

CLAIMS

1. A method for normalising quantitative data obtained by amplification of nucleic acids from a cell, organism, or virus, the method comprising:
- 5 (i) obtaining quantitative data
- from amplification of genomic DNA from a first gene and RNA transcribed from the first gene, and
 - from amplification of a sequence of non-transcribed DNA in the cell, organism, or virus; and
- 10 (ii) using the quantitative data to derive a normalised value (nV) representative of the relative amounts of:
- said genomic DNA and RNA transcripts of the first gene, to
 - said genomic DNA that is not transcribed,
- present within the nucleic acid sample prior said amplification.
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2. The method of claim 1, wherein the quantitative data is amplicon copy number, and the method comprises:
- obtaining a value A (vA) representing total amplicon number generated from the amplification of said genomic DNA and RNA transcripts of the first gene, and a value
- 20 B (vB) representing total amplicon number generated from the sequence of non-transcribed DNA;
- calculating a normalised value (nV) using the equation:
$$vA/vB = nV$$

or an equivalent form thereof.
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3. The method of claim 1, wherein the quantitative data is amplicon copy number, and the method comprises:
- obtaining a value X (vX) representing total amplicon number generated from: the amplification of genomic DNA and RNA transcripts from the first gene, and
- 30 amplification of genomic DNA and RNA transcripts from at least one additional gene;
- obtaining a value B (vB) representing total amplicon number generated from the sequence of non-transcribed DNA,
 - calculating a normalised value (nV) using the equation:
$$vX / (vB \times (X+1)) = nV$$

or an equivalent form thereof,
- 35 wherein X is the number of said additional gene(s).

4. The method of claim 2 or claim 3, wherein the amplification is digital polymerase chain reaction (dPCR).
5. The method of claim 1, wherein the quantitative data is threshold value (Ct), and the method comprises:
- obtaining a cycle threshold value CtA from the amplification of said genomic DNA and RNA transcripts of the first gene,
 - obtaining a cycle threshold value CtB from the amplification of the sequence of non-transcribed DNA; and
 - calculating a normalised value (nV) using the equation:
$$2^{CtB - CtA} = nV$$
or an equivalent form thereof.
6. The method of claim 1, wherein the quantitative data is threshold value (Ct), and the method comprises:
- obtaining a cycle threshold value CtX from: the amplification of said genomic DNA and RNA transcripts of the first gene, and from amplification of genomic DNA and RNA transcripts from at least one additional gene;
 - obtaining a cycle threshold value CtB from the amplification of the sequence of non-transcribed DNA; and
 - calculating a normalised value (nV) using the equation:
$$2^{CtB - CtX} / (X + 1) = nV$$
or an equivalent form thereof,
- wherein X is the number of said additional gene(s).
7. The method of claim 5 or claim 6, wherein the amplification is quantitative polymerase chain reaction (qPCR).
8. The method of any one of claims 1 to 7, wherein the sequence of non-transcribed DNA is genomic DNA.
9. The method of any one of claims 1 to 8, further comprising conducting said amplification of the nucleic acids from the cell, organism, or virus.

10. The method of any one of claims 1 to 9, wherein the nucleic acids from the cell, organism, or virus are an extract of total nucleic acids.

11. The method of any one of claims 1 to 10, wherein any said amplification is
5 conducted using: polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), strand displacement amplification (SDA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), recombinase polymerase amplification (RPA), helicase dependant amplification (HDA), strand invasion based amplification (SIBA), transcript-mediated amplification (TMA), self-
10 sustained sequence replication (3SR), nucleic acid sequence based amplification (NASBA), or any combination thereof.

12. The method of any one of claims 1 to 11, further comprising using the normalised value (nV) to assess the level of transcriptional activity in the cell, organism, or virus.

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13. The method of any one of claims 1 to 12, further comprising:

- obtaining a transcription-negative normalised value (nV-) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known not to have transcriptional activity; and
- 20 - comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to the transcription-negative normalised value (nV-), to thereby assess the level of transcriptional activity in the cell, organism, or virus.

25 14. The method of claim 13, wherein the transcription-negative normalised value (nV-) is a mean value generated from said series of said normalised values (nV).

15. The method of claim 13 or claim 14, wherein:

- the transcription-negative normalised value (nV-) is used as a base value for
30 assessing a presence or an absence of transcriptional activity in the cell, organism or virus; and
- an absence of transcriptional activity is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is equal to or below the transcription-negative normalised value (nV-); or

- transcriptional activity is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is above the transcription-negative normalised value (nV-).

