

Non-Patent Document

[0008]

Non-Patent Document 1: Kageyama T, et al., Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR, J Clin Microbiol, 2003 Apr; 41(4): 1548-57

Non-Patent Document 2: Inspection and Safety Division of Food Safety Department of Pharmaceutical and Food Safety Bureau in Ministry of Health, Labor and Welfare, MHLW Notice No. 1105001 (November 5, 2003) Appendix "Norovirus Detection Method," Final Revision: MHLW Notice No. 0514004 (May 14, 2007)

Non-Patent Document 3: Inspection and Safety Division of Food Safety Department of Pharmaceutical and Food Safety Bureau in Ministry of Health, Labor and Welfare, MHLW Notice No. 1105001 (November 5, 2003) Appendix "Norovirus Detection Method," Final Revision: MHLW Notice No. 1022 Article 1 (October 22, 2013)

Non-Patent Document 4: Nishimura N, et al., Detection of noroviruses in fecal specimens by direct RT-PCR without RNA purification, J Virol Methods., 2010 Feb; 163(2): 282-286

~~SUMMARY OF THE INVENTION~~

PROBLEMS TO BE SOLVED BY THE INVENTION

[0009]

An object of the present invention is to provide a simple RNA virus detection method. Specifically, a method is provided,

in which RNA is, without thermal treatment, extracted from an RNA virus particle such as norovirus by means of one or more types of surfactant and a virus detection process is subsequently easily performed by RT-PCR reaction of released RNA. Moreover, a simpler RNA virus detection method is provided in such a manner that RNA extraction from the RNA virus particle and the RT-PCR are performed in the same container without opening/closing a lid of the container.

SOLUTIONS TO THE PROBLEMS

[0009a] In one aspect, the present disclosure refers to a method for detecting RNA virus in a specimen, comprising:

a step (1) of suspending the specimen in distilled water, saline, or buffer solution;

a step (2) of extracting centrifugal supernatant from suspension generated in the step (1);

a step (3) of mixing the centrifugal supernatant extracted in the step (2) with specimen treatment solution containing one or more types of surfactant;

a step (4) of mixing a mixture obtained in the step (3) with one-step RT-PCR reaction solution containing reverse transcriptase and DNA polymerase to induce RT-PCR; and

a step (5) of detecting a RT-PCR product wherein the specimen treatment solution contains hydroxide.

[0010]

The object of the present ~~invention~~disclosure is achieved by the following ~~aspects~~examples of the ~~invention~~disclosure.

[1]

The method for detecting RNA virus in a specimen, including

the step (1) of suspending the specimen in distilled water, saline, or buffer solution,

the step (2) of extracting centrifugal supernatant from suspension generated in the step (1),

the step (3) of mixing the centrifugal supernatant extracted in the step (2) with specimen treatment solution containing one or more types of surfactant,

the step (4) of mixing a mixture obtained in the step (3) with one-step RT-PCR reaction solution containing reverse

CLAIMS

1. A method for detecting RNA virus in a specimen,
comprising:

a step (1) of suspending the specimen in distilled water,
saline, or buffer solution;

a step (2) of extracting centrifugal supernatant from
suspension generated in the step (1);

a step (3) of mixing the centrifugal supernatant
extracted in the step (2) with specimen treatment solution
containing one or more types of surfactant;

a step (4) of mixing a mixture obtained in the step (3)
with one-step RT-PCR reaction solution containing reverse
transcriptase and DNA polymerase to induce RT-PCR; and

a step (5) of detecting a RT-PCR product

wherein the specimen treatment solution contains
hydroxide.

2. The method according to claim 1, wherein the RNA virus
is selected from a group consisting of norovirus, rotavirus,
rhinovirus, coronavirus, human immunodeficiency virus,
hepatitis C virus, Japanese encephalitis virus, and dengue
virus.

3. The method according to claim 1, wherein the RNA virus
is norovirus.

4. The method according to claim 3, wherein a norovirus genotype is Genogroup I (GI) or Genogroup II (GII).

5. The method according to any one of claims 1 to 4, wherein the specimen is derived from a sample selected from a group consisting of a biological sample, a biologically-derived sample, an environmental sample, and an environmentally-derived sample.

