

Isolation and identification of pathogenic bacteria associated with fish and fishery product- *escherichia coli*

Scope:

The International Standard describes a method for the enumeration of *E. coli* and coliform bacteria. It is based on membrane filtration and subsequent culture on a Chromogenic Coliform Agar (CCA) medium, and calculation of the target organism in the sample. The method is especially suitable for waters with low bacterial numbers producing less than 100 total colonies on CCA, such as drinking water, disinfected water, or water after appropriate treatment. It is not suitable for surface waters or shallow well-waters, due to possible background growth on the agar medium interfering with the reliable enumeration of *E. coli* and coliform bacteria.

Principle:

Filtration of a test portion of the sample through a membrane filter, which retains the organisms, and placement of the membrane filter on a chromogenic coliform agar plate. After incubation of the membrane filter at 36°C ($\pm 2^\circ\text{C}$) for 21°C ($\pm 3^\circ\text{C}$) h β -D-galactosidase positive colonies (pink to red) are counted as presumptive coliform bacteria that are not *E. coli*. To avoid false-positive results, the presumptive colonies shall be confirmed by a negative oxidase reaction. β -D-galactosidase and β -D-glucuronidase positive colonies (dark-blue to violet) are counted as *E. coli*. Total coliform bacteria are the sum of oxidase negative colonies with pink to red colour and all dark-blue to violet colonies.

Equipment and glassware:

- a) Autoclave
- b) Incubator at 36°C ($\pm 2^\circ\text{C}$)
- c) pH meter
- d) Membrane filtration equipment
- e) Membrane filters (pore size 0.45 μm ; sterile)
- f) Sterile forceps with rounded ends
- g) Sterile loop

Media/reagents:

- a) Chromogenic Coliform Agar

b) Oxidase reagent

c) Tryptone Soy Agar (TSA)

Sampling handling:

Samples have to be transported and stored at 5°C ($\pm 3^\circ\text{C}$). Under special circumstances, samples may be kept at 5°C ($\pm 3^\circ\text{C}$) for up to 24 h. In this case the storage time must be mentioned in the test report.

Procedure:

Water samples (100 ml or more) are filtered through membrane filters, which are then placed on the required media. Following the standard method, filters are placed on CCA and incubated at 36°C ($\pm 2^\circ\text{C}$) for 21h (± 3 h).

Colonies giving rise to a pink to red coloration of the medium are likely coliform bacteria that are not E.coli (positive reaction). Colonies giving a dark-blue to violet reaction are counted as E.coli. An oxidase test is performed with all, or at least 10 of the pink or red colonies selected (ISO 8199 (see footnote 15), by use of a commercial oxidase test or fresh oxidase reagent to confirm that the coliform bacteria are not E.coli.

By use of fresh oxidase reagent, a representative number of colonies (at least 10) is transferred to a pre-treated filter paper by use of a loop. A positive oxidase reaction is shown by appearance of dark blue colour within 30 s and is identified as E.coli. This is not observed for coliform bacteria since they are oxidase negative.

Subcultures are prepared where too many colonies are grown, because closeness of colonies or too small colonies will not ensure that the oxidase test is carried out with pure cultures. Subcultures would be prepared onto a non-selective agar, e.g. TSA at 36°C ($\pm 2^\circ\text{C}$) for 21h (± 3 h).

Performance criteria, quality assurance:

The use of positive controls, negative controls, and blanks is part of the test. Performance testing of the CCA should be carried out by use of control strains. The International Standard provides performance characteristics for the CCA. Data for the calculation of performance characteristics are usually collected from tests with potable water, thus it may be necessary for laboratories to carry out their own validation, depending on the type of water.

Expression of result:

Confirmed colonies are counted on the membrane filter, and calculated as numbers of coliforms and *E. coli* present in 100 ml water (or other filtered volume). The sum of all oxidase negative pink to red colonies, plus all dark-blue to violet colonies, is the count of coliform bacteria. *E. coli* are all dark-blue to violet colonies.

Result and Comment: