## Sampling and processing of samples for microbiological investigation

Samples must be handled and labelled in such a way as to guarantee both their legal and analytical validity. For official control, it is important that the laboratory receives a sample truly representative of the product that has not been damaged or changed during transport or storage. Incorrect sampling can lead to false negative or false positive results. Whenever possible, samples are submitted to the laboratory in the original unopened containers, or representative portions are transferred to sterile containers under aseptic conditions. Sample taking is always by use of sterile sampling equipment and use of aseptic technique. Containers used in sampling must be clean, dry, leak-proof, wide-mouthed, sterile, and of a size suitable for samples of the product. Sterile plastic bags (for dry, unfrozen materials only) or plastic bottles are useful containers for line samples. Each sample unit should be identified with a properly marked strip of masking tape. Whenever possible, at least 100 g for each sample unit should be obtained. Open and closed controls of sterile containers should be submitted with the sample. The samples should be delivered promptly to the laboratory, with the original storage conditions maintained as nearly as possible. For transport of samples, they should be kept under conditions which prevent alteration in the number of microorganism present. The fastest means of transport should be preferred. Frozen or refrigerated products are transported in approved insulated containers of rigid construction, so that they will arrive at the laboratory unchanged. Frozen samples are collected in pre-chilled containers. Refrigerated samples should be cooled in ice at 0-4°C, and transported in a sample chest with suitable refrigerant, capable of maintaining the sample at 0-4°C until arrival at the laboratory. When collecting liquid samples, an additional sample as a temperature control should be taken. The temperature of the control sample should be checked at the time of collection, and on receipt at the laboratory. The times and dates of collection and of arrival of all samples at the laboratory should be recorded. Dry or canned foods that are not perishable and are collected at ambient temperatures need not be refrigerated.

The following storage temperature should be observed:

Fresh and refrigerated products between 0 and 4°C, frozen or deep frozen products below -18°C fresh fish and sensitive products between 0 and 2°C, spoiled stable units between 0 and 4°C and transport in closed packaging.

**Microbiological methods:**

**Principle:**

Decimal dilutions of the sample are used to prepare pour plates in plate count agar (PCA). The Petri dishes are incubated at 30°C for 72 h after which the colonies growing in the medium are counted.

**Equipment:**

a) Autoclave

b) Incubator at 30ºC (±1°C)

c) Petri dishes

d) Pipettes (1 ml)

e) Water bath (capable of operating at 44–47°C) f) Colony counter

g) pH meter

h) Test tubes, flasks or bottles

**Media/Diluents:**

**(a) Use the diluent as specified in ISO 6887**

**General diluents**
Peptone salt solution
Buffered peptone water
The addition of 3.5–4.0% sodium chloride (isotonic with sea water) is recommended when examining raw, unprocessed marine fish for their natural marine microbial flora.
**Diluents for special purposes**
Peptone-salt solution with bromocresol purple solution may be used with certain acidic products so that adjustment of pH can be carried out without the use of a sterile pH probe. Peptone solution may be used for bivalve molluscs, gastropods and other shellfish.

**(b) Plate count agar**

**Sample preparation:**

- Use a 50g analytical unit of liquid or dry food to determine aerobic plate count value and MPN of coliforms.
- Add dilution water to blender jar containing 50 g analytical unit and blend 2 min. Make dilutions of original homogenate promptly, using pipettes that deliver required volume accurately. Not more than 15 min should elapse from the time.
- Prepare decimal dilutions, as appropriate, of food homogenate by transferring 10 ml of previous dilution to 90 ml of diluent. Shake all dilutions

**{Sample preparation is to be performed according to appropriate parts (applicable to fish and seafood) of:**

**EN ISO 6887-1:** Microbiology of food and animal feeding stuffs - Preparation of the test samples, of initial suspension and of decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions

**ISO 6887-3:** Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 3: Specific rules for the preparation of fish and fishery products

**ISO 7218:** Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.**}**

**Procedure:**

Having prepared decimal dilutions in the appropriate diluents, 1 ml aliquots of each appropriate dilution are transferred to two Petri dishes.

Dilutions are selected that will give colony counts of between 15 and 300 colonies per plate. Some 12–15 ml PCA that has been cooled to 44 – 47°C is poured into each Petri dish. The time between preparing the sample and pouring the medium into the Petri dishes should be no longer than 45 min. The contents of the Petri dishes are carefully mixed and then allowed to solidify on a level surface. If it is suspected that the colonies will overgrow the surface of the medium, about 4 ml of an overlay medium at 44 – 47°C can be poured on to the surface of the solidified, inoculated medium. The overlay is allowed to solidify. The Petri dishes are incubated inverted at 30ºC (±1°C) for 72 h (±3 h). Dishes should not be stacked more than six high, and must be separated from one another, and from the walls and top of the incubator. After incubation, the number of colonies present on each plate is counted and used to calculate the number of CFU per g or ml sample.

**Expression of results:**

The number of colonies present on each plate is counted and used to calculate the number of CFU per g or ml sample.

**Result and Comment:**