6. The method according to any one of claims 1 to 4, wherein the specimen is derived from a sample selected from a group consisting of an excrement sample, an excrement-derived sample, a vomit sample, and a vomit-derived sample.

7. The method according to any one of claims 1 to 6, wherein the surfactant is an anion surfactant.

8. The method according to claim 7, wherein the anion surfactant is one or more types of anion surfactant selected from a group consisting of alkyl sulfate, alkyl ether sulfate, docusate, a sulfonate fluoro surfactant, alkyl benzen sulphonate, alkyl aryl ether phosphate, alkyl ether phosphate, alkyl carboxylate, sodium lauroyl sarcosinate, a carboxylate fluoro surfactant, sodium cholate, and sodium deoxycholate.

9. The method according to claim 7, wherein the anion surfactant is alkyl sulfate.

10. The method according to claim 9, wherein the alkyl sulfate is sodium dodecyl sulfate or ammonium dodecyl sulfate.

11. The method according to any one of claims 1 to 10, wherein a concentration of the surfactant is 0.02% to 0.5% (w/v).

~~12. The method according to any one of claims 1 to 11, wherein the specimen treatment solution contains hydroxide.~~

~~13~~12. The method according to any one of claims 1 to 11~~claim 12~~, wherein the hydroxide is sodium hydroxide or potassium hydroxide.

~~14~~13. The method according to any one of claims 1 to 12~~claim 12 or 13~~, wherein a concentration of the hydroxide is 10 to 100 mM.

~~15~~14. The method according to any one of claims 1 to ~~14~~13, wherein a mixture ratio between the specimen and the specimen treatment solution in the step (3) is 1:3 to 6 in terms of a volume ratio.

~~16~~15. The method according to any one of claims 1 to ~~15~~14, wherein the reverse transcriptase is selected from a group consisting of AMV reverse transcriptase, MMLV reverse transcriptase, HIV reverse transcriptase, and variants thereof.

~~17~~16. The method according to any one of claims 1 to ~~16~~15, wherein the DNA polymerase is selected from a group consisting of Taq DNA polymerase, Tth DNA polymerase, KOD DNA polymerase, Pfu DNA polymerase, and variants thereof.

~~18~~17. The method according to any one of claims 1 to ~~17~~16, wherein the step (5) is performed by real-time measurement.

~~19~~18. The method according to any one of claims 1 to ~~18~~17, wherein the step (3) is performed under a temperature of 1°C to 60°C.

~~20~~19. The method according to any one of claims 1 to ~~19~~18, wherein the steps (3) to (5) are performed in an identical container.

~~21~~20. The method according to any one of claims 1 to ~~20~~19, wherein in the step (5), an amplification curve of the RT-PCR

product is measured using a fluorescence filter, and it is determined whether a presence of the RNA virus in the specimen is positive or negative.

~~22. An RNA virus detection kit comprising:
—— specimen treatment solution containing one or more types of surfactant; and
—— one-step RT-PCR reaction solution containing reverse transcriptase and DNA polymerase.~~

~~23. The kit according to claim 22, wherein the RNA virus is selected from a group consisting of norovirus, rotavirus, rhinovirus, coronavirus, human immunodeficiency virus, hepatitis C virus, Japanese encephalitis virus, and dengue virus.~~

~~24. The kit according to claim 22, wherein the RNA virus is norovirus.~~

~~25. The kit according to claim 24, wherein a norovirus genotype is Genogroup I (GI) or Genogroup II (GII).~~

~~26. The kit according to any one of claims 22 to 25, wherein the surfactant is an anion surfactant.~~

~~27. The kit according to claim 26, wherein the anion surfactant is one or more types of anion surfactant selected from a group consisting of alkyl sulfate, alkyl ether sulfate, docusate, a sulfonate fluoro surfactant, alkyl benzenesulphonate, alkyl aryl ether phosphate, alkyl ether phosphate, alkyl carboxylate, sodium lauroyl sarcosinate, a carboxylate fluoro surfactant, sodium cholate, and sodium deoxycholate.~~

~~28. The kit according to claim 26, wherein the anion surfactant is alkyl sulfate.~~

~~29. The kit according to claim 28, wherein the alkyl sulfate is sodium dodecyl sulfate or ammonium dodecyl sulfate.~~

~~30. The kit according to any one of claims 22 to 29, further comprising:~~

~~— a kit instruction manual.~~