcripts of SPATS2L in the biological sample. Those skilled in the art will be familiar with methods for measuring the expression level of a gene in a biological sample.

In a second aspect of the present disclosure, therefore, there is provided a method of quantifying the level of expression of SPATS2L in a subject at risk of developing severe symptoms of disease and/or becoming contagious following exposure, or possible exposure, to a respiratory virus, comprising:

a) obtaining a biological sample from the subject; and

b) measuring a level of expression of SPATS2L in the sample by RNA transcriptomics analysis.

The method may further comprise assessing the subject to be at risk of developing severe symptoms of disease and/or becoming contagious based on the measured expression level of SPATS2L in the sample.

In some embodiments, for example, the amount of SPATS2L mRNA in the biological sample may be measured by capturing SPATS2L transcripts from the sample on a microarray. Suitably, the SPATS2L transcripts from the sample may be captured using one or more complementary oligonucleotide probes for SPATS2L, which are immobilised on a surface, e.g. a plate or bead. A high or low density format may be employed. In some embodiments, a plurality of different probes (typically up to about 20, e.g. about 11) may be used to quantify the amount of SPATS2L. Suitably, each probe may comprise 15-30 bases, e.g. about 25 bases. The quantity of bound mRNA can be determined by suitably labelling the transcripts with a fluorophore and measuring the intensity of fluorescence. Suitably, the measured amounts of mRNA transcripts may be normalised by reference to one or more maintenance genes, e.g. GADPH, beta-Actin, ISGF-3 (STAT1).

Thus, in some embodiments, for example, the mRNA transcripts in the biological sample may be quantified by RNA transcriptomics analysis performed by Almac Diagnostics using the GeneChip™ Human Genome U133 Plus 2.0 Array (which is available from Affymetrix, Voyager Mercury Park, Wycombe Lane, High Wycombe, HP10 0HH, UK). Additional details of the GeneChip™ Human Genome U133 Plus 2.0 Array are provided in the *GeneChip™ Human Genome Arrays* Data Sheet, Part No. 701484, Rev. 4, the contents of which are incorporated herein in their entirety.

In some embodiments, the expression levels of SPATS2L in the biological sample may be quantified using an RNA sequencing (RNA-Seq) method. Transcripts may be converted to cDNA, fragmented and attached to suitable adapters containing functional moieties that permit

sequencing; e.g. an amplification element (which facilitates clonal amplification of the fragments) and a primary sequencing priming site. Following amplification and size selection, the cDNA library may be analysed, e.g. by next generation sequencing (NGS), which produces short sequences that correspond to all or part of the fragments from which they are derived. Sequencing may comprise either single-end or paired-end sequencing methods.

In some embodiments, step (b) may comprise subtracting a baseline level of expression of SPATS2L from the level of expression of SPATS2L measured in step (a) to obtain a measured value for the change in the level of expression of SPATS2L.

By "baseline level" herein is meant a level of expression of SPATS2L in a biological sample obtained from the subject prior to exposure, or possible exposure, of the subject to the respiratory virus, or a level of expression of SPATS2L in a biological sample obtained from one or more uninfected control subjects.

In some embodiments, the analysing step (b) may comprise comparing the measured change in the level of expression of SPATS2L with a reference threshold change, wherein when the measured change in the level of expression of SPATS2L is above the reference threshold change, the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

In some embodiments, the reference threshold change may be determined by: measuring the baseline levels of expression of SPATS2L in biological samples obtained from a group of persons uninfected with respiratory virus; measuring the levels of expression of SPATS2L in biological samples obtained from the group of persons after inoculation with respiratory virus; ascertaining the change in the level of expression of SPATS2L for each person from uninfected to after inoculation; classifying the persons in the group according to their risk of developing severe symptoms of disease and/or becoming contagious; and setting the reference threshold change to be a change in the level of expression of SPATS2L which discriminates between persons in the group who are classified as at risk and persons in the group who are not classified as at risk, according to a desired measure of test performance.

Suitably, the reference levels of expression of one or more house keeping genes (of the kind known in the art) in the biological samples obtained from the group of persons may also be measured. These reference levels of expression of the one or more house keeping genes may

provide suitable stable standards for scaling the measured change in the level of expression of SPATS2L of a subject where appropriate, in accordance with the present disclosure.

As used herein (and as normally used in the art) "test performance" may mean the ability of a method to discriminate between subjects having a relevant characteristic (herein, being at risk of developing severe symptoms of disease and/or becoming contagious) and subjects lacking the relevant characteristic. Measures of test performance which are well known to those skilled in the art include specificity, sensitivity, accuracy, positive predictive value (PPV) and negative predictive value (NPV). One or more of the measures of test performance may be used in the methods of the present disclosure.

By "inoculation" as used herein may be meant exposure of a subject on purpose to respiratory virus; for example by intranasal inoculation with virus.

Suitably, the biological samples obtained from the group of persons after inoculation with respiratory virus may be obtained about 30 to about 90 hours after inoculation; such as about 40 to about 50 hours after inoculation, for example about 45 hours after inoculation. It has been found that upregulation of SPATS2L may begin about 30 hours (for example, about 40 hours, such as about 45 hours) after inoculation with a respiratory virus, and may last for a period of up to several days thereafter, in a person at risk of developing severe symptoms of disease and/or becoming contagious. Accordingly in some embodiments, the biological samples may be obtained from the group of subjects about 1 to about 7 days after inoculation, for example about 1 to about 5 days after inoculation. Suitably, in some embodiments, the biological samples may be obtained about 30, 40 or 45 hours or more after inoculation.

In some embodiments, persons may be classified as being at risk of developing severe symptoms of disease and/or becoming contagious if, for example, they have a high viral load after inoculation; such as if their peak viral load falls in a top quantile of the group for peak viral load. Those skilled in the art will be familiar with methods for measuring viral load, such, for example, as by qPCR.

Further or alternatively, persons may be classified as being at risk of developing severe symptoms of disease and/or becoming contagious if they display one or more relevant symptoms and/or signs after inoculation; such, for example, as one or more of sneezing, cough, runny nose, stuffy nose and sore throat. Those skilled in the art will be familiar with methods for identifying relevant symptoms and/or signs. For example, persons may be classified as such

if they are in a top quantile of the group after inoculation for a peak combined Visual Analogue Score (VAS) or Categorical Score (e.g. a score on a standard categorical five-grade scale, 5GS) for sneezing, cough, runny nose, stuffy nose and sore throat (which may in some embodiments be determined by VAS self-reported score card).

Suitably, in some embodiments, a top quantile as disclosed herein may be the top tertile or quartile, but other quantiles may be used depending on how it is desired to define a person who is at risk of severe symptoms of disease and/or becoming contagious for a particular application.

By "after inoculation" in the classification step herein may be meant within a period of assessment post inoculation. By way of example, the period of assessment may be up to 7 or 8 days post inoculation, and in some cases up to 15 or even 28 days, although it will be appreciated that persons will generally display peak viral loads and/or peak symptoms or signs of infection much sooner, typically within 3-5 days, especially those falling in the top quantile. In some embodiments, persons may be classified as being at risk of developing severe symptoms of disease and/or becoming contagious if they have a peak viral load and/or peak symptoms or signs in the top quantile for the group within about 36 to about 84 hours after inoculation.

In accordance with the present disclosure, numerical values for the change in the level of expression of SPATS2L are given for a subject whose measured expression level H_m of at least one house keeping gene in **Table 1** below, is substantially the same as the reference expression level H_{ref} of at least one house keeping gene as reported in **Table 1** below. In a subject whose measured level of expression H_m of at least one house keeping gene is significantly different from the reference value H_{ref} reported in **Table 1**, their measured change E_m in the level of expression of SPATS2L may be scaled accordingly (E_S). It will be appreciated by persons skilled in the art that otherwise, comparison of their measured change E_m in the level of expression of SPATS2L to a numerical value for a reference threshold change in the level of expression of SPATS2L disclosed herein, may not be a comparison of like for like.

Thus, in some embodiments, the measured change in the level of expression of SPATS2L may be scaled by reference to the level of expression of at least one house keeping gene measured in the biological sample of the subject and a reference level of expression of the

at least one house keeping gene which is associated with reference threshold levels as disclosed herein, before being compared to the reference threshold change in the level of expression of SPATS2L.

The house keeping gene may, in accordance with the disclosure of **Table 1**, be selected from: Actin Beta (ACTB), Beta-2-Microglobulin (B2M), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Glucuronidase Beta (GUSB), Hypoxanthink Phosphoribosyltransferase 1 (HPRT1), Phosphoglycerate Kinase 1 (PGK1), TATA Box Binding Protein (TBP) and Transferrin Receptor (TFRC). **Table 1** discloses reference expression levels for the house keeping genes which are the expression levels of those genes associated with the numerical values for the threshold levels of SPATS2L disclosed herein. While the house keeping gene may be selected from this list, other suitable house keeping genes areknown to those skilled in the art.

Table 1

House keeping gene	Reference expression level (H_{ref})
ACTB	14.550
B2M	13.773
GAPDH	13.538
GUSB	11.775
HPRT1	9.386
PGK1	11.682
TBP	10.491
TFRC	9.737

In some embodiments, the scaled change in the level of expression of SPATS2L may be calculated according to the formula:

$$E_s = E_m \times \frac{H_{ref}}{H_m}$$

wherein E_S is the scaled value of the measured change in the level of expression of SPATS2L in the subject, E_m is the measured change in the level of expression of SPATS2L in the subject, H_{ref} is the reference level of expression of the housekeeping gene, for example as reported in **Table 1** above, and H_m is the expression level of the housekeeping gene measured in the biological sample of the subject. In accordance with the present disclosure, H_{ref} is associated with one or more reference threshold levels, such as those reported in **Table 1** above.

It will be appreciated by those skilled in the art that, instead of scaling the measured change E_m in the level of expression of SPATS2L of a subject to enable the comparison of like for like, the reference threshold change in the level of expression of SPATS2L may be scaled. Notably, where the reference threshold SPATS2L expression level is scaled in this way, the numerical values and ranges for measures of test performance associated with the threshold (such as, for example, sensitivity, specificity, PPV and NPV) do not change.

In some embodiments, the reference threshold change in the level of expression of SPATS2L may be about 0.4 to about 0.9. For example, the reference threshold change may be about 0.4; about 0.6; or about 0.8, particularly when mRNA transcripts in the biological sample are quantified by a microarray RNA transcriptomics analysis of the kind described above; for example of the kind that is commercially available from Almac Diagnostics using the GeneChip™ Human Genome U133 Plus 2.0 Array.

In some embodiments, the probability that a subject who will go on to develop severe symptoms of disease and/or become contagious is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious (sensitivity) may be in a range of about 0.4 to about 0.7; for example about 0.45 to about 0.55; such as about 0.5.

In some embodiments, the probability that a subject who will not go on to develop severe symptoms of disease and/or become contagious is assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious (specificity) may be in a range of about 0.7 to about 0.9; for example about 0.75 to about 0.85; such as about 0.8.

In accordance with the present disclosure, it has been found that a scaled change in the level of expression of SPATS2L of greater than the reference threshold change may correspond to a positive predictive value (PPV) in a range of about 0.4 to about 0.5; for example about 0.45 to about 0.55; such as a PPV of about 0.5. The term "positive predictive value" as used herein may mean the probability that the assessment of a subject as being at risk of developing severe symptoms of disease and/or becoming contagious is correct (i.e., correctly identifies a subject truly at risk of developing severe symptoms of disease and/or becoming contagious).

Meanwhile, a scaled change in the level of expression of SPATS2L of less than the reference threshold change may correspond to a negative predictive value (NPV) of greater than about 0.75; for example greater than about 0.85; greater than about 0.9; or greater than about

0.95. The term "negative predictive value" as used herein may mean the probability that the assessment of a subject as not being at risk of developing severe symptoms of disease and/or becoming contagious is correct (i.e., correctly identifies a subject truly not at risk of developing severe symptoms of disease and/or becoming contagious).

In some embodiments, analysing step (b) of the method of the first aspect of the present disclosure may comprise comparing the measured level of expression of SPATS2L to a reference threshold for the level of expression of SPATS2L, wherein when the measured level of expression of SPATS2L is above the reference threshold, the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

In some embodiments, the reference threshold for the level of expression of SPATS2L may be determined by: measuring the levels of expression of SPATS2L in biological samples obtained from a group of persons after inoculation with respiratory virus; classifying the persons in the group according to their risk of developing severe symptoms of disease and/or becoming contagious; and setting the reference threshold to be the level of expression of SPATS2L which discriminates between persons in the group who are classified as at risk and persons in the group who are not classified as at risk, according to a desired measure of test performance.

Suitably, the reference levels of expression of one or more house keeping genes (of the kind known in the art) in the biological samples obtained from the group of persons may also be measured. These reference levels of expression of the one or more house keeping genes may provide suitable stable standards for scaling the measured level of expression of SPATS2L of a subject where appropriate, in accordance with the present disclosure.

Suitably, the biological samples obtained from the group of persons after inoculation with respiratory virus may be obtained at the time points after inoculation disclosed herein.

In accordance with the present disclosure, the measured level of expression of SPATS2L may be scaled by reference to the level of expression of at least one house keeping gene measured in the biological sample of the subject and a reference level of expression of the at least one house keeping gene which is associated with reference threshold levels as disclosed herein, before being compared to the reference threshold level of expression of SPATS2L.

The house keeping gene may be selected from those disclosed herein, or from other suitable house keeping genes known to those skilled in the art.

In some embodiments, the scaled level of expression of SPATS2L may be calculated according to the formula:

$$E_s = E_m \times \frac{H_{ref}}{H_m}$$

wherein E_S is the scaled value of the measured level of expression of SPATS2L in the subject, E_m is the measured level of expression of SPATS2L in the subject, H_{ref} is the reference level of expression of the housekeeping gene, for example as reported in **Table 1** above, and H_m is the level of expression of the housekeeping gene measured in the biological sample of the subject. In accordance with the present disclosure, H_{ref} is associated with one or more reference threshold levels, such as those reported in **Table 1** above.

In accordance with the present disclosure, instead of scaling the measured level of expression of SPATS2L of a subject to enable the comparison of like for like, the reference threshold level of expression of SPATS2L may be scaled.

The reference threshold for the level of expression of SPATS2L may be about 4.9; for example about 5; about 5.2; about 5.5; or about 5.6, particularly when mRNA transcripts in the biological sample are quantified by a microarray RNA transcriptomics analysis of the kind described above, for example of the kind that is commercially available from Almac Diagnostics using the GeneChip™ Human Genome U133 Plus 2.0 Array.

In some embodiments, the probability that a subject who will go on to develop severe symptoms of disease and/or become contagious is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious (sensitivity) may be in a range of about 0.3 to about 0.7; for example about 0.4 to about 0.6; such as about 0.5.

In some embodiments, the probability that a subject who will not go on to develop severe symptoms of disease and/or become contagious is assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious (specificity) may be in a range of about 0.6 to about 0.8; for example about 0.65 to about 0.8; such as about 0.75.

In accordance with the present disclosure, it has been found that a scaled level of expression of SPATS2L above a reference threshold for the level of expression of SPATS2L may correspond to a positive predictive value (PPV) in a range of about 0.4 to about 0.5; for example about 0.45 to about 0.5; such as a PPV of about 0.45.

Meanwhile, a scaled level of expression of SPATS2L below a reference threshold for the level of expression of SPATS2L may correspond to a negative predictive value (NPV) in a range of more than about 0.75; more than about 0.85; more than about 0.9; or more than about 0.95.

It will be appreciated by those skilled in the art that the prevalence of a relevant characteristic in a group of persons, such, for example, as the prevalence of persons having severe symptoms of disease and/or being contagious, may vary. By "prevalence" herein may be meant the frequency of the relevant characteristic, for example expressed as a percentage of the group. This may affect a positive predictive value and/or a negative predictive value of a threshold disclosed herein. The "positive predictive value" of a threshold disclosed herein (such as, in various embodiments, a threshold change in the expression level of SPATS2L or a threshold expression level of SPATS2L) may accordingly be defined as (sensitivity of threshold x prevalence) / [(sensitivity of threshold x prevalence) + ((1 – specificity of threshold) x (1 – prevalence))]. Meanwhile, the term "negative predictive value" of a threshold disclosed herein (such as, in various embodiments, a threshold change in the expression level of SPATS2L or a threshold expression level of SPATS2L) may be defined as (specificity of threshold x (1 – prevalence)) / [(specificity of threshold x (1 – prevalence)) + ((1 – sensitivity of threshold) x prevalence)]. It will further be appreciated that where the prevalence of the relevant characteristic varies, the sensitivity and/or specificity of a threshold disclosed herein (such as, in various embodiments, a threshold change in the expression level of SPATS2L or a threshold expression level of SPATS2L) may remain invariant.

In some embodiments, the prevalence of persons having severe symptoms of disease and/or being contagious may be in a range of about 10 to about 50 % of the group; for example about 10 to about 30 %; about 15 to about 30 %; or about 25 to about 30 %.

In some embodiments, the level of expression of SPATS2L in the subject may be measured two or more times, and the subject may be assessed to be at risk of developing severe symptoms and/or becoming contagious if the at least one or more than one of the measured levels, when assessed, indicates so, optionally wherein successive measurements are separated

by a time interval of at least about 12 hours; for example at least about 24 hours; at least about 36 hours; or at least about 48 hours.

In some embodiments, the thresholds against which the two or more measurements are assessed may be different, the threshold for the assessment of at least one measurement being configured to minimise false positives, and the threshold for the assessment of at least another measurement being configured to have fewer false negatives than the one measurement.

In some embodiments, the biological sample may have been obtained from the subject at least about 30 to about 90 hours after exposure, or possible exposure, to the respiratory virus; such as about 40 to about 50 hours after exposure, or possible exposure, for example about 45 hours after exposure, or possible exposure, to the respiratory virus. As disclosed herein, it has been found that upregulation of SPATS2L may begin about 30 hours (for example, about 40 hours, such as about 45 hours) after (deliberate) inoculation with a respiratory virus, and may last for a period of up to several days thereafter, in a person at risk of developing severe symptoms of disease and/or becoming contagious. Accordingly in some embodiments, the biological sample may be obtained about 1 to about 7 days after exposure, or possible exposure, to the respiratory virus, for example about 1 to about 5 days after exposure, or possible exposure, to the respiratory virus. Suitably, in some embodiments, the biological sample may be obtained about 30, 40 or 45 hours or more after exposure, or possible exposure.

In some embodiments, the subject may have had one or more positive diagnostic tests for respiratory viral disease, presents with symptoms of respiratory viral disease, and/or has had prolonged exposure to at least one other person who is infected with a respiratory virus.

In some embodiments, the one or more diagnostic tests for respiratory viral disease may comprise the use of reverse transcriptase polymerase chain reaction (RT-PCR) in a diagnostic test for the presence of viral RNA, carried out on a nose and/or throat or upper airway sample, such, for example, as a nasopharyngeal sample, collected from the subject.

Further or alternatively, the one or more diagnostic tests for respiratory viral disease may comprise a lateral flow test for the presence of one or more viral antigens, carried out on a nose and/or throat or upper airway sample, such, for example, as a nasopharyngeal sample, collected from the subject.

In some embodiments, the subject may present with one or more of nasal congestion, sneezing, rhinorrhoea, pyrexia (fever) and cough and sputum production.

In some embodiments, the respiratory virus may be respiratory syncytial virus (RSV), parainfluenza virus (HPIV), metapneumovirus (HMPV), rhinovirus (HRV), coronavirus such as SARS-CoV (for example, SARS-CoV-1 or SARS-CoV-2), adenovirus (HAdV), enterovirus (EV), bocavirus (HBoV), parechovirus (HPeV) or an influenza virus. In particular embodiments, the respiratory virus may be an influenza virus, such, for example, as a seasonal strain of influenza, such as seasonal strains of human influenza A viruses and human influenza B viruses, such, for example, as influenza A subtype H3N2. In particular embodiments, the respiratory virus may be a non-pandemic strain. In other particular embodiments, the respiratory virus may be an HRV virus.

In some embodiments, the biological sample may be or may comprise a blood sample. In some embodiments, the blood sample may comprise a minimum of about 1.5 μg RNA, at a minimum concentration of about 250ng/ μl. Further or alternatively, the biological sample may be or may comprise a sputum sample, nasal wash (lavage) sample, nasopharyngeal sample, nasal aspirate sample, oral swab sample, saliva sample, tissue biopsy sample, peritoneal fluid sample, or pleural fluid sample. The biological sample may be obtained from the subject using any clinically acceptable method.

In some embodiments, the expression level of SPATS2L and/or the expression levels of one or more housekeeping genes, may be measured by quantifying mRNA transcripts of SPATS2L in the biological sample. Suitable methods for this are well known to those skilled in the art.

For example, in some embodiments, mRNA transcripts in the biological sample may be quantified by a polymerase chain reaction (PCR)- based method. The mRNA may be amplified by polymerase chain reaction (PCR). The mRNA is then examined by, e.g., hybridisation with oligonucleotides specific for the mRNA, optionally immobilized on a substrate (e.g., an array or microarray). Selection of suitable probes and primers specific for the mRNA, and selection of hybridisation and PCR conditions, are within the ordinary skill of those skilled in the art. Binding of the mRNA to one or more oligonucleotide probes specific for the mRNA may allow identification and quantification of the mRNA. In some embodiments, the probes used may comprise the probe set 241812_at (SEQ ID NO: 1) which is commercially

available from Messrs. Thermo Fisher Scientific Inc. (Waltham, MA) as part of the Affymetrix Human Genome U133 Plus 2.0 Array.

In some embodiments, the polymerase chain reaction (PCR)- based method may be RT-qPCR.

Thus, in some preferred embodiments, mRNA transcripts in the biological sample may be quantified by a microarray RNA transcriptomics analysis of the kind described above, for example of the kind commercially available from Almac Diagnostics using the GeneChip™ Human Genome U133 Plus 2.0 Array.

In some embodiments, the subject may be administered one or more medicinal products before or after exposure or possible exposure to the respiratory virus.

In some embodiments, the method of the first aspect of the present disclosure may comprise administering one or more medicinal products to the subject, if the subject is assessed to be at risk of developing severe symptoms and/or becoming contagious.

In some embodiments, the one or more medicinal products may be selected from one or more antiviral agents and/or one or more immunomodulatory agents.

Suitable medicaments may thus include one or more immunomodulators, such, for example, as those described in WO 2018/007788 or WO 2019/122909 (the contents of each of which are incorporated by reference in their entirety); for example UR-13870 or POLB 001; antiviral agents (such as oseltamivir); antibiotics; and other drugs. It will be appreciated that where an antiviral agent is referred to herein, the antiviral agent may be any one, or more, of the following or pharmaceutically acceptable salts thereof: amantadine; rimantadine; ribavirin; idoxuridine; trifluridine; vidarabine; acyclovir; ganciclovir; foscarnet; zidovudine; didanosine; zalcitabine; stavudine; famciclovir; valaciclovir; antitussives; mucolytics; expectorants; antipyretics; analgesics and/or nasal decongestants. In particular, the antiviral agent may be a neuraminidase inhibitor, such as: oseltamivir (which may be in the form of oseltamivir phosphate), zanamivir, peramivir and/or laninamivir. In certain embodiments, the antiviral agent may be molnupiravir.

It will be appreciated that further or alternatively to one or more suitable medicinal products, a subject may be subjected to one or more surgical or non-surgical interventions. For example, the subject may be hospitalised or at least allocated a hospital bed in anticipation of

their condition worsening. In some embodiments, the subject may be administered supplementary oxygen, such, for example, as by means of a ventilator, or by means of extracorporeal membrane oxygenation.

Viral transmission may occur through the spread of virions (intact whole viral particles) in droplets via coughing, sneezing or even breathing (whereby air is expelled from a person's lungs through their nose or mouth). An increase in viral load, and thus in viral shedding, may therefore increase the quantity of virions in droplets spread by a given subject, and is likely to increase the contagiousness of the subject. Likewise, any signs of disease that could increase the spread of droplets would also increase the chances of being contagious.

Accordingly, by "contagious" herein may be meant any person who releases virions into the surrounding atmosphere; optionally wherein the person is capable of releasing virions over a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days. For example, the person may be capable of releasing virions over a period of from 1 to 15 days, such as from 5 to 15 days.

Further or alternatively, a contagious person may be a person who transmits virions to other persons, optionally wherein the person is capable of transmitting virions over a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days. For example, the person may be capable of releasing virions over a period of from 1 to 15 days, such as from 5 to 15 days.

In some embodiments, a contagious person may release and/or transmit infectious virions by one or more of: coughing, sneezing, and transferring virions from a body surface to an object.

In accordance with the present disclosure, a person may be defined as contagious if they have a high viral load and/or exhibit symptoms or signs which are calculated to spread virions, for example one or more of the following symptoms: sneezing, cough, runny nose, stuffy nose and sore throat.

In some embodiments, the subject may be an infant (i.e. younger than one year old) or elderly (i.e. 65 years old or more) or may be a pregnant woman. It will be appreciated that viral infection can lead to especially severe symptoms and/or contagiousness in children (i.e. younger than ten years old) or infants (i.e. younger than one year old). For example, RSV infection is thought to lead to particularly severe symptoms and/or contagiousness in children or infants of less than two years old.

In some embodiments, a human subject may have one or more underlying comorbidities that predispose the subject to severe symptoms of disease. For example, the subject may be immunocompromised, or may suffer from COPD, severe genetic anaemia, asthma or diabetes, chronic hepatic or renal insufficiency, obesity or a cardiovascular disorder or condition. For example, where the virus is an HRV virus, the subject may suffer from asthma.

In some embodiments, the subject may have been vaccinated against a relevant respiratory virus. Vaccination may lower the probability of infection following exposure to respiratory virus, and/or of a subject developing severe symptoms of disease following such exposure. Nonetheless, subjects who, despite having been vaccinated, may go on to develop severe symptoms of disease and/or become contagious, may be assessed, in methods of the first aspect of the present disclosure, as at risk of developing severe symptoms of disease and/or becoming contagious.

In some embodiments, the subject may be serologically naïve, i.e. seronegative for circulating antibodies or T cells to the respiratory virus. By way of example, this may be the case for many subjects in the first 1, 2, 3, 4, 5 or 6 months of a respiratory virus pandemic, when it may be expected that a majority of a local or worldwide population (for example, more than 50, 70, or 90 % of a population) are serologically naïve to the virus.

It will be appreciated that where a majority of a local or worldwide population (for example, more than 50, 70, or 90 % of a population) are serologically naïve, it may be preferable to use medicaments sparingly. By way of example, sparing use of medicaments (such as antiviral agents, immunomodulators and/or antibiotics, disclosed herein) may be preferable where there are local or worldwide shortages. For such reasons, in determining which subjects to treat, a physician may sometimes find it necessary to be more selective than would be normal practice. In some embodiments, the physician may administer one or more suitable medicaments only to subjects who are assessed to be at risk of developing severe symptoms of disease and/or becoming contagious. Alternatively, only such subjects may be permitted to self-administer the one or more suitable medicaments. Additionally or alternatively, where there is a shortage of hospital facilities, only such subjects may be referred (where they present at a primary care facility, or are visited at home by a physician) to hospital.

In a pandemic, implementation of the method of the first aspect of the present disclosure may, in some embodiments, comprehend: post-exposure assessment (or assessment following

suspected exposure to virus); assessment when a subject develops symptoms suspected to indicate viral infection; event-triggered assessment, such as when a subject boards or disembarks a train, plane, ship or other mode of public transport; and/or regular assessment of healthcare workers and certain other staff, for example all staff in a hospital or primary care facility, over a period of time. Successive assessments over a period of time may, for example, be separated by a time interval of at least about 12 hours; for example at least about 24 hours; at least about 36 hours; or at least about 48 hours.

In some embodiments, the subject may be a patient who is resident in a hospital. It will be appreciated that an outbreak of one or more respiratory viruses may sometimes occur amongst patients who may be resident in the hospital for reasons other than viral infection. For example, the subject may be on a ward of patients considered by a physician to be at high risk of morbidity and mortality following viral infection (or one or more secondary infections associated with a primary viral infection) such as may be found in a hospital high dependency unit, or intensive care unit.

Following exposure, or possible exposure, to a respiratory virus, the risk of the subject (patient) developing severe symptoms of disease and/or becoming contagious may be assessed in accordance with the method of the first aspect of the present disclosure, in order to determine whether or not to administer one or more suitable medicaments to the subject. Where the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious, one or more suitable medicaments may be administered to the subject. For example, a physician may administer the one or more suitable medicaments to the subject. Alternatively, the subject may self-administer the one or more suitable medicaments.

In some embodiments, therefore, the method of the first aspect of the present disclosure may further comprise administering one or more medicinal products to the subject prophylactically, if the subject is assessed to be at risk of developing severe symptoms and/or becoming contagious.

Suitable medicaments may include one or more immunomodulators; antiviral agents (such as oseltamivir); antibiotics; and other drugs, such as those disclosed herein.

Implementation of the method of the first aspect of the present disclosure may, in some embodiments, be triggered by: an outbreak in the ward, or in one or more nearby wards; the onset of a period of risk, for example the ‘flu season’ and/or winter (notably, the ‘flu season’

and winter may, or may not, overlap); exposure to another subject who is known to be infected with a respiratory virus; and very early symptoms suggesting infection with a respiratory virus. The method may thus provide an advance 'early warning system' to assess the risk of severity and/or contagiousness. A subject (patient) may have regular scheduled assessments over time. For example, a subject may be assessed at regular scheduled intervals during the 'flu season', during winter, and/or when there is or has recently been an outbreak in the hospital, or the surrounding local area. Successive scheduled assessments may be separated by a time interval of at least about 12 hours; for example at least about 24 hours; at least about 36 hours; or at least about 48 hours.

It will be appreciated that a proportion of the population may have mild (even asymptomatic) infections. Treating everyone having an infection, including those subjects assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious, may mean exposing subjects unnecessarily to drugs with side effects. This may be especially undesirable, for example, for subject groups that are more susceptible to side effects, such as may be the case amongst patients who are resident in hospital (particularly patients who may be resident in the hospital for reasons other than viral infection) and/or infants (i.e. younger than one year old) children (i.e. younger than ten years old) elderly subjects (i.e. 65 years old or more) and pregnant women.

