APPENDIX I

ENCINA WASTEWATER AUTHORITY LABORATORY

MICROBIOLOGICAL AND TOTAL SUSPENDED SOLIDS

QUALITY CONTROL DOCUMENT

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ENCINA WASTEWATER AUTHORITY LABORATORY
MICROBIOLOGICAL QUALITY CONTROL

This is a summary of the microbiological quality control procedures as defined by the Encina Wastewater Authority Laboratory Analytical Quality Assurance Program and In-house Laboratory Methods Manual.

STANDARD TOTAL COLIFORM MEMBRANE FILTER PROCEDURE (Standard Method 9222 B.)

Procedures:

Step 1 Use sterile forceps to place a membrane filter on the porous plate of the receptacle. Carefully place a matched funnel unit over the receptacle and lock it in place.

Step 2. Filter 100 ml of a well mixed sample shaken 25 times. It is recommended that sand and algal clumps be allowed to settle out of the well mixed sample (5 seconds settling) before measuring filtration volumes. Any unused sample should be stored at 4^{o} C. until a preliminary determination of countability can be made. (usually within 20 hours) Although not generally recommended, filtration of a smaller volume of a highly contaminated sample may be performed at that time.

Step 3 With the filter still in place, rinse the funnel and the cylinder with three 20 ml portions of sterile dilution water.Using sterile forceps that have been dipped in ethanol, flamed and cooled, transfer the filter with a rolling motion avoiding entrapment of air to the selected medium.

For details regarding the procedure for the enrichment technique consult the Quality Assurance Program and In-house Laboratory Methods Manual.

Definition: All bacteria that produce a red colony with a metallic sheen within 24 hours incubation at 35^{o} C. on an ENDO type medium are considered members of the coliform group. The sheen may cover the entire colony, or may appear only in a central area, or on the periphery.

Counting

a. Use the fluorescent magnifying lamp and a hand tally to count colonies. Use the grid marks on the surface of the filter to track position. Count all discreet sheen producing colonies and total the cfu/100 ml. Count each filter twice when numbers exceed 10 per filter.
b. Try holding the agar bed at various angles incident to the light source to be sure you are counting all fluorescing colonies.
c. You may want to refrigerate cultures with high background counts for 30 to 60 minutes before counting to deter the spread of confluence while aiding sheen discernment.
d. Organisms from undisinfected sources may produce sheen in 16 to 18 hours which subsequently may fade. Be sure to check colony development early in the day and monitor periodically for potential changