TITLE: MICROBIOLOGICAL ANALYSIS OF WATER FOR USE AND CONSUMPTION

INTRODUCTION

The microbiological analysis of the waster water includes, as basic determinations, the total microorganisms, total coliforms and faecal coliforms. There are specific culture mediums on the market for each one of these determinations, such as those provided by Millipore. A routine method based on these commercial culture mediums is described below.

BASIS

It is a question of separating the microorganisms from the water by filtration through specific filter membranes and then depositing the membranes with the residue in Petri dishes that contain a specific culture medium for growing the microorganisms that need to be determined, on a filter paper base. All the material that is utilized must be sterilized so that there is no external contamination. The sterilization of the material is carried out in an autoclave at 121ºC for 20 minutes.

The proposed culture mediums are liquids as these mediums are better at penetrating membrane pores and covering their surface.

REAGENTS

Culture Mediums

The commercial names of the culture mediums sold by Millipore are here indicated:

- Culture medium for the counting of total microorganisms: TGE (Tryptone Glucose Extract Broth).

- Culture medium for the counting of total coliforms: m-Endo Total Coliform Broth.

- Culture medium for the counting of faecal coliforms: m-FC Broth.

Instruments

Membrane filtration equipment

Millipore filters (filter disc of cellulose esters with 0.45 µm pore diameter)

Millipore membrane

Kitasato vacuum flask

Petri dishes

PROCEDURE

Membrane filtration methodology is followed, utilizing the appropriate culture medium for the counting of different types of organisms. In order to do so, 10ml of waste water is taken initially, it is put into a 100ml flask and filled to volume with sterile water. The dilution to be made of the sample depends on the degree of expected contamination. The stages necessary for the microbiological analysis are as follows:

Preparation of the Petri dishes

- Open the Petri dish, which contains a sterile absorbent base.

- Open a 2ml vial of the appropriate medium and pour onto the absorbent base, distributing it over the whole surface.

Sample filtration

Carried out in a glass vacuum flask (kitasato) onto which a plastic filter holder with a cellulose ester filter disc (0.45 μm pore diameter) is placed.

- The membrane is placed in the filter using sterilized tweezers.

- Take 10ml of the properly diluted sample to the filter holder.

- Connect the vacuum pump to filter the sample. The possible microorganisms will stay trapped in the filter.

- Disconnect the vacuum pump. With the flamed tweezers, take the filter and place in the Petri dish prepared for the microbiological determination.

<u>Incubation and colony count</u>

- The Petri dish containing the filter disc with the residue is taken to the oven, thermostatized to 37ºC, for the determination of total microorganisms and total coliforms; or to 44.5º for faecal coliforms, for a period of 24 hours.

- After the incubation, proceed to counting the colonies formed in each filter disc, expressing the results in millions of microorganisms per litre of water.

The colour of the colonies developed in the indicated mediums vary according to the microorganism:

- Total microorganisms: yellowish colour colonies.

- Total coliforms: reddish colonies with metallic green shine.

- Faecal coliforms: bluish colour colonies.