It will be appreciated that, rather than being a patient who is resident in a hospital, the subject may in some embodiments be a resident of a care home. Care homes may accommodate elderly (i.e. 65 years old or more) and/or frail subjects. In some embodiments, these subjects (residents) may be at high risk of morbidity and mortality as a result of viral infection, or secondary infections associated with a primary viral infection. In some embodiments, one or more underlying comorbidities, for example those mentioned above, may predispose such subjects (residents) to severe viral infection. It will be appreciated that features described in relation to a patient who is resident in a hospital may apply in relation to a resident of a care home and *vice versa*.

In some embodiments, the method of the first aspect of the present disclosure may comprise assessing the effect of a given treatment, for example a medicament, on the recovery of a subject from infection with a respiratory virus. It may be preferable to determine whether a subject may develop a (potentially severe) 'rebound' infection, or make a (full) recovery. This may sometimes be especially preferable where the subject is a child (of younger than ten years old) an infant (i.e. younger than one year old) or elderly (i.e. 65 years old or more) or a pregnant

woman. It may also sometimes be especially preferable where one or more underlying comorbidities predispose the subject to severe viral infection.

In embodiments where a subject has been treated successfully, for example with a medicament, the assessment may be that the subject is not or is no longer at risk of developing severe symptoms of disease and/or becoming contagious.

In embodiments where treatment has not (yet) succeeded, the assessment may be that the subject remains at risk of developing severe symptoms of disease and/or becoming contagious. This may form the basis for a clinical decision to begin a new treatment, or an escalation of treatment until the assessment is that the subject is not at risk of developing severe symptoms of disease and/or becoming contagious. In some embodiments, therefore, the subject may be treated, for example administered one or more suitable medicaments, for example those disclosed herein, until the assessment is that the subject is no longer at risk of developing severe symptoms of disease and/or becoming contagious. In some embodiments, the subject may be treated with a suitable medicament to which the subject is naïve (i.e. which the subject has not previously received as treatment for the present infection); for example one or more suitable medicaments as disclosed herein, and/or their treatment with one or more suitable medicaments (which the subject has already received, or been receiving, as treatment for the present infection) may be escalated (for example, the frequency of dosing may be increased, and/or the dose may be increased) until the subject is assessed to no longer be at risk of developing severe symptoms of disease and/or becoming contagious. In some embodiments, a physician may administer the one or more suitable medicaments to the subject. Alternatively, the subject may self-administer the one or more suitable medicaments.

In some embodiments, the subject may have a clinical management plan which is under review by a physician. The physician may be considering whether one or more changes should be made to a clinical management plan of the subject, for example other than adapting medication. In some embodiments, the physician may be considering whether the patient should be monitored more or less frequently over time; and/or whether the patient should enter, remain in, or leave quarantine.

Where the subject is assessed as being at risk of developing severe symptoms of disease and/or becoming contagious, the clinical management plan of the subject may be adapted in

accordance with the present disclosure, such that the subject is monitored more frequently, and/or such that the subject remains in, or enters, quarantine.

Where the subject is assessed as not being at risk of developing severe symptoms of disease and/or becoming contagious, the clinical management plan of the subject may be adapted in accordance with the present disclosure, such that the subject is monitored less frequently, and/or such that the subject leaves, or does not enter, quarantine.

In some embodiments, the subject may be a person, such as an outpatient, presenting in a primary care or outpatient facility. A physician in such a facility may use the method of the present disclosure to anticipate progression to severe illness, possibly involving referral to hospital for treatment.

By "having severe symptoms of disease" herein may be meant that a person requires hospitalisation, and optionally referral to a hospital high dependency unit, or even an intensive care unit. This may be, for example, the view of one or more than one physician examining the person. A person may be diagnosed (for example, by examination by one or more than one physician) to have severe symptoms of disease based on one or more of their respiratory rate, blood oxygen level, and chest radiograph (chest X-ray or CXR).

In some embodiments, severe symptoms of disease may be symptoms or signs indicative of lower respiratory tract disease and/or lower respiratory tract inflammation and/or hypercytokinemia (e.g. lung or systemic), especially in response to infection with a seasonal strain (e.g. H3N2, H1N1), as opposed to a highly pathogenic strain (e.g. H5N1). They may comprise one or more of: tachypnoea, hypoxemia, an arterial oxygen saturation of $\leq 92\%$ on room air by a transcutaneous method and radiological pulmonary infiltrates.

Further or alternatively, severe symptoms of disease may be distinct from clinically tolerable symptoms and signs such, for example, as nasal congestion, sneezing, rhinorrhoea, pyrexia (fever) and cough and sputum production, from which a patient normally recovers naturally without the need for therapeutic intervention.

However, it will be appreciated that different persons with severe symptoms may present with a wide range of different severe symptoms of disease, and may additionally present with more clinically tolerable symptoms.

In some embodiments, severe symptoms of disease may accordingly comprise tachypnoea (respiratory rate ≥ 30 for ages ≥ 12 years, rate ≥ 40 for ages 6 to 12 years, rate ≥ 45 for ages 3 to 6 years, rate ≥ 50 for ages 1 to 3 years).

In some embodiments, the person may have or show signs of discomfort with breathing or dyspnoea (unable to speak full sentences, appear breathless, using accessory respiratory muscles).

Further, or alternatively, severe symptoms may comprise abnormal levels of fatigue and/or lethargy.

Further or alternatively, severe symptoms of disease may comprise hypoxemia and/or cardiopulmonary insufficiency. In some embodiments, the person may have an arterial oxygen saturation of ≤ 92% on room air by a transcutaneous method. Typically, hypoxemia or cardiopulmonary insufficiency may comprehend one or more of dyspnoea, tachypnoea, cyanosis, low blood pressure (designated as below normal range for age and sex) and tachycardia. In some cases, hypoxemia and/or cardiopulmonary insufficiency may be such as to require the administration of supplementary oxygen, such, for example, as by means of a ventilator, or by means of extracorporeal membrane oxygenation.

Further, or alternatively, severe symptoms of disease may comprise the presence of radiological pulmonary infiltrate; for example as determined by chest radiograph (chest X-ray or CXR).

Accordingly, in particular embodiments, severe symptoms of disease may comprise tachypnoea, an arterial oxygen saturation of ≤ 92% on room air by a transcutaneous method, and radiological pulmonary infiltrates.

In some embodiments, a person with severe symptoms of disease may have significantly higher absolute neutrophil counts than a person with mild or moderate symptoms of disease. Typically, a person with severe symptoms may have a neutrophil count in the range 2.1-24.5 x 103 /µl (as compared with a person with moderate symptoms, who may have a neutrophil count in the range 0.62-10.88 x 103 /µl or a person with mild symptoms of disease, who may have a neutrophil count in the range 0.5-6.5 x 10^3 /µl). In some embodiments, the absolute platelet count may be significantly lower in persons with severe symptoms of disease, e.g. 27-250 x 103 /µl (as compared with a person with moderate symptoms, who may have a

platelet count in the range 55-345 x 103 /μl or a person with mild symptoms, who may have a platelet count in the range 79-370 x 103 /μl).

A person having severe symptoms of disease may, in some embodiments, have one or more secondary infections (for example, one or more bacterial infections) associated with a primary viral infection.

In some embodiments, implementation of the method of the first aspect of the present disclosure may be triggered by: the subject (e.g. an outpatient) being considered by a physician to be at risk of severe infection, for example where the subject (e.g. outpatient) is an infant (i.e. younger than one year old); elderly (i.e. 65 years old or more); a pregnant woman; and/or where the subject has one or more underlying comorbidities predispose the subject to severe viral infection.

In some embodiments, for example by way of precaution, an otherwise healthy subject (e.g. outpatient) may be assessed using the method of the present disclosure. This may comprehend an inclusionary approach to assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus.

In some embodiments, where the subject (e.g. outpatient) is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious, one or more suitable medicaments disclosed herein may be administered to the subject. In some embodiments, a physician may administer one or more suitable medicaments to the subject (e.g. outpatient). Alternatively, the subject may self-administer one or more suitable medicaments.

Additionally or alternatively, in embodiments where the subject (e.g. outpatient) is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious, the subject may be referred to hospital.

In some embodiments, the subject may be military personnel. It will be appreciated that in a military setting, maintaining the health of subjects (personnel) may be especially preferable, to enable proper execution of their duties. As soon as infection or symptoms are suspected, a

subject may, in some embodiments, be assessed and optionally treated and/or managed in an appropriate way to limit the impact of infection on the subject and/or their close contacts.

Where the subject (personnel) is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious, one or more suitable medicaments disclosed herein may be administered to the subject. In some embodiments, a physician may administer one or more suitable medicaments to the subject. Alternatively, the subject may self-administer one or more suitable medicaments. Additionally or alternatively, where the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious, the subject may be referred to hospital.

Implementation of the method of the present disclosure may, in some embodiments, be triggered by: outbreaks in the surrounding local area; the onset of a period of risk, for example the 'flu season' and/or winter; exposure to another subject who is known to be infected with a respiratory virus; and very early symptoms suggesting infection with a respiratory virus.

In some embodiments, a subject (personnel) may have regular scheduled assessments over time. For example, a subject may be assessed at regular scheduled intervals during the 'flu season', during winter, and/or when there is or has recently been an outbreak in the surrounding local area. Successive scheduled assessments may be separated by a time interval of at least about 12 hours; for example at least about 24 hours; at least about 36 hours; or at least about 48 hours. It will be appreciated that the risk of viral infection may be higher in military settings such as training camps, ships and aircraft, so in these settings the performance of assessments at regular scheduled time intervals may be particularly appropriate.

Identifying subjects assessed to be at risk of developing severe symptoms of disease and/or becoming contagious, and initiating early treatment and/or management, may reduce both the impact of the disease on the subject, and their risk of transmitting it to other persons. It may sometimes be preferable to be able to avoid moving personnel about to develop severe symptoms of disease and/or become contagious into a high risk area (such as an area with a civilian population, and/or an area of active combat).

It will be appreciated that the assessment of whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, may in some embodiments assist with a decision to quarantine a subject away

from other personnel, to reduce the impact of an outbreak, e.g. in training camps or on board ships.

It will also be appreciated that the considerations disclosed herein with reference to military personnel apply in similar environments, for example amongst sports teams, restaurant or other hospitality staff, factory workers, building site staff, and aeroplane or ship crew.

In some embodiments, one or more (for example all) steps of the method of the present disclosure may be carried out at home. In a home environment, subjects may be assessed promptly if they have been exposed, or possibly exposed, to a respiratory virus.

Implementation of one or more steps of the method of the present disclosure in a home environment may, in some embodiments, be triggered by: outbreaks in the surrounding local area; the onset of a period of risk, for example the 'flu season' and/or winter; exposure to another subject who is known to be infected with a respiratory virus; and very early symptoms suggesting infection with a respiratory virus. A subject may have regular scheduled assessments over time. For example, a subject may be assessed at regular scheduled intervals during the 'flu season', during winter, and/or when there is or has recently been an outbreak in the surrounding local area. Successive assessments may be separated by a time interval of at least about 12 hours; for example at least about 24 hours; at least about 36 hours; or at least about 48 hours.

Subjects for whom at-home assessment may be particularly appropriate include subjects already considered by a physician to be at risk of severe infection and/or contagiousness; for example where the subject has one or more underlying comorbidities, such as those disclosed herein, that predispose the subject to severe viral infection. By way of example, at-home assessment may sometimes be particularly appropriate where the subject is a pregnant woman, or where the subject is elderly (i.e. 65 years old or more); a child (of younger than ten years old); or an infant (of younger than one year old).

For children and infants, notably, the risk of viral infection may cause particular concern for parents or guardians. It may therefore be useful to assess whether a child or infant is at risk of developing severe symptoms of disease and/or becoming contagious by implementing a method disclosed herein, thus identifying a confident threshold of seriousness before the child

or infant should be treated with one or more medicaments disclosed herein, and/or referred to hospital.

In some embodiments, one or more (for example all) steps of the method of the present disclosure may be carried out in a home environment for an otherwise-healthy subject. While some viral infections may resolve without treatment, in other instances the condition of a subject may deteriorate, sometimes rapidly. If a risk of deterioration is suspected, at-home assessment may provide additional data to inform a decision on whether to present at a primary care facility, or hospital. By way of example, in embodiments wherein the subject is a health service employee then, having performed the assessment at home, they could seek treatment as soon as possible, and thereby return to work more quickly. This may reduce the impact of absence from work of health service employees or certain other staff during the 'flu season', and/or during winter, when health service employees and certain other staff are in greater demand. For this reason, a subject such as a health service employee may have regular scheduled assessments over time. In some embodiments, a subject may be assessed at regular scheduled intervals during the 'flu season', during winter, and/or when there is or has recently been an outbreak in the surrounding local area. Successive assessments may be separated by a time interval of at least about 12 hours; for example at least about 24 hours; at least about 36 hours; or at least about 48 hours.

Where the members of a household include one or more at-risk member(s) (for example, having one or more underlying comorbidities leading to a predisposition to severe viral infection) then a household member assessed, by a method disclosed herein, to be at risk of developing severe symptoms of disease and/or becoming contagious, may reduce the likelihood of transmitting the viral infection to the at-risk member(s) by seeking early treatment. In some embodiments, one or more suitable medicaments disclosed herein may be administered to the household member assessed to be at risk of developing severe symptoms of disease and/or becoming contagious. For example, a physician may administer the one or more suitable medicaments. Thus, in some embodiments, the household member may self-administer the one or more suitable medicaments. Additionally or alternatively, where the household member is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious, they may be referred to hospital.

In some embodiments, the method of the first aspect of the present disclosure may comprise assessing which participants of a study on viral transmission are at risk of becoming contagious.

These subjects (study participants) may be under review for supervised transmission to volunteers, and/or selection for aerosol generation analysis.

In some embodiments, where the subject (study participant) is assessed as being at risk of becoming contagious, the subject may be managed for supervised transmission to volunteers, and/or selected for aerosol generation analysis.

In some embodiments, where the subject is assessed as not being at risk of becoming contagious, the subject (study participant) may not be managed for transmission to volunteers, and/or may therefore not be selected for aerosol generation analysis.

In a third aspect of the present disclosure, therefore, there is provided a method of conducting a clinical trial or field study, comprising:

(a) measuring levels of expression of SPATS2L in biological samples obtained from a plurality of subjects;

(b) analysing the measured level of expression of SPATS2L in each sample to assess whether a respective subject is at risk of developing severe symptoms of disease and/or becoming contagious; and

(c) including or excluding a subject who is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious in the clinical trial or field study or in a subgroup of the clinical trial or field study.

Suitably, the levels of expression of SPATS2L in the biological samples may be measured by RNA transcriptomics analysis using one or more oligonucleotide probes specific for SPATS2L which are immobilised on a surface (e.g. a microarray) and labelling the transcripts with a detectable label, or by RNA-seq in which transcripts in the samples are converted to cDNA, fragmented, provided with suitable adapters to facilitate amplification and sequencing and then sequenced.

In some embodiments, a subject who is included in the clinical trial or field study, or in the subgroup of the clinical trial or field study, may be administered one or more medicinal products; for example an investigational medicament.

In some embodiments, all subjects included in the clinical trial or field study may be administered one or more medicinal products; for example an investigational medicament. The

response of all subjects to the medicinal product may be analysed, and the response of those included in the subgroup may be compared to those of subjects excluded from the subgroup.

Suitably, the methods of the present disclosure may be carried out using one or more computers. For example, a computer may receive input data representing a measured level of expression of SPATS2L in a biological sample obtained from a subject. The same computer may, for example, analyse the data to assess the likelihood that the subject will develop severe symptoms of disease and/or become contagious; or, alternatively, may transmit that data to a further computer for analysis. An output may then be generated by the first or further computer, or by a yet further computer, representing the likelihood of the subject developing severe symptoms of disease and/or becoming contagious.

In a fourth aspect of the present disclosure, therefore, there is provided a computer-implemented method of assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, comprising:

(a) receiving by a computer input data representing a measured level of expression of SPATS2L in a biological sample obtained from the subject;

(b) processing by a computer the input data to analyse the level of expression to assess the likelihood that the subject will develop severe symptoms of disease and/or become contagious; and

(c) outputting by a computer output data representing the likelihood of the subject developing severe symptoms of disease and/or becoming contagious.

Suitably, the level of expression of SPATS2L in the biological sample may be measured by RNA transcriptomics analysis using one or more oligonucleotide probes specific for SPATS2L mRNA transcripts which are immobilised on a surface (e.g. a microarray) and labelling the transcripts with a detectable label, or by RNA-seq in which SPATS2L mRNA transcripts in the sample are converted to cDNA, fragmented, provided with suitable adapters to facilitate amplification and sequencing and then sequenced.

In some embodiments, said outputting step (c) comprises displaying the output data on a display in a form which can be understood by a human being, or transmitting the output data to a remote computer.

In a fifth aspect of the present disclosure, therefore, there is provided a computer program comprising instructions which, when carried out by a computer, cause the computer to carry out step (b) of the method of the first, second or third aspect of the present disclosure.

In a sixth aspect of the present disclosure, there is provided a method of providing electronic data representing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, comprising:

(a) measuring a level of expression of SPATS2L in a biological sample obtained from the subject;

(b) receiving by a computer the measured level of expression and encoding by the computer the measured level of expression in computer-readable form, and

(c) transmitting by a computer the encoded measured level of expression to a remote computer for evaluation in accordance with a method of the third aspect of the present disclosure.

In a seventh aspect of the present disclosure, there is provided a computer network comprising:

(a) at least one server;

(b) at least one other computing device; and

(c) a data communications link between the server and the other device;

wherein the at least one other device is configured to transmit data to the at least one server, the data comprising the level of expression of SPATS2L in a biological sample obtained from a subject; and the at least one server is configured to analyse the level of expression of SPATS2L to assess whether the subject is at risk of developing severe symptoms of disease and/or becoming contagious by executing a method of the third aspect of the present disclosure.

In some embodiments, the at least one server may be configured to analyse the level of expression of SPATS2L before relaying a result to the other device, the result concerning whether the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

In some embodiments, the network may further comprise at least one further computing device, wherein the at least one server is configured to analyse the level of expression of SPATS2L before relaying a result to the at least one further computing device (the result concerning whether the subject is assessed to be at risk of developing severe symptoms of

disease and/or becoming contagious) for example for display by the at least one additional computing device. The at least one additional computing device may be a tablet, laptop, mobile phone, or the like.

In an eighth aspect of the present disclosure, there is provided a kit for assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, the kit comprising at least one reagent that allows quantitation of a SPATS2L oligonucleotide.

Suitably, the at least one reagent may comprise a probe or primer specific for SPATS2L mRNA transcripts, which enables quantification of the amount of SPAT2L mRNA transcripts in the same by microarray or RNA-seq transcriptomics techniques. Thus, in some embodiments, the kit may comprise, in separate containers but packaged together, an oligonucleotide which is the reagent that allows quantitation of a SPATS2L oligonucleotide, and which hybridises to a SPATS2L oligonucleotide (e.g., mRNA encoded by SPATS2L) and further reagents, such as reagents for performing a PCR-based quantitative assay, such as RT-qPCR.

In some embodiments, the kit may comprise molecular standards and/or positive and/or negative control formulations for the oligonucleotides to be screened.

In some embodiments, the kit may comprise suitable primers for amplification of mRNA encoded by SPATS2L. A person of ordinary skill in the art knows how to design primers for amplifying a nucleic acid of interest, as well as oligonucleotide probes for sequence-specific hybridisation to a desired nucleic acid. By "sequence-specific hybridization" herein is meant that the probe(s) preferentially bind to a nucleic acid sequence encoding a target nucleic acid. Sequence-specific hybridization, if present, is detected using any suitable detection method, for example detection of fluorescence by a fluorescent moiety, as will be familiar to those skilled in the art.

In some embodiments, the kit may comprise buffers, enzymes, enzyme substrates, wash reagents, and the like, packaged together or in separate containers.

In embodiments where a solid support comprising one or more probes (e.g., an array) is employed, the solid support (e.g. array) may comprise a plurality of oligonucleotide probes coupled to a surface of a substrate (e.g., plastic, complex carbohydrate, or acrylic resin) in different known locations. A person of ordinary skill in the art knows how to produce solid

supports (e.g. arrays). The location of probes specific to a particular gene transcript may be catalogued, hybridization to the immobilized probe may be detected (e.g. by detection of fluorescence, as disclosed herein) and the polynucleotide may be identified by the location of the hybridization on the array.

Thus, in some embodiments, the kit may comprise a microarray, the microarray comprising an oligonucleotide which is the reagent that allows detection of a SPATS2L oligonucleotide, and which hybridises to a SPATS2L oligonucleotide.

In some embodiments, the kit may comprise instructions for assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus.

In some embodiments, the kit may comprise one or more tools for collecting a biological sample, such as a blood sample collection tube or nasopharyngeal swab.

Further, it will be appreciated that analysis can be carried out in a variety of physical formats, and the format of the kit will depend on the setting of use. For example, assay plates, large arrays, or bead arrays may be suitable for processing large numbers of biological samples. Alternatively, single sample formats, and/or formats such as Loop Mediated Isothermal Amplification- (LAMP-) based PCR, may be suitable for point of care settings, home settings, and/or testing a small number of biological samples.

It will be appreciated that features described in relation to one aspect of the present disclosure, may be incorporated into any other aspect of the present disclosure, and *vice versa*.

Description of the Drawings

Embodiments of the present disclosure will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a visual analogue scale (VAS) subject self-reported symptom diary card, as used in Example 1 below;

Figure 2 is a plot of the mean SPATS2L expression level over time (from before inoculation, at day -1, to day 28 after inoculation) for 25 subjects, 9 of whom were classified in accordance with the present disclosure to be at risk (line labelled (1)); and 16 of whom were

classified as not at risk (line labelled (2)) of developing severe symptoms of disease and/or becoming contagious;

Figure 3 is a ROC (receiver operating characteristic) curve for RNA probe set 241812_at (SEQ ID NO: 1) in SPATS2L at 45 hours, with no adjustment for baseline;

Figure 4 is a ROC curve for RNA probe set 241812_at (SEQ ID NO: 1) in SPATS2L at 45 hours, minus the expression level at baseline;

Figure 5 is a flow chart showing how a subject may be processed through a typical decision process, as in Example 2 below; and

Figure 6 is a schematic drawing showing a network comprising a device configured to transmit data to a server; optionally, the network further comprises an additional device.

Examples

Example 1 –Test thresholds for SPATS2L

The present example investigates the use of the expression level of SPATS2L in humans to assess whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus. The example particularly investigates the impact of varying a reference "threshold" SPATS2L expression level (i.e. the SPATS2L expression level above which a subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious) both (i) with and (ii) without adjusting for a "baseline" level of expression. In the present example, the "baseline" level of expression of SPATS2L is the level of expression of SPATS2L in a biological sample obtained from the subject prior to inoculation of the subject with a respiratory virus. It will be appreciated that in other implementations, a baseline level of expression of SPATS2L may be the level of expression of SPATS2L in a biological sample obtained from an uninfected control subject. Where change from baseline is measured, the threshold may be a threshold change from baseline.

Table 2 below lists definitions of terms used in determining the utility of a selected threshold SPATS2L expression level. In determining the utility of a threshold, a decision must be made to maximise either true positives (for example, by maximising PPV) or true negatives (for example, by maximising NPV). Only a perfect test would allow simultaneous

maximisation of both of these. It is noted that as the prevalence of a condition of interest varies, PPV and NPV may also vary.

Table 2: Definitions

Term	Definition
Case	A subject who goes on to be severe/contagious
Control	A subject who does not go on to be severe/contagious
True positive	A positive obtained for a case
True negative	A negative obtained for a control
Sensitivity *	Probability that a case receives a positive result
Specificity *	Probability that a control receives a negative result
Positive Predictive Value (PPV) *	Probability that a positive result denotes a case
Negative Predictive Value (NPV) *	Probability that a negative result denotes a control
Accuracy *	(true positives + true negatives)/(cases + controls)

* Measures of test performance.

Procedure

After completing standard influenza human viral challenge screening assessments and meeting standard influenza human viral challenge eligibility criteria, in addition to having no detectable or low levels of pre-existing serological antibodies against GMP challenge virus strain A/Perth/16/2009 H3N2 (hVIVO, Queen Mary Bioenterprises Innovation Centre, 42 New Road, London, E1 2AX, UK) as determined by hemagglutination inhibition assay. 56 subjects entered quarantine 1 to 2 days prior to inoculation. During this time, baseline samples were collected.

Subjects were inoculated intranasally with the GMP challenge virus via pipette. Virus inoculation day was termed "day 0" and the subjects were monitored closely from day 1 to day 8, when they were discharged from quarantine. Subjects returned for a follow-up visit on day 15 (± 1) and a final discharge visit on day 28 (± 3).

Subjects were monitored by completion of Visual Analogue Score (VAS) diary cards three times daily for self-reported symptoms. A copy of a VAS diary card used in the present Example is shown in **Figure 1** of the accompanying drawings, but it will be understood that the precise layout/format of the scorecard is immaterial, and different presentations may be used to collect the same or similar information. As shown in **Figure 1**, subjects reported signs and symptoms including: runny nose, stuffy nose, sneezing, sore throat, earache, malaise

(tiredness), headache, muscle and/or joint ache, chilliness/feverishness, cough, chest tightness, shortness of breath, and wheeze, assessed on a 100 mm scale of from "not at all bothered" to "severely bothered." A subject's mark along the 100 mm scale was measured to the closest mm by clinic staff (giving a symptom score for each symptom of from 0 to 100).

Analysis for viral shedding was conducted by RT-qPCR from nasopharyngeal swabs (NPS) collected several (e.g. two or three) times daily. NPS sample collection commenced on day 1 post-inoculation, as it is thought that sampling earlier may interfere with the establishment of infection post-inoculation. NPS samples were collected throughout the duration of the quarantine phase and at follow up visits, and were processed by centrifugation, followed by separation of cell pellets from the supernatant. The supernatant was used for RT-qPCR viral load assessments, in accordance with standard procedures in the art and in line with US Food and Drug Administration Good Clinical Practice and Clinical Trials Regulations.

Viral transmission often occurs through the spread of virions (intact whole viral particles) in droplets via coughing, sneezing or even breathing. An increase in viral load, and thus in viral shedding, may therefore increase the quantity of virions in droplets spread by a given subject, and is likely to increase the contagiousness of a subject. Likewise, any signs of infection that could increase the spread of droplets (e.g. by expulsion of air through the nose or mouth) would also increase the chances of being contagious. In the present example, subjects were defined as being contagious if they entered the top tertile for peak viral load (based on qPCR titre) during the period of the study after inoculation and/or the top tertile for a peak combination of five cold-like symptoms and signs (sneezing, cough, runny nose, stuffy nose and sore throat)during the period of the study after inoculation.

For the purposes of this analysis, subjects were similarly defined as having, or being at risk of developing, severe signs of disease if they entered the top tertile for a combination of five self-reported cold-like symptoms (sneezing, cough, runny nose, stuffy nose and sore throat) during the period of the study after inoculation and/or the top tertile for peak viral load during the period of the study after inoculation (based on qPCR titre).

Of the study participants, 15 individuals were thus classified as contagious/ severe after inoculation, whereas 41 individuals were classified as non-contagious/ non-severe/ uninfected after inoculation.

Blood samples were collected in Paxgene RNA tubes (PreAnalytiX GmbH, Feldbachstrasse, CH-8634, Hombrechtikon, Switzerland) twice daily. Automated RNA extraction using QIAsymphony PAXgene Blood RNA Kit (PreAnalytiX GmbH) was performed on the collected blood samples. RNA concentration and purity were measured using Nanodrop™ spectrophotometric (ThermoFisher Scientific, 168 Third Avenue, Waltham, MA 02451, USA) quality control and Bioanalyzer (Agilent, Unit 610 Wharfedale Road, Wokingham, RG41 5TP, UK) quality control were performed to ensure integrity of the extracted RNA sample. A minimum of 1.5 μg RNA at a minimum concentration of 250ng/μl was required for samples to proceed to downstream analysis. NuGEN Ovation® RNA Amplification System V2 with Ovation® Whole Blood Solution (both Tecan, Seestrasse 103, 8708 Männedorf, Switzerland) was used for Microarray processing of the RNA samples which passed the quality control criteria. RNA transcriptomics analysis was performed by Almac Diagnostics using the GeneChip™ Human Genome U133 Plus 2.0 Array (Affymetrix, Voyager Mercury Park, Wycombe Lane, High Wycombe, HP10 0HH, UK).

Those skilled in the art will know how to prepare suitable target-specific PCR primers for SPATS2L mRNA; for example using an *in silico* platform such, for example, as Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast. Ye, J., Coulouris, G., Zaretskaya, I. et al. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13, 134 (2012). https://doi.org/10.1186/1471-2105-13-134).

The probe set used for transcriptomics analysis was 241812_at, which has a target consensus sequence as disclosed by SEQ ID NO: 1 which is based on 14 reference sequences for SPATS2L and comprises 11 probes as follows:

SEQ ID NO	Probe Sequence (5'-3')	Probe X	Probe Y	Probe Interrogation Position	Target Strandedness
2	GAGTGGGAACGTATTCTTCCCCTGA	999	651	74	Antisense
3	TCCCCTGAAGTACTGCCATCAAGCA	1108	987	91	Antisense
4	AAGCAGTCCCGCTAAAGTCAAGCTG	545	249	111	Antisense
5	GGCTTGAGAGTGGGTCTTGGCCAGG	212	879	313	Antisense
6	CTCTCAGTCTCTACAGAATGTCTCA	778	381	381	Antisense
7	TAGAGGATCATCATTACTCTGCCCC	271	1069	419	Antisense
8	GCTTCTGTTTTATTCTGCTATAGTT	712	517	446	Antisense
9	TGTCTTACACTTCTGTAACCTCTCT	846	923	515	Antisense
10	GTAACCTCTCTTTCAATATCATTTT	529	739	529	Antisense
11	AACAGTCCACTATTCTTTTAGGATT	797	229	573	Antisense
12	AATAATTTTATCTGCCTGACCTCAT	489	281	598	Antisense

It will be appreciated that in different embodiments of the disclosure other suitable probes and primers may be used.