5 16. The method of any one of claims 13 to 15, wherein said transcription-negative normalised value (nV-):

- incorporates statistical variation in said series of normalised values (nV) from individuals of the population of the cells, organisms, or viruses known not to have transcriptional activity; and/or

10 - is provided with a confidence interval that said transcription-negative normalised value (nV-) is predictive of a presence or an absence of transcriptional activity in the cell, organism or virus.

17. The method of claim 16, wherein the confidence interval is more than 90%, or more
15 than 95%.

18. The method of any one of claims 13 to 17, further comprising:

- obtaining a transcription-positive normalised value (nV+) generated using a series of said normalised values (nV) obtained from individuals of a population of the
20 cells, organisms, or viruses known to have transcriptional activity; and

- comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to the transcription-positive normalised value (nV+), to thereby assess the level of transcriptional activity in the cell, organism, or virus.

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19. The method of claim 18, wherein the transcription-positive normalised value (nV+) is a mean value generated from said series of said normalised values (nV).

20. The method of claim 18 or claim 19, wherein:

30 - the transcription-positive normalised value (nV+) is used as a base value for transcriptional activity in the cell, organism or virus; and

- a lack or absence of transcriptional activity is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is below the transcription-positive normalised value (nV+); or

- transcriptional activity is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is equal to or above the transcription-positive normalised value (nV+).

5 21. The method of any one of claims 18 to 20, wherein said transcription-positive normalised value (nV+):

- incorporates statistical variation in said series of normalised values (nV) from individuals of the population of the cells, organisms, or viruses known to have transcriptional activity; and/or

10 - is provided with a confidence interval that said transcription-positive normalised value (nV+) is predictive of a presence or an absence of transcriptional activity in the cell, organism or virus.

22. The method of claim 21, wherein the confidence interval is more than 90%, or more
15 than 95%.

23. The method of claim 13, further comprising:

- obtaining a transcription-negative normalised value (nV-) generated using a series of said normalised values (nV) obtained from individuals of a population of the
20 cells, organisms, or viruses known not to have transcriptional activity;

- obtaining a transcription-positive normalised value (nV+) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known to have transcriptional activity; and

- comparing the normalised value (nV) obtained by said amplification of
25 nucleic acids from the cell, organism, or virus to:

(i) the transcription-negative normalised value (nV-) and to the transcription-positive normalised value (nV+), or

(ii) to a combined transcription normalised value (nV[±]) intermediate to the transcription-negative normalised value (nV-) and the transcription-positive
30 normalised value (nV+),

to thereby assess the level of transcriptional activity in the cell, organism, or virus.

24. The method of claim 23, wherein the combined transcription normalised value (nV[±]) is calculated using the equation:

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$$(nV+) + (nV-) / 2 = (nV^{\pm})$$

or an equivalent form thereof.

25. The method of claim 24, wherein said combined transcription normalised value ($nV^{\pm}$):

- incorporates statistical variation in said series of transcription-negative normalised value (nV^{-}) and/or said transcription-positive normalised value (nV^{+}); and/or
- 5 - is provided with a confidence interval that said combined transcription normalised value ($nV^{\pm}$) is predictive of a presence or an absence of transcriptional activity in the cell, organism or virus.

26. The method of claim 25, wherein the confidence interval is more than 90%, or more
10 than 95%.

27. The method of any one of claims 12 to 26, wherein the level of transcriptional activity in the cell, organism, or virus is assessed for the purpose of determining any one or more of:

- 15 - viability of the test cell or the test organism;
- whether the test cell, organism or virus is alive or dead;
- transcriptional perturbation within the test cell, organism, or virus.

28. The method of any one of claims 1 to 11, further comprising using the normalised value (nV) to assess the level of drug resistance or drug sensitivity in the cell, organism,
20 or virus, wherein:

- said cell, organism, or virus has been treated with a drug prior to said amplification of nucleic acids, and
- said normalised value (nV) is compared to a control normalised value (cnV)
25 generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known to be:

- (i) resistant to the drug; or
- (ii) sensitive to the drug,

to thereby assess the level of drug resistance or drug sensitivity in the cell, organism, or
30 virus.

29. The method of any one of claims 1 to 11 or 28, further comprising:

- obtaining a drug-sensitive normalised value (dsV) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells,
35 organisms, or viruses known to be sensitive to the drug; and

- comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to the drug-sensitive normalised value (dsV), to thereby assess the level of drug resistance or drug sensitivity or in the cell, organism, or virus, wherein the cell, organism, or virus has been treated with a drug prior to said amplification of nucleic acids.

30. The method of claim 29, wherein the drug-sensitive normalised value (dsV) is a mean value generated from said series of said normalised values (nV).

