CLAIMS

[Claim 1]

A method for detecting a target nucleic acid contained in a dried blood spot in a filter paper by real-time PCR, including the steps of (A) to (D) below:

(A) a step of adding the dried blood spot in a filter paper to a PCR reaction tube or plate, wherein the filter paper is a circular punched piece with a diameter of 1.2 mm to 2.0 mm or a punched piece containing whole blood in an amount of 0.95 v/v% to 6.6 v/v% based on the total amount of the reaction solution;

(B) a step of adding 20 to 50 μL of a PCR reagent to the PCR reaction tube or plate;

(C) a step of performing PCR reaction in the tube or plate which contains the PCR reagent and the dried blood spot in a filter paper and which is sealed with a cap; and

(D) a step of sequentially and optically detecting the target nucleic acid amplified by the PCR reaction.

[Claim 2]

The method for detecting a target nucleic acid according to claim 1, wherein the step of (B) is conducted after the step of (A).

[Claim 3]

The method for detecting a target nucleic acid according to claim 1 or 2, wherein the PCR reaction tube or plate is a tube in an eight-tube strip or a 96-well plate for PCR reaction.

[Claim 4]

The method for detecting a target nucleic acid according to any of claims 1 to 3, wherein the PCR reagent contains at least a primer, a polymerase, dNTPs and an

intercalator or a fluorescently labeled probe.

[Claim 5]

The method for detecting a target nucleic acid according to claim 4, wherein the amplified target nucleic acid is detected through detection of fluorescence emitted by irradiation of the intercalator with an excitation light.

[Claim 6]

The method for detecting a target nucleic acid according to claim 4, wherein the fluorescently labeled probe has a fluorescent substance and a quencher and includes a partial sequence complementary to a template of the nucleic acid amplification reaction, and the amplified target nucleic acid is detected through detection of fluorescence emitted by irradiation of the fluorescent substance with an excitation light.

[Claim 7]

The method for detecting a target nucleic acid according to any of claims 1 to 6, wherein the target nucleic acid is one or more gene fragments and/or genes selected from the group consisting of TRECs, KRECs and *SMN1*.

[Claim 8]

A method for quantifying a target nucleic acid by the method for detecting a target nucleic acid by real-time PCR according to any of claims 1 to 7, wherein the target nucleic acid is quantified using a standard below;

(1) a standard which contains an artificial nucleic acid including the sequence of the target nucleic acid and which is not contained in the dried blood spot in a filter paper, wherein the standard is dissolved directly in a reaction container and used.

[Claim 9]

Use of a quantification kit in a method for quantifying a target nucleic acid by the method for detecting a target nucleic acid by real-time PCR according to any of claims

1 to 7, wherein the quantification kit includes at least the following:

(1) a standard which contains an artificial nucleic acid including the sequence of the target nucleic acid and which is not contained in the dried blood spot in a filter paper, wherein the standard is dissolved directly in a reaction container and used.