Final data were supplied in the form of CEL files. Analysis-ready data were obtained via normalisation to correct for background, the removal of outliers, log-transformation, and correcting for batch effects. The analysis below is based upon processed RNA data.

The data set obtained for each subject thus comprised: (i) a classification as to whether they have, or are at risk of developing, severe signs of disease; and/or are, or are at risk of becoming, contagious; (ii) their measured baseline, pre-exposure SPATS2L expression level; and (iii) their measured SPATS2L expression levels at regular time intervals following exposure to virus.

As shown in **Figure 2**, it was found that measured SPATS2L expression levels may begin to be elevated shortly after inoculation (such as by 30 hours after inoculation) in subjects who have, or are at risk of developing, severe signs of disease; and/or are, or are at risk of

becoming, contagious. Measured SPATS2L expression levels may remain high for up to six or seven days after inoculation. The mean measured SPATS2L expression level over time for these subjects is shown by the line labelled (1). Meanwhile, the mean measured SPATS2L expression level over time for subjects not having, or not at risk of developing, severe signs of disease; and/or not being, or not at risk of becoming, contagious, is shown by the line labelled (2).

The following analysis was carried out on SPATS2L expression levels measured in samples obtained 45 hours after inoculation.

No adjustment for baseline (absolute values)

Figure 3 shows a receiver operating characteristic (ROC) curve for the RNA probe set 241812_at (SEQ ID NO: 1) in SPATS2L in samples obtained 45 hours after inoculation, with no adjustment for baseline (i.e., based on absolute values for the measured SPATS2L expression level). This demonstrates the impact of varying a reference "threshold" SPATS2L expression level (i.e. the SPATS2L expression level above which a subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious) without adjusting for a reference measured "baseline" level of expression of SPATS2L. The ROC curve displays the probe sensitivity versus (1 – probe specificity) at different reference thresholds for the reference measured level of expression of SPATS2L.

Those skilled in the art will appreciate that a perfect test, with a sensitivity of 1.0 (no false negatives) and a specificity of 1.0 (no false positives) would have an area under the ROC curve (AUC) of 1.0. A test of no predictive value would have an AUC of 0.5.

The area under the curve (AUC) of **Figure 3** is 0.74, indicating good predictive value for the expression level of the SPATS2L gene.

Table 3 below shows the test performance measures arising from the application of various thresholds for the level of expression of SPATS2L (column 1) at 45 hours after inoculation.

Table 3: Test performance measures for 241812_at (SEQ ID NO: 1) (no adjustment for baseline)

RNA Expression	Percentile	Sensitivity	Specificity	Accuracy	PPV	NPV
7.24	90	0.20	0.93	0.73	0.50	0.76

5.55	80	0.27	0.80	0.66	0.33	0.75
5.16	70	0.53	0.78	0.71	0.47	0.82
4.91	60	0.67	0.68	0.68	0.43	0.85
4.77	50	0.80	0.59	0.64	0.41	0.89
4.62	40	0.87	0.49	0.59	0.38	0.91
4.46	30	0.93	0.37	0.52	0.35	0.94
4.29	20	1.00	0.27	0.46	0.33	1.00
4.14	10	1.00	0.12	0.36	0.29	1.00

Additionally, **Figure 3** illustrates, by way of example, that at 45 hours after inoculation, for a threshold level of expression of SPATS2L of 7.59, the sensitivity is 0.20, the specificity is 0.98, and the accuracy is 0.77.

Change from baseline

Figure 4 shows a ROC curve for RNA probe 241812_at (SEQ ID NO: 1) for SPATS2L in samples obtained 45 hours after inoculation, minus expression level at baseline (i.e. prior to inoculation of the subject with GMP challenge virus strain A/Perth/16/2009). These data have undergone standard processing steps for Affymetrix RNA data (see 'Procedure' above). It is noted that a slight improvement in performance is seen as a result of the adjustment for baseline, in that the AUC is increased from 0.74 in **Figure 3** to 0.78 in **Figure 4**.

Table 4 below shows the test performance measures arising from the application of various thresholds for the change in the level of expression of SPATS2L (column 1) at 45 hours after inoculation.

Table 4: Test performance measures for 241812_at (SEQ ID NO: 1) (change from baseline)

Change from baseline	Percentile	Sensitivity	Specificity	Accuracy	PPV	NPV
2.62	90	0.20	0.93	0.73	0.50	0.76
0.85	80	0.40	0.85	0.73	0.50	0.80
0.71	70	0.60	0.80	0.75	0.53	0.85
0.36	60	0.73	0.71	0.71	0.48	0.88
0.24	50	0.87	0.61	0.68	0.45	0.93
0.07	40	0.93	0.51	0.62	0.41	0.95
-0.01	30	0.93	0.37	0.52	0.35	0.94
-0.17	20	1.00	0.27	0.46	0.33	1.00
-0.55	10	1.00	0.12	0.36	0.29	1.00

As indicated in **Figure 4**, by way of example, at 45 hours after inoculation, for a threshold change in the level of expression of SPATS2L of 3.37, the sensitivity is 0.20, the specificity is 1.00, and the accuracy is 0.79.

Example 2 - Identifying which patients will become severe and/or contagious in a clinical study

In designing a clinical study, it may sometimes be useful to be able to identify pre-emptively study participants who are at risk of developing severe symptoms of disease and/or becoming contagious, thereby allowing selective dosing of these subjects with an investigational or licensed medicament (drug/vaccine) or placebo at the earliest opportunity. This may improve the ability to detect a clinically relevant reduction in disease in response to the medicament by only evaluating the effects of the medicament in individuals who will/would have developed severe symptoms of disease and/or become contagious. This may also reduce unnecessary exposure of subjects to an investigational medicament, which may have unknown and/or unpleasant side effects. This may also reduce the amount of medicament required.

In the present example, subjects are screened for eligibility for the evaluation of efficacy of an investigational medicament in a clinical study. Eligible subjects arrive at the clinic and blood samples and symptom scores are taken at a known time (e.g. in accordance with the procedure of Example 1 above) before subjects are exposed to virus in accordance with the study protocol (e.g. by inoculation, such as inoculation with GMP challenge virus strain A/Perth/16/2009 H3N2, see Example 1). Baseline values for the level of expression of SPATS2L are measured in the pre-exposure blood samples (e.g. in accordance with the procedure of Example 1).

Blood samples are taken regularly at specific time-points after virus exposure (e.g. twice, three times a day, or more often) alongside symptom scores. Values for the level of expression of SPATS2L are measured in the blood samples (e.g. in accordance with the procedure of Example 1). The clinical study operator may refer to the absolute value of SPATS2L at a given time-point, or alternatively the measured baseline SPATS2L expression level (i.e. a subject's pre-exposure SPATS2L expression level) may be subtracted from the SPATS2L expression level measured post exposure to virus for each subject.

The levels of expression of one or more house keeping genes are also measured in all blood samples as standard procedure, thereby providing a means of scaling measured SPATS2L expression values, where appropriate, as disclosed herein.

In a clinical trial or field study, a reference threshold SPATS2L expression level as disclosed herein may be selected to minimise false positives, such as by maximising PPV; for example by increasing the reference threshold SPATS2L expression level above which the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious. Alternatively, the reference threshold SPATS2L expression level may be selected to minimise false negatives, such as by maximising NPV; for example by decreasing the threshold SPATS2L expression level above which the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious. In a clinical trial, it may sometimes be especially preferred to minimise false positives. It will be appreciated that this may be useful where the investigational medicament has one or more side effects, where its side effects are unknown, and/or where the investigational medicament is expensive and/or in short supply.

Where a subject is assessed as being at risk of developing severe symptoms of disease and/or becoming contagious (by reference to the selected threshold, e.g. a threshold selected to minimise false positives, or to minimise false negatives) this may immediately trigger dosing of the subject with an investigational medicament (drug/vaccine/placebo). Medicaments could include one or more immunomodulators; antiviral agents; antibiotics; and other drugs, as disclosed herein.

Other actions that may be triggered (additionally or as an alternative to dosing with a medicament) include increasing the frequency of observations/ samples/ measurements in those assessed as being at risk of developing severe symptoms of disease and/or becoming contagious, or reducing observations/samples/measurements in those who are assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious.

Using methods of the present disclosure as part of a decision-making procedure in a clinical trial may enable the trial operator to select only those subjects who are assessed to be likely to develop severe symptoms of disease and/or become contagious for inclusion in statistical analysis of the efficacy of an investigational medicament. It will be appreciated that this may be useful where the investigational medicament has one or more side effects, where its side effects are unknown, and/or where the investigational medicament is expensive and/or

in short supply. Another benefit is that the analysis of the efficacy of the investigational medicament is likely to be more accurate, since it will (mostly) be used to treat those who develop severe symptoms of disease and/or become contagious, rather than all subjects (encompassing uninfected subjects and those with mild infections). It will be appreciated that the decision to minimise false positives, or minimise false negatives, may accordingly turn on these considerations.

In some instances, where a subject is assessed as not being at risk of developing severe symptoms of disease and/or becoming contagious, the subject may be dosed in any event at a predetermined time point post exposure or inoculation (e.g. day 4, 5 or 6). Such subjects may then form a further subgroup for analysis.

A typical decision process is summarised with reference to the flowchart of **Figure 5**.

The process commences by **(i)** measuring the level of expression of SPATS2L in a first biological sample obtained from a subject.

The process operator may then proceed to **(ii)** input the level of expression of SPATS2L into an algorithm, which analyses the level of expression of SPATS2L. The output is entered into a decision tree. The subject is accordingly assigned either to a group 'at risk of developing severe symptoms of disease and/or becoming contagious' or a group 'not at risk'.

Optionally, the operator may **(ia)** reassess the subject's risk, by measuring the level of expression of SPATS2L in a further biological sample (or further biological samples); for example a sample (or samples) obtained at a time interval (or intervals) after the first sample. The time interval (or intervals) may be in a range of at least about 12 hours; for example at least about 24 hours; at least about 36 hours; or at least about 48 hours. Optionally, the operator may themselves **(iv)** compare the level of expression of SPATS2L measured in the further biological sample(s) to the level of expression of SPATS2L measured in the first biological sample, before proceeding to step **(ii)**.

Where the subject is assigned to the group 'not at risk', steps **(i)** and **(ii)**, and optionally one or both of steps **(ia)** and **(iv)** may optionally be repeated.

Where the subject is assigned to the group 'at risk of developing severe symptoms of disease and/or becoming contagious', then **(v)** a medicament and/or other treatment may be

administered to the subject, and/or the subject may be quarantined. Optionally, before proceeding to step **(v)**, **(vi)** the operator may combine the assessment with additional diagnostic/clinical evidence to further assess the risk level of the subject.

Example 3 - Identifying which patients will become severe and/or contagious in a sports team

In a sports team, it may sometimes be useful to be able to identify pre-emptively subjects who are at risk of developing severe symptoms of disease and/or becoming contagious, thereby allowing quarantine of such subjects away from other members of the team, and dosing of these subjects with one or more suitable medicaments (drug/vaccine) at the earliest opportunity. It will be appreciated that in a sports team, maintaining the health of subjects may be especially preferable in view of the impact of even a minor and/or temporary deterioration in health on sporting performance. As soon as infection or symptoms are suspected, a subject may be assessed in accordance with methods of the present disclosure, and optionally treated and/or managed in an appropriate way to limit the impact of infection on the subject and/or their close contacts (e.g. other members of the team).

In the present example, blood samples and symptom scores are taken from subjects (e.g. in accordance with the procedure of Example 1) at a time when they are known to not have been exposed to virus. Baseline values for the level of expression of SPATS2L are measured in these pre-exposure blood samples (e.g. in accordance with the procedure of Example 1).

Blood samples are taken regularly at specific time-points thereafter (e.g. once a day, or more or less often). Values for the level of expression of SPATS2L are measured in the blood samples (e.g. in accordance with the procedure of Example 1). A managing physician may refer to the absolute value for the measured level of expression of SPATS2L at a given time-point for a subject, or alternatively the baseline SPATS2L expression level (i.e. a subject's pre-exposure SPATS2L expression level) may be subtracted from the measured SPATS2L expression level at a given time-point for a subject.

The levels of expression of one or more house keeping genes are also obtained from all blood samples as standard procedure, thereby providing a means of scaling SPATS2L expression values, where appropriate, as disclosed herein.

In the context of a sports team, a reference threshold SPATS2L expression level as disclosed herein may be selected to minimise false positives, such as by maximising PPV; for

example by increasing the reference threshold SPATS2L expression level above which the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious. Alternatively, the threshold SPATS2L expression level may be selected to minimise false negatives, such as by maximising NPV; for example by decreasing the reference threshold SPATS2L expression level above which the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious. In a sports team, it may sometimes be especially preferred to minimise false negatives in view of the impact of incorrectly assessing a subject to not be at risk of developing severe symptoms of disease and/or becoming contagious on the health and thus sporting performance of that subject, and/or on the health and thus sporting performance of the remaining members of the team. It will be appreciated that in a sports team, an inclusionary approach to medicating subjects may be preferable.

Where a subject is assessed as being at risk of developing severe symptoms of disease and/or becoming contagious (by reference to the selected reference threshold, e.g. a reference threshold selected to minimise false negatives) this may immediately trigger dosing of the subject with a medicament (drug/vaccine). Medicaments could include one or more immunomodulators; antiviral agents; antibiotics; and other drugs, as disclosed herein.

Other actions that may be triggered (additionally or as an alternative to dosing with a medicament) include increasing the frequency of observations, taking samples and/or measurements in those assessed as being at risk of developing severe symptoms of disease and/or becoming contagious, or reducing observations/samples/measurements in those who are assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious.

In some instances, where a subject is assessed as not being at risk of developing severe symptoms of disease and/or becoming contagious, the subject may be dosed in any event; for example dosed with a vaccine. This may be preferable where the subject is about to travel to a location having a higher prevalence of viral disease (e.g., when travelling to a sporting event).

It will be appreciated that the considerations of this example apply in similar environments, such, for example, as amongst military personnel, restaurant or other hospitality staff, factory workers, building site staff, and aeroplane or ship crew.

Example 4 – Assessing risk of severe disease/contagiousness in a subject presenting with symptoms and/or signs of infection

An individual subject presents in a primary care facility or hospital with symptoms and/or signs of viral infection, e.g. fever, sneezing, cough, runny nose, stuffy nose and/or sore throat. It may be unknown how much time has elapsed since the subject may have been exposed to a respiratory virus (or whether exposure has occurred at all). The SPATS2L expression level of the individual subject prior to exposure, or possible exposure, of that subject to the respiratory virus may also be unknown.

When the subject arrives at the primary care facility or hospital, blood samples and symptom scores are taken, e.g. in accordance with the procedure of Example 1 above. The expression level of SPATS2L is measured in the blood sample, e.g. in accordance with the procedure of Example 1.