31. The method of claim 29 or claim 30, wherein:

- the drug-sensitive normalised value (dsV) is used as a base value for assessing a presence or an absence of resistance or sensitivity to the drug in the cell, organism or virus; and
- resistance to the drug is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is above the drug-sensitive normalised value (dsV); or
- sensitivity to the drug is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is equal to or below the drug-sensitive normalised value (dsV).

32. The method of any one of claims 29 to 31, wherein said drug-sensitive normalised value (dsV):

- incorporates statistical variation in said series of normalised values (nV) from individuals of the population of the cells, organisms, or viruses known to be sensitive to the drug; and/or
- is provided with a confidence interval that said drug-sensitive normalised value (dsV) is predictive of a presence or an absence of:
 - (i) resistance to the drug in the cell, organism or virus; or
 - (ii) sensitivity to the drug in the cell, organism or virus.

33. The method of claim 32, wherein the confidence interval is more than 90%, or more than 95%.

34. The method of any one of claims 1 to 11 or 28, further comprising:

- obtaining a drug-resistant normalised value (drV) generated using a series of said normalised values (nV) obtained from individuals of a population of the cells, organisms, or viruses known to be resistant to the drug; and

- comparing the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus to the drug-resistant normalised value (drV), to thereby assess the level of drug resistance or drug sensitivity or in the cell, organism, or virus, wherein the cell, organism, or virus has been treated with a drug prior to said amplification of nucleic acids.

35. The method of claim 34, wherein the drug-resistant normalised value (drV) is a mean value generated from said series of said normalised values (nV).

36. The method of claim 34 or claim 35, wherein:

- the drug-resistant normalised value (drV) is used as a base value for assessing a presence or an absence of resistance or sensitivity to the drug in the cell, organism or virus; and

- resistance to the drug is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is equal to or above the drug-resistant normalised value (drV); or

- sensitivity to the drug is indicated when the normalised value (nV) obtained by said amplification of nucleic acids from the cell, organism, or virus is below the drug-resistant normalised value (drV).

37. The method of any one of claims 34 to 36, wherein said drug-resistant normalised value (drV):

- incorporates statistical variation in said series of normalised values (nV) from individuals of the population of the cells, organisms, or viruses known to be resistant to the drug; and/or

- is provided with a confidence interval that said drug-resistant normalised value (drV) is predictive of a presence or an absence of:

- (i) resistance to the drug in the cell, organism or virus; or
- (ii) sensitivity to the drug in the cell, organism or virus.

38. The method of claim 37, wherein the confidence interval is more than 90%, or more than 95%.

39. The method of one of claims 1 to 11, further comprising using the normalised value (nV) to assess the level of drug resistance or drug sensitivity in the cell, organism, or virus, wherein:
- a first population of said cell, organism, or virus which has been treated with a drug prior to said amplification of nucleic acids, is used to generate a first said normalized value (nV),
 - a second population of said cell, organism, or virus which has not been treated with a drug prior to said amplification of nucleic acids, is used to generate a second said normalized value (nV),
 - said first normalized value (nV) and said second normalized value (nV) are compared to assess the level of transcriptional activity in the cell, organism, or virus with or without drug treatment, and thereby assess the level of drug resistance or drug sensitivity in the cell, organism, or virus.
40. The method of claim 39, wherein said drug sensitivity is indicated when said first normalized value (nV) is lower than said second normalized value (nV).
41. The method of any one of claims 27 to 40, wherein the drug is an antimicrobial.
42. The method of any one of claims 27 to 41, wherein the drug is an antimicrobial of a class selected from: Aminoglycosides, ansamycins, carbacephem, carbapenems, cephalosporins, glycopeptides, macrolidespenicillins, monobactams, polypeptides, quinolones, sulphonamides, tetracyclines.
43. The method of any one of claims 27 to 42, wherein the drug is Ciprofloxacin, Azithromycin, Rifampicin, or Doxycycline.
44. The method of any one of claims 27 to 43, wherein the first gene is a gene from a Chlamydia species (e.g. Chlamydia trachomatis), a Gonorrhea species, or a mycoplasma species (e.g. Mycoplasma genitalium).
45. The method of any one of claims 1 to 44, wherein said genomic DNA from the first gene and RNA transcribed from the first gene, and said sequence of non-transcribed DNA are co-amplified in the same reaction.

46. The method of claim 45, wherein said reaction comprises using reverse transcriptase.

47. The method of any one of claims 1 to 46, wherein the cell is a mammalian cell, a
5 human cell, an animal cell, a plant cell, a bacterial cell, a host cell infected by viruses, or a
host cell of infected by bacteria.

48. The method of any one of claims 1 to 47, wherein the organism is a mammal, a
human, a plant, a bacterium, a virus, a fungus, an alga, an archaeon or a protozoan.