

CLAIMS

[Claim 1]

A method for examining microorganisms to measure an
5 amount of microorganisms in a sample taken from the ballast
water containing microorganisms delivered from the ship and
collected into a transparent sample container, the
measurement of the amount of microorganisms in the sample
comprising a sampling preparation step of stirring and
10 mixing the sample and a fluorescence staining reagent to
prepare a sampling, and a calculation step of an amount of
microorganisms, which is a step of calculating an amount of
microorganisms from the number of light emissions in
fluorescence emission obtained by irradiation of the
15 sampling with excitation light of a specific wavelength,
wherein

the sampling preparation step comprises a fluorescence
staining step of stirring and mixing certain amounts of the
sample and the fluorescence staining reagent, a still
20 standing step of leaving a solution after the fluorescence
staining step to still stand for a certain time, and a
dilution step of diluting the solution after the still
standing step with a liquid that emits no fluorescence,

the fluorescence staining step comprises loading,
25 into the sample container, 1 to 10% by volume of the sample
based on the total volume of the sample container and also
loading, into the sample container, 1% by volume of the
fluorescence staining reagent based on the volume of the
sample, and stirring and mixing the sample and the
30 fluorescence staining reagent, and

a dilution liquid of 90 to 99% by volume of the
sample container based on the total volume thereof is

loaded in the dilution step into the sample container to make dilution.

[Claim 2]

5 The method for examining microorganisms according to claim 1, wherein FDA is used as the fluorescence staining reagent, and is added so that a concentration thereof in the sample before dilution is 0.01 mM.

10 [Claim 3]

 A method for examining microorganisms to measure an amount of microorganisms in a sample taken from the ballast water containing microorganisms delivered from the ship and collected into a transparent sample container, the
15 measurement of the amount of microorganisms in the sample comprising a sampling preparation step of stirring and mixing the sample and a fluorescence staining reagent to prepare a sampling, and a calculation step of an amount of microorganisms, which is a step of calculating an amount of
20 microorganisms from the number of light emissions in fluorescence emission obtained by irradiation of the sampling with excitation light of a specific wavelength, wherein

 the sampling preparation step comprises a fluorescence
25 staining step of adding, stirring, and mixing certain amounts of the sample and the fluorescence staining reagent to achieve the concentration of the reagent within the sampling of 0.01mM, a still standing step of leaving a solution after the fluorescence staining step to still
30 stand for a certain time, and a pH adjustment step of adding a pH adjuster to the solution after the still standing step, and

the pH adjustment step comprises adding the pH adjuster when a pH of the solution in the fluorescence staining step is 8.0, so that the pH is 6.0 in the pH adjustment step, wherein

- 5 FDA is used as the fluorescence staining reagent in the sampling preparation step.