Since the SPATS2L expression level of the individual subject prior to exposure, or possible exposure, of that subject to the respiratory virus is unknown (unless this is in the patient's medical records) it may not be possible to analyse the measured SPATS2L expression level of the individual subject minus a baseline SPATS2L expression level which is the pre-exposure SPATS2L expression level measured in the same individual subject. However, it may be possible to analyse the measured SPATS2L expression level of the individual subject minus a baseline SPATS2L expression level which is the level of expression of SPATS2L measured in an uninfected control subject of the same or a similar phenotype (age, gender, BMI, ethnicity, underlying conditions, etc.). One may assess whether the subject is at risk of developing severe symptoms of disease and/or becoming contagious using the absolute value of the SPATS2L expression level measured for the individual subject, scaled, where appropriate, by reference to the level of expression of at least one house keeping gene measured in the biological sample of the subject and a reference level of expression of the at least one house keeping gene which is associated with a reference threshold level, in accordance with Example 5 below.

The measured level of expression of SPATS2L for the individual subject, or the change (from baseline) in the measured level of expression of SPATS2L for the individual subject, may be assessed against a reference threshold level of expression of SPATS2L, or against a reference threshold change in the level of expression of SPATS2L, defining the minimum level of expression of SPATS2L, or the minimum change in the level of expression of SPATS2L, required for the subject to be assessed as being at risk of developing severe symptoms of disease and/or becoming contagious, as described in the following paragraph.

The reference threshold may, for example, be set to maximise PPV or NPV (see **Tables 2 and 3** of Example 1). If the reference threshold is set to maximise PPV, there may be fewer false positives (i.e. a smaller probability of the subject mistakenly being assessed as at risk of developing severe symptoms of disease and/or becoming contagious). It will be appreciated that this may be useful where it is preferable to avoid treating everyone having an infection, for example if treatment entails exposing subjects to medicaments with harmful side effects, or where suitable medicament(s) is/are expensive, and/or in short supply. If the reference threshold is set to maximise NPV, then there may be fewer false negatives (i.e. a smaller probability of the subject mistakenly being assessed as not at risk of developing severe symptoms of disease and/or becoming contagious). This may be useful where it is preferable to identify everyone having an infection, for example if treatment entails exposing subjects to an inexpensive medicament in abundant supply with minimal side effects, and/or where an inclusionary quarantine policy is in place in the local area.

It will be appreciated that the assessment may optionally be supplemented with a different diagnostic test that confirms the individual subject has the relevant respiratory viral infection (e.g. a viral test).

Where the subject is assessed as being at risk of developing severe symptoms of disease and/or becoming contagious, this may trigger dosing of the subject with a suitable medicament (drug/vaccine). Medicaments could include one or more immunomodulators, such as those described in WO 2018/007788 or WO 2019/122909, for example UR-13870, antiviral agents such as oseltamivir, antibiotics, and other drugs, such as those disclosed herein.

The subject may undergo assessment two or more times, and may be considered to be at risk of developing severe symptoms and/or becoming contagious if at least one or more than one of the assessments concludes that the subject is at risk of developing severe symptoms of disease and/or becoming contagious. Optionally, successive assessments of the two or more assessments may be based on blood samples collected at the same time. Optionally, successive assessments of the two or more assessments may be based on blood samples collected at different times, for example separated by a time interval of at least about 12 hours; for example at least about 24 hours; at least about 36 hours; or at least about 48 hours. The threshold SPATS2L expression level above which the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious may be set to be different for the two or more assessments; for example, the threshold for at least one assessment being configured

to maximise PPV and the threshold for at least one other assessment being configured to maximise NPV (see **Tables 2 and 3** of Example 1).

Other actions that may be triggered alongside or instead of dosing with a medicament include increasing the observations/samples/measurements in a subject assessed to be at risk of developing severe symptoms of disease and/or becoming contagious, or reducing observations/samples/measurements in a subject assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious; and/or subjecting the subject to one or more surgical or non-surgical interventions (e.g. a ventilator, or extracorporeal membrane oxygenation).

Suitably, the method may be implemented using one or more computers. **Figure 6** shows a network **(N)** used in a computer-implemented method of assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus according to the present disclosure. The network **(N)** comprises at least one server **(S)**, and at least one other computing device **(1)** for example, a desktop computer, laptop, or mobile device such as a tablet. There is a data communications link **(L)** between server **(S)** and device **(1)**. Link **(L)** typically comprises at least one transmission medium (e.g., the Internet, or a local area network) that carries data traffic between source and destination, data communication equipment, communication protocols and software. Device **(1)** is configured to transmit input data to server **(S)** (which may be remote from device **(1)**, for example in a different country) the input data representing the level of expression of SPATS2L in a biological sample obtained from a subject. Server **(S)** is configured to execute a computer program comprising instructions which cause the server to receive the input data; analyse the input data to assess the likelihood that the subject will develop severe symptoms of disease and/or become contagious, in accordance with Example 1, i.e. perform a comparison with a threshold SPATS2L expression level; and output data representing the likelihood of the subject developing severe symptoms of disease and/or becoming contagious (which may be accompanied by an indication of the specificity, sensitivity, PPV and NPV of the selected threshold). Server **(S)** may then relay a result to device **(1)**, the result concerning whether the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious. The network further comprises at least one additional device **(2)**. Server **(S)** may be configured to relay the result via link **(L)** to additional device **(2),** for example for display by the at least one additional computing device. The at least one additional computing device may be a tablet, laptop, mobile phone, or the like.

Example 5 - House-keeping genes

When the level of expression of SPATS2L is measured as part of the methods of the present disclosure (for example by detection of fluorescence by a fluorescent moiety during RT-qPCR) routine processing steps will usually be carried out on the raw data in order to obtain processed data which is suitable for analysis. These may include steps known to those skilled in the art, such, for example, as normalisation to correct for background radiation, the removal of outliers, log transformation, and correcting for batch effects. However, the experimental conditions under which the data are collected may nonetheless influence the values measured, even when said processing steps are carried out.

The expression levels of house keeping genes are known to display substantially no (or little) variability under a range of physiological conditions, while the expression levels of other genes may fluctuate. Suitably, therefore, the expression levels of house keeping genes may provide a stable standard against which to scale the measured expression level of a gene (such as SPATS2L) which, in contrast to a house keeping gene, may fluctuate under different physiological conditions. As used herein, the term "house keeping gene" may therefore refer to a gene, such as, for example, a constitutive gene (transcribed continually) which is expressed at substantially constant rates both under physiological conditions associated with not being at risk of developing severe symptoms of disease and/or becoming contagious, and under physiological conditions associated with being at risk of developing severe symptoms of disease and/or becoming contagious. The level of expression of a house keeping gene may remain substantially the same, for a given subject, both before and after deliberate inoculation of the subject with respiratory virus, for example inoculation under the procedure of Example 1 above; even if that subject goes on to be at risk of developing severe symptoms of disease and/or becoming contagious.

Table 5 below shows the mean reference levels of expression (and the standard deviations, SD) for a series of known house keeping genes, which were measured using the probes listed in the first column. These mean reference expression levels were calculated from house keeping gene expression levels of the blood samples collected from subjects in Example 1, quantified by RNA transcriptomics analysis performed by Almac Diagnostics using the GeneChip™ Human Genome U133 Plus 2.0 Array. They were therefore measured under the same experimental and physiological conditions (and underwent the same processing steps,

namely normalisation, the removal of outliers, log-transformation, and correcting for batch effects) as the SPATS2L expression levels set out in Example 1 above.

The probes of column 1 of **Table 5** are available to persons of skill in the art.

Table 5: Reference expression levels in known house keeping genes

Probe.Set.ID	Mean	Std Dev	CV*	N.Valid	Gene.Symbol
200801_x_at	14.551	0.053	0.004	56	ACTB
213867_x_at	14.548	0.048	0.003	56	ACTB
201891_s_at	14.151	0.106	0.008	56	B2M
216231_s_at	13.395	0.149	0.011	56	B2M
212581_x_at	13.542	0.136	0.010	56	GAPDH
213453_x_at	13.557	0.141	0.010	56	GAPDH
217398_x_at	13.516	0.143	0.011	56	GAPDH
202605_at	11.775	0.208	0.018	56	GUSB
202854_at	9.386	0.267	0.028	56	HPRT1
200737_at	11.435	0.156	0.014	56	PGK1
200738_s_at	12.749	0.105	0.008	56	PGK1
217356_s_at	11.140	0.169	0.015	56	PGK1
217383_at	11.405	0.160	0.014	56	PGK1
203135_at	10.491	0.254	0.024	56	TBP
207332_s_at	9.258	0.348	0.038	56	TFRC
208691_at	10.215	0.350	0.034	56	TFRC

*CV is the coefficient of variation (= standard deviation/mean).

It will be seen from **Table 5** that where different probes are used to detect the (reference) expression level of the same housekeeping gene, the mean values for the (reference) expression level detected by the different probes differ very little from each other. By way of example, 200801_x_at and 213867_x_at detect (mean) ACTB reference expression levels of 14.551 and 14.548, respectively (in the same blood samples of Example 1).

In this connection, it will be appreciated that if a suitable probe other than probe set 241812_at (SEQ ID NO: 1) were to be used for the detection of SPATS2L, there would similarly be minimal variation between the value for the expression level of SPATS2L detected by that suitable other probe, and the value for the expression level detected by probe set 241812_at (SEQ ID NO: 1).

No adjustment for baseline (absolute values) - scaling

In accordance with the present disclosure, a scaled level of expression of SPATS2L may be calculated according to the formula:

$$E_s = E_m \times \frac{H_{ref}}{H_m}$$

wherein E_S is the scaled value of the measured expression level of SPATS2L in the subject, E_m is the measured expression level of SPATS2L in the subject, H_{ref} is the reference expression level of the housekeeping gene (associated with a reference threshold expression level of SPATS2L, i.e. a threshold suitable for the subjects from whom a data set was originally obtained, such as the subjects of Example 1), and H_m is the measured expression level of the housekeeping gene in the subject.

By way of example, a reference expression level of a house keeping gene may be 10. A reference threshold expression level of SPATS2L may be 4.

For a subject having a measured expression level of SPATS2L of 3 and a measured expression level of a house keeping gene of 20, the scaled value of their measured SPATS2L expression level may be 3 x [20/10] = 6. The scaled SPATS2L expression level of 6 may then be compared with the reference threshold of 4. Since 6 is greater than 4, the subject would be assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

Alternatively, the reference SPATS2L expression level of 4 may be scaled: 4 x [10/20] = 2. The measured SPATS2L level of 3 may then be compared with the scaled threshold of 2. Since 3 is greater than 2, the subject would again be assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

Change from baseline - scaling

In accordance with the present disclosure, a scaled change in the level of expression of SPATS2L may be calculated according to the formula:

$$E_s = E_m \times \frac{H_{ref}}{H_m}$$

wherein E_S is the scaled value of the measured change in the expression level of SPATS2L in the subject, E_m is the measured change in the expression level of SPATS2L in the subject, H_{ref} is the reference expression level of the housekeeping gene (associated with a

reference threshold change in the expression level of SPATS2L, i.e. a threshold suitable for subjects from whom a data set was originally obtained, such as the subjects of Example 1), and H_m is the measured expression level of the housekeeping gene in the subject.

By way of example, a reference expression level of a house keeping gene may be 10. A reference threshold change in the expression level of SPATS2L may be 1.

For a subject having a measured change in the SPATS2L expression level of 0.5 and a measured expression level of a house keeping gene of 40, the scaled value of their measured change in the expression level of SPATS2L may be 0.5 x [40/10] = 2. The scaled change in the expression level of SPATS2L of 2 may then be compared with the reference threshold change of 1. Since 2 is greater than 1, the subject would be assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

Alternatively, the reference threshold SPATS2L expression level of 1 may be scaled: 1 x [10/40] = 0.25. The measured change in the level of expression of SPATS2L of 0.5 may then be compared with the scaled threshold of 0.25. Since 0.5 is greater than 0.25, the subject would be again assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

Where in the foregoing description, integers or elements are mentioned which have known, obvious or foreseeable equivalents, then such equivalents are herein incorporated as if individually set forth. Reference should be made to the claims for determining the true scope of the present disclosure, which should be construed so as to encompass any such equivalents. It will also be appreciated by the reader that integers or features of the disclosure that are described as preferable, advantageous, convenient or the like are optional and do not limit the scope of the independent claims. Moreover, it is to be understood that such optional integers or features, whilst of possible benefit in some embodiments of the disclosure, may not be desirable, and may therefore be absent, in other embodiments.

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Claims

1. A method of assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, comprising:
   a) measuring a level of expression of SPATS2L (spermatogenesis-associated serine-rich 2-like) in a biological sample obtained from the subject; and
   b) analysing the measured level of expression of SPATS2L to assess whether the subject is at risk of developing severe symptoms of disease and/or becoming contagious.

2. A method of quantifying the level of expression of SPATS2L in a subject at risk of developing severe symptoms of disease and/or becoming contagious following exposure, or possible exposure, to a respiratory virus, comprising:
   a) obtaining a biological sample from the subject; and
   b) measuring a level of expression of SPATS2L in the sample by RNA transcriptomics analysis.

3. The method of claim 2, wherein the level of expression of SPATS2L in the biological sample is measured by RNA transcriptomics analysis using one or more oligonucleotide probes that are specific for SPATS2L mRNA transcripts and are immobilised on a surface and labelling the transcripts with a detectable label.

4. The method of claim 2, wherein the level of expression of SPATS2L in the biological sample is measured by RNA-seq; wherein SPATS2L mRNA transcripts in the sample are converted to cDNA, fragmented, provided with suitable adapters to facilitate amplification and sequencing and then sequenced.

5. A method of conducting a clinical trial or field study, comprising:
   a) measuring levels of expression of SPATS2L in biological samples obtained from a plurality of subjects;
   b) analysing the measured level of expression of SPATS2L in each sample to assess whether a respective subject is at risk of developing severe symptoms of disease and/or becoming contagious; and
   c) including or excluding a subject who is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious in the clinical trial or field study or in a subgroup of the clinical trial or field study.

6. A computer-implemented method of assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, comprising:
   a) receiving by a computer input data representing a measured level of expression of SPATS2L in a biological sample obtained from the subject;
   b) processing by a computer the input data to analyse the level of expression to assess the likelihood that the subject will develop severe symptoms of disease and/or become contagious; and
   c) outputting by a computer output data representing the likelihood of the subject developing severe symptoms of disease and/or becoming contagious.

7. A method of providing electronic data representing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, comprising:
   a) measuring a level of expression of SPATS2L in a biological sample obtained from the subject;
   b) receiving by a computer the measured level of expression and encoding by the computer the measured level of expression in computer-readable form, and
   c) transmitting by a computer the encoded measured level of expression to a remote computer for evaluation in accordance with a method of claim 6.

8. The method of claim 6, wherein said outputting step (c) comprises displaying the output data on a display in a form which can be understood by a human being, or transmitting the output data to a remote computer.

9. The method of any of claims 1, 5, 6 or 8, wherein analysing step (b) comprises subtracting a baseline level of expression of SPATS2L (which is the level of expression of SPATS2L in a biological sample obtained from the subject prior to exposure, or possible exposure, of the subject to the respiratory virus) from the level of expression of SPATS2L measured in step (a), to obtain a measured value for the change in the level of expression of SPATS2L.

10. The method of any of claims 1, 5, 6 or 8, wherein analysing step (b) comprises subtracting a baseline level of expression of SPATS2L (which is the level of expression of SPATS2L in a biological sample obtained from one or more uninfected control subjects) from the

level of expression of SPATS2L measured in step (a), to obtain a measured value for the change in the level of expression of SPATS2L.

11. The method of claim 9 or claim 10, wherein analysing step (b) further comprises comparing the measured change in the level of expression of SPATS2L with a reference threshold change, wherein when the measured change in the level of expression of SPATS2L is above the reference threshold change, the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

12. The method of claim 11, wherein the reference threshold change is determined by:
- measuring the baseline levels of expression of SPATS2L in biological samples obtained from a group of persons uninfected with respiratory virus;
- measuring the levels of expression of SPATS2L in biological samples obtained from the group of persons after inoculation with respiratory virus;
- ascertaining the change in the level of expression of SPATS2L for each person from uninfected to after inoculation;
- classifying the persons in the group according to their risk of developing severe symptoms of disease and/or becoming contagious; and
- setting the reference threshold change to be a change in the level of expression of SPATS2L which discriminates between persons in the group who are classified as at risk and persons in the group who are not classified as at risk, according to a desired measure of test performance.

13. The method of any of claims 1, 5, 6 or 8, wherein analysing step (b) comprises comparing the measured level of expression of SPATS2L to a reference threshold for the level of expression of SPATS2L, wherein when the measured level of expression of SPATS2L is above the reference threshold, the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

14. The method of claim 13, wherein the reference threshold for the level of expression of SPATS2L is determined by:
- measuring the levels of expression of SPATS2L in biological samples obtained from a group of persons after inoculation with respiratory virus;
- classifying the persons in the group according to their risk of developing severe symptoms of disease and/or becoming contagious; and
- setting the reference threshold to be the level of expression of SPATS2L which discriminates between persons in the group who are classified as at risk and persons

in the group who are not classified as at risk, according to a desired measure of test performance.

15. The method of claim 11 or claim 12, wherein the measured change in the level of expression of SPATS2L is scaled by reference to the level of expression of at least one house keeping gene measured in the biological sample of the subject and a reference level of expression of the at least one house keeping gene which is associated with a reference threshold level, before being compared to the reference threshold change in the level of expression of SPATS2L; or the method of claim 10 or claim 11, wherein the measured level of expression of SPATS2L is scaled by reference to the level of expression of at least one house keeping gene measured in the biological sample of the subject and a reference level of expression of the at least one house keeping gene which is associated with a reference threshold level, before being compared to the reference threshold level of expression of SPATS2L.

16. The method of claim 15, wherein the scaled change in the level of expression of SPATS2L or the scaled level of expression of SPATS2L is calculated according to the formula:

$$E_s = E_m \times \frac{H_{ref}}{H_m}$$

wherein E_S is the scaled value of the measured change in expression level of SPATS2L or the scaled value of the measured level of expression of SPATS2L in the subject, E_m is the measured change in the expression level of SPATS2L or the measured level of expression of SPATS2L in the subject, H_{ref} is the reference level of expression of the housekeeping gene which is associated with one or more reference threshold levels, and H_m is the expression level of the housekeeping gene measured in the biological sample of the subject.

17. The method of claim 15 or claim 16, wherein the house keeping gene is selected from: ACTB, B2M, GAPDH, GUSB, HPRT1, PGK1, TBP and TFRC.

18. The method of claim 17 when dependent on claim 16, wherein the reference expression level of the housekeeping gene H_{ref} is as follows:

House keeping gene	Expression level
ACTB	14.550
B2M	13.773
GAPDH	13.538

GUSB	11.775
HPRT1	9.386
PGK1	11.682
TBP	10.491
TFRC	9.737

19. The method of any of claims 15 to 18 when dependent on claim 11or claim 12, wherein the reference threshold change is about 0.4 to about 0.9, when mRNA transcripts in the biological sample are quantified by microarray RNA transcriptomics analysis in which SPATS2L transcripts from the sample are captured using one or more complementary oligonucleotide probes for SPATS2L immobilised on a surface.

20. The method of claim 19, wherein the probability that a subject who will go on to develop severe symptoms of disease and/or become contagious is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious is in a range of about 0.4 to about 0.7.

21. The method of claim 19 or claim 20, wherein the probability that a subject who will not go on to develop severe symptoms of disease and/or become contagious is assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious is in a range of about 0.7 to about 0.9.

22. The method of any of claims 19 to 21, wherein a scaled change in the level of expression of SPATS2L above the reference threshold change corresponds to a positive predictive value (PPV) in a range of about 0.4 to about 0.5.

23. The method of any of claims 19 to 22, wherein a scaled change in the level of expression of SPATS2L below the reference threshold change corresponds to a negative predictive value (NPV) in a range of greater than about 0.75.

24. The method of any of claims 15 to 18 when dependent on claim 13 or claim 14, wherein the reference threshold for the level of expression of SPATS2L is about 4.9 to 5.6, when mRNA transcripts in the biological sample are quantified by RNA transcriptomics analysis which SPATS2L transcripts from the sample are captured using one or more complementary oligonucleotide probes for SPATS2L immobilised on a surface.

25. The method of claim 24, wherein the probability that a subject who will go on to develop severe symptoms of disease and/or become contagious is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious is in a range of about 0.3 to about 0.7.

26. The method of claim 24 or claim 25, wherein the probability that a subject who will not go on to develop severe symptoms of disease and/or become contagious is assessed not to be at risk of developing severe symptoms of disease and/or becoming contagious is in a range of about 0.6 to about 0.8.

27. The method of any of claims 24 to 26, wherein a scaled level of expression of SPATS2L above the reference threshold corresponds to a positive predictive value (PPV) in a range of about 0.4 to about 0.5.

28. The method of any of claims 24 to 27, wherein a scaled level of expression of SPATS2L below the reference threshold corresponds to a negative predictive value (NPV) in a range of more than about 0.75.

29. The method of any of claims 1,5, 6 or 8 to 28, wherein the level of expression of SPATS2L in the subject is measured two or more times, and the subject is assessed to be at risk of developing severe symptoms and/or becoming contagious if the at least one or more than one of the measured levels, when assessed, indicates so, optionally wherein successive measurements are separated by a time interval of at least about 12 hours.

30. The method of claim 29, wherein the thresholds against which the two or more measurements are assessed are different, the threshold for the assessment of at least one measurement being configured to minimise false positives, and the threshold for the assessment of at least another measurement being configured to have fewer false negatives than the one measurement.

31. The method of any of claims 1 to 30, wherein the biological sample was obtained (or, when dependent on claim 2, wherein step (b) comprises obtaining the biological sample) from the subject at least about 45 hours after exposure, or possible exposure, to the respiratory virus.

32. The method of any preceding claim, wherein the subject has had a positive diagnostic test for respiratory viral disease, presents with symptoms of respiratory viral disease, and/or has had prolonged exposure to at least one other person who is infected with a respiratory virus.

33. The method of any preceding claim, wherein the respiratory virus is respiratory syncytial virus (RSV), parainfluenza virus (HPIV), metapneumovirus (HMPV), rhinovirus (HRV), coronavirus such as SARS-CoV (for example, SARS-CoV-1 or SARS-CoV-2), adenovirus (HAdV), enterovirus (EV), bocavirus (HBoV), parechovirus (HPeV) or an influenza virus

34. The method of claim 33, wherein the respiratory virus is an influenza virus.

35. The method of any preceding claim, wherein the biological sample is a blood sample.

36. The method of any preceding claim, wherein the expression level of SPATS2L and/or the expression level of one or more house keeping genes, is or are measured by quantifying mRNA transcripts of SPATS2L in the biological sample.

37. The method of claim 36, wherein the mRNA transcripts in the biological sample are quantified by a PCR-based method, such as RT-qPCR.

38. The method of any preceding claim, wherein the subject is administered one or more medicinal products, before or after exposure or possible exposure to the respiratory virus.

39. The method of claim 5 or any of claims 9 to 37 when dependent on claim 5, wherein a subject who is included in the clinical trial or field study, or in the subgroup of the clinical trial or field study, is administered one or more medicinal products, and/or is subjected to one or more surgical or non-surgical interventions.

40. The method of claim 1, or the method of any of claims 9 to 37 when dependent on claim 1, further comprising administering one or more medicinal products to the subject, and/or subjecting the subject to one or more surgical or non-surgical interventions, if the subject is assessed to be at risk of developing severe symptoms and/or becoming contagious.

41. The method of any of claims 38 to 40, wherein the one or more medicinal products are selected from one or more antiviral agents (e.g. oseltamivir) and/or one or more immunomodulatory agents.

42. The method of any of claims 39 to 41, wherein the one or more surgical or non-surgical interventions are selected from hospitalising the subject, allocating the subject a hospital bed in anticipation of their condition worsening; administering oxygen to the subject, e.g. by means of a ventilator or by extracorporeal membrane oxygenation.

43. A computer program comprising instructions which, when carried out by a computer, cause the computer to carry out step (b) of the method of any of claims 1, 5, 6 or 8 to 37.

44. A computer network comprising:

a) at least one server;

b) at least one other computing device; and

c) a data communications link between the server and the other device;

wherein the at least one other device is configured to transmit data to the at least one server, the data comprising the level of expression of SPATS2L in a biological sample obtained from a subject; and the at least one server is configured to analyse the level of expression of SPATS2L to assess whether the subject is at risk of developing severe symptoms of disease and/or becoming contagious by executing the method of claim 6, or by executing the method of any of claims 8 to 37 when dependent on claim 6.

45. The computer network of claim 44, wherein the at least one server is configured to analyse the level of expression of SPATS2L before relaying a result to the other device, the result concerning whether the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

46. The computer network of claim 44 or claim 45, further comprising at least one further computing device, wherein the at least one server is configured to analyse the level of expression of SPATS2L before relaying a result to the at least one further computing device, the result concerning whether the subject is assessed to be at risk of developing severe symptoms of disease and/or becoming contagious.

47. A kit for assessing whether a subject is at risk of developing severe symptoms of disease and/or becoming contagious after exposure, or possible exposure, to a respiratory virus, the kit comprising a reagent that allows quantitation of a SPATS2L oligonucleotide.

48. The kit of claim 47, wherein the kit comprises a microarray, the microarray comprising an oligonucleotide which is the reagent that allows quantitation of a SPATS2L oligonucleotide, and which hybridises to a SPATS2L oligonucleotide.

49. The method, kit or network of any preceding claim, wherein a subject having severe symptoms of disease is any subject who requires hospitalisation; and/or wherein severe symptoms of disease comprise one or more of: tachypnoea, hypoxemia, an arterial oxygen

saturation of ≤ 92% on room air by a transcutaneous method and radiological pulmonary infiltrates.

50. The method, kit or network of any preceding claim, wherein the subject is a mammal, optionally wherein the subject is a human, or a non-human mammal.

Quarantine Subject Symptom Diary Card
Protocol: RDO-CS-004

Subject Number: _____ Subject Initials: ___

Date: ___ ___ ____ (dd mmm yyyy) Time: __ : __ (hh mm)

Morning ☐ Afternoon ☐ Evening ☐

How much are your symptoms bothering you?

Symptoms Please report the symptoms you are experiencing at the moment	Mark the scale with a single line as demonstrated before: Not at all bothered ☺ — Severely bothered ☹	*hVIVO Staff use only* Line Length (cm): ___.___ Length of mark (cm): ___.___
Runny Nose		
Stuffy Nose		
Sneezing		
Sore Throat		
Earache		
Malaise (tiredness)		
Headache		
Muscle and/or Joint Ache		
Chillness / Feverishness		
Cough		
Chest Tightness		
Shortness of breath		
Wheeze		

Subject's Initials _____

Physician's Initials _____ ___ ___ ____ (dd mmm yyyy)

FIG. 1

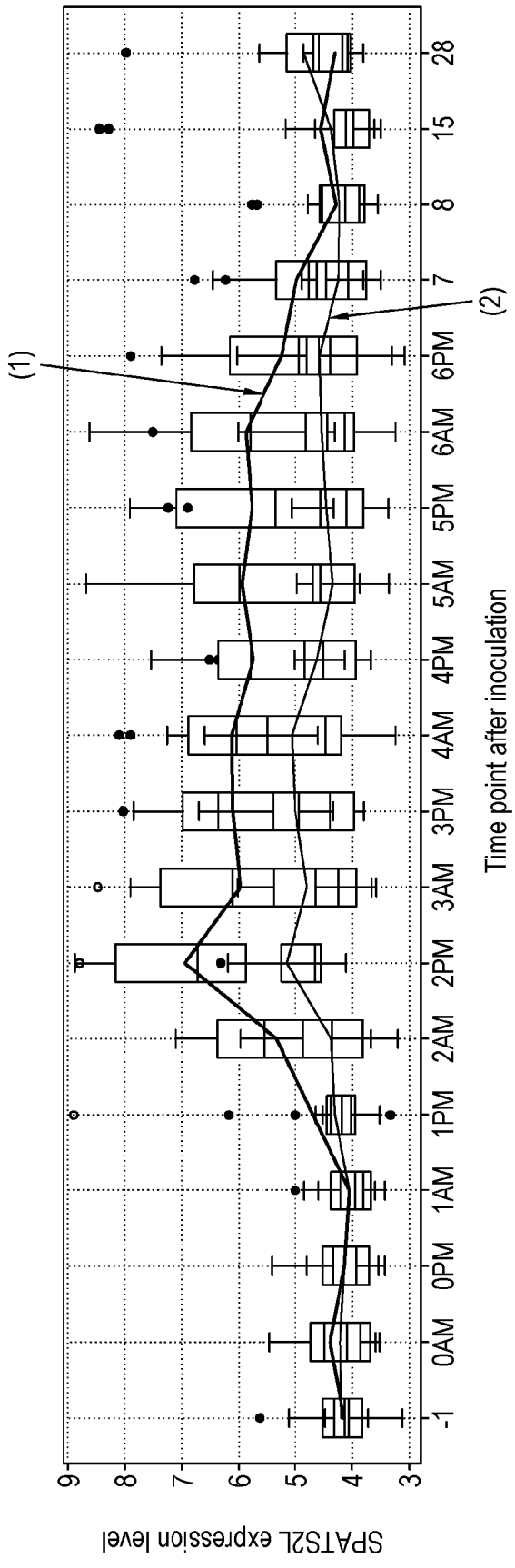
SPATS2L expression level
9
8
7
6
5
4
3
-1
0AM
0PM
1AM
1PM
2AM
2PM
3AM
3PM
4AM
4PM
5AM
5PM
6AM
6PM
7
8
15
28
Time point after inoculation
(1)
(2)

FIG. 2

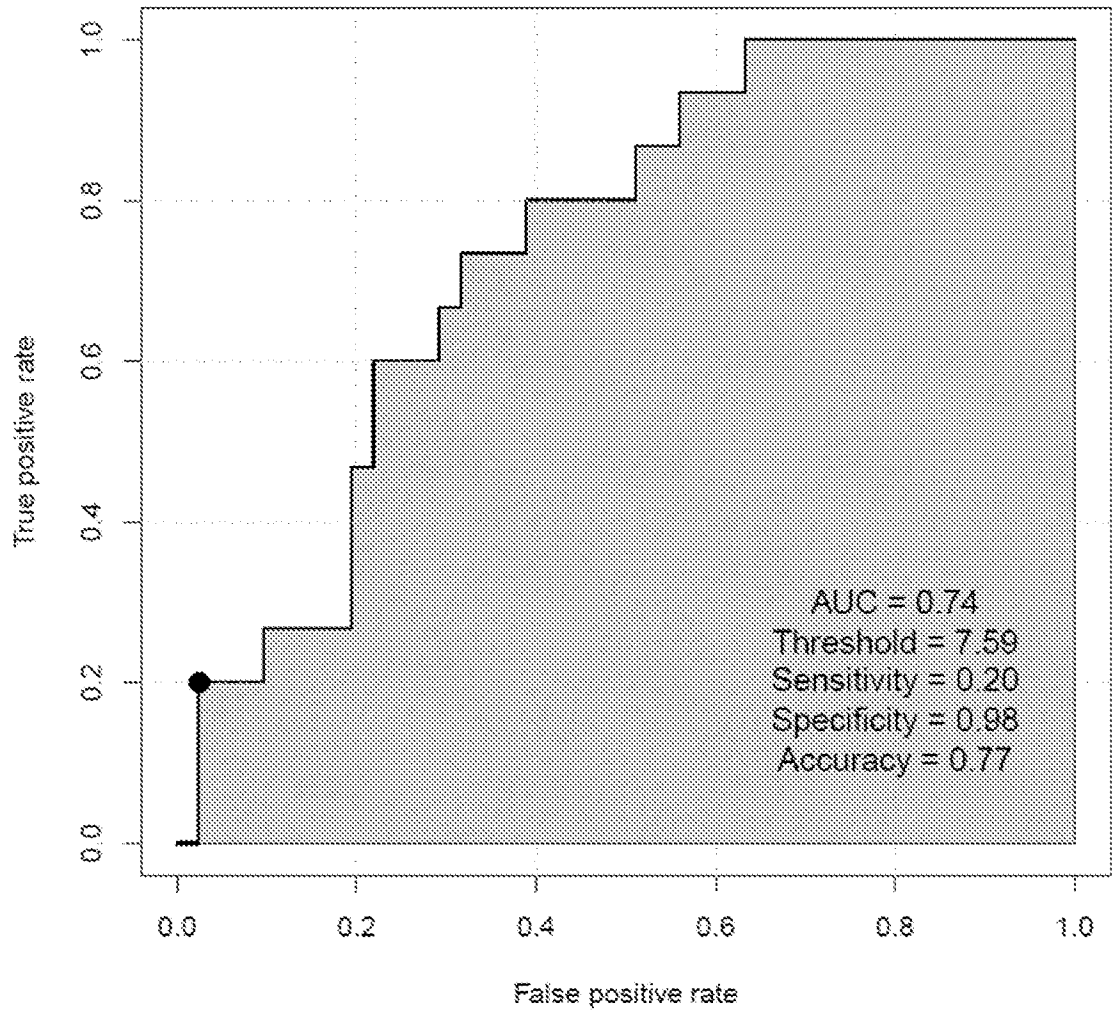
True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0
AUC = 0.74
Threshold = 7.59
Sensitivity = 0.20
Specificity = 0.98
Accuracy = 0.77

Fig. 3

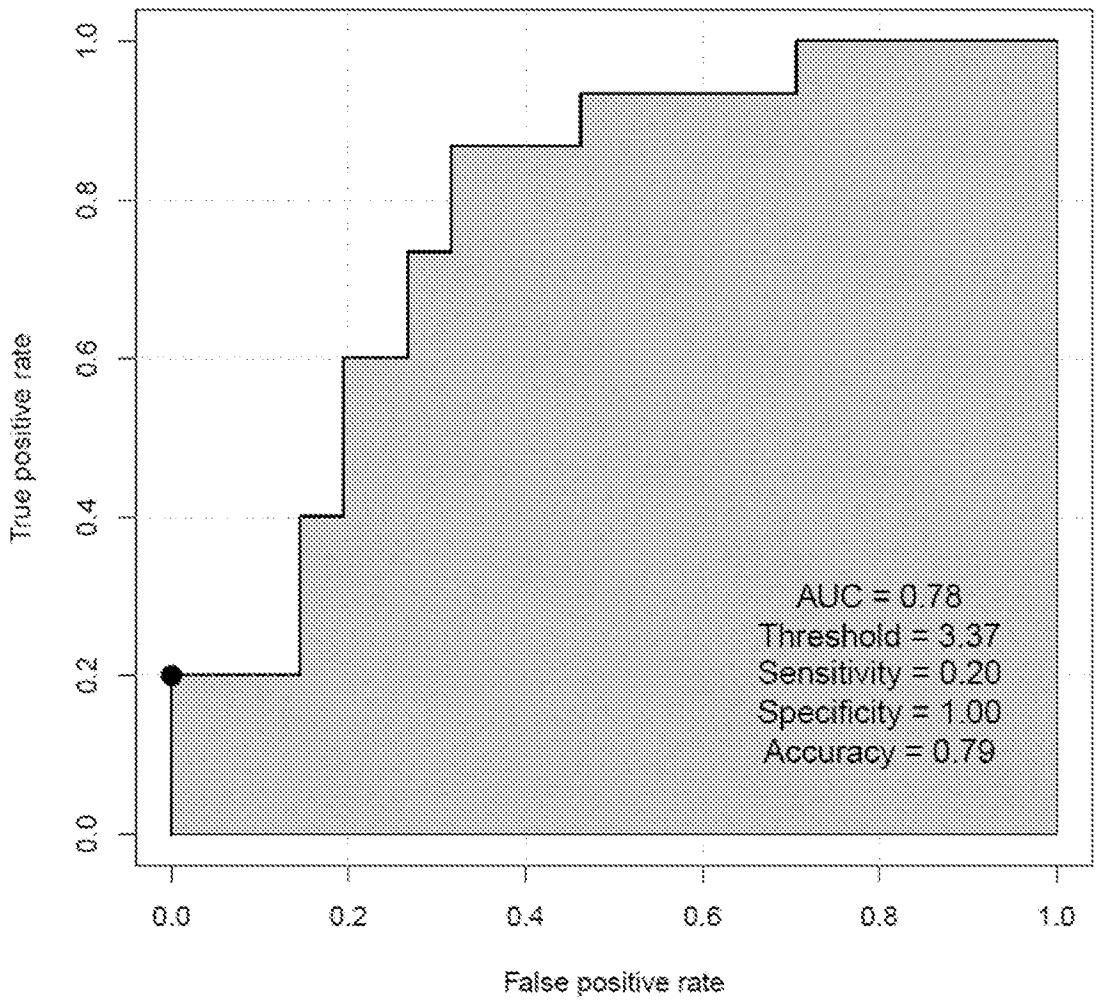
True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0
AUC = 0.78
Threshold = 3.37
Sensitivity = 0.20
Specificity = 1.00
Accuracy = 0.79

Fig. 4

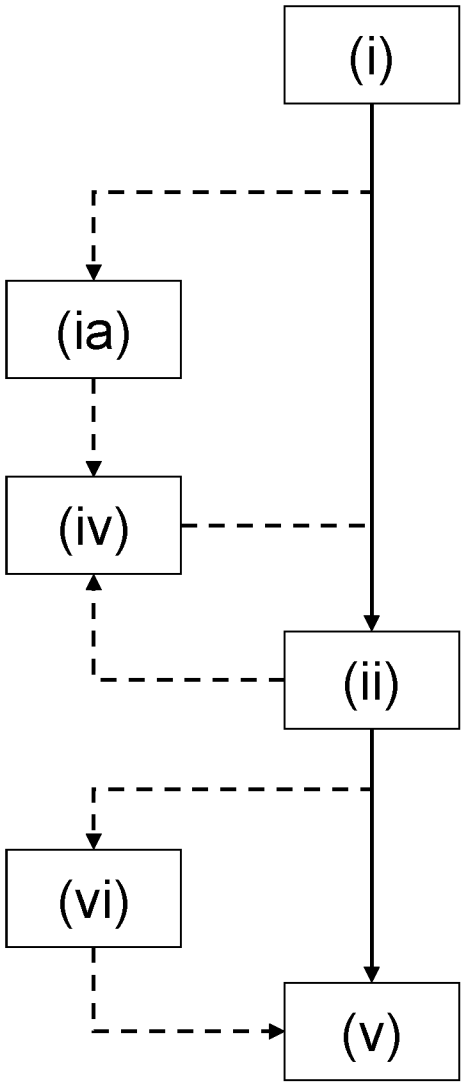

Fig. 5

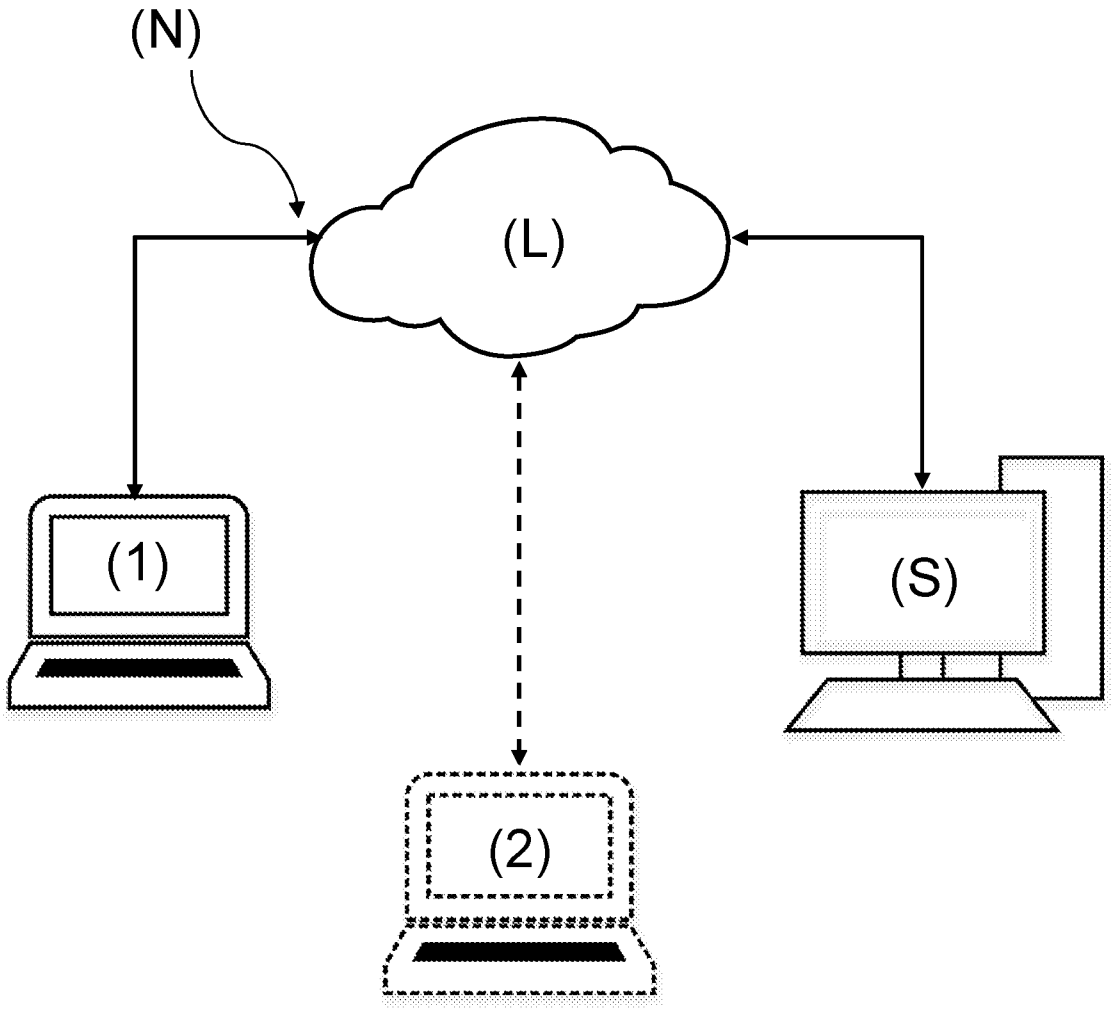

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2022/052609

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6883
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FAN JINGHAN ET AL: "Comparative transcriptome analysis reveals involvement of TLR-2 signaling in the pathogenesis of intracranial aneurysm", JOURNAL OF CLINICAL NEUROSCIENCE, CHURCHILL LIVINGSTONE, GB, vol. 47, 21 October 2017 (2017-10-21), pages 258-263, XP085307175, ISSN: 0967-5868, DOI: 10.1016/J.JOCN.2017.07.016	47
Y	page 36, line 13	3,4, 8-42,45, 46,48-50

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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 January 2023	30/01/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Leber, Thomas

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2022/052609

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAVENPORT EMMA E ET AL: "Transcriptomic profiling facilitates classification of response to influenza challenge", JOURNAL OF MOLECULAR MEDICINE, SPRINGER BERLIN HEIDELBERG, BERLIN/HEIDELBERG, vol. 93, no. 1, 28 October 2014 (2014-10-28), pages 105-114, XP035416346, ISSN: 0946-2716, DOI: 10.1007/S00109-014-1212-8 [retrieved on 2014-10-28]	1,2,5-7, 43,44
Y	title; Fig. 1; Fig. 3, legend; Fig. 3, SPATS2L is the 20th marker from the top in Fig. 3;	3,4, 8-42,45, 46,48-50
X	JULIUS MULLER ET AL: "Development of an objective gene expression panel as an alternative to self-reported symptom scores in human influenza challenge trials", JOURNAL OF TRANSLATIONAL MEDICINE, vol. 15, no. 1, 8 June 2017 (2017-06-08), XP055478297, DOI: 10.1186/s12967-017-1235-3 cited in the application	1,2,6,7, 43,44
Y	Fig. 2a and b; Fig. 3b and 4b; whole document	3,4, 8-42,45, 46,48-50
A	BARTON AMBER J. ET AL: "Transcriptomics in Human Challenge Models", FRONTIERS IN IMMUNOLOGY, vol. 8, 18 December 2017 (2017-12-18), pages 1-12, XP093012703, DOI: 10.3389/fimmu.2017.01839 the whole document	1-50
A	CHRISTOPHER W. WOODS ET AL: "A Host Transcriptional Signature for Presymptomatic Detection of Infection in Humans Exposed to Influenza H1N1 or H3N2", PLOS ONE, vol. 8, no. 1, 1 January 2013 (2013-01-01) , page e52198, XP055472707, DOI: 10.1371/journal.pone.0052198 cited in the application the whole document	1-50

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2022/052609

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Zan Anna ET AL: "DeepFlu: a deep learning approach for forecasting symptomatic influenza A infection based on pre-exposure gene expression", bioRxiv, 20 May 2021 (2021-05-20), pages 1-9, XP093012710, DOI: 10.1101/2020.12.02.407940 Retrieved from the Internet: URL:www [retrieved on 2023-01-10] the whole document -----	1-50

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2022/052609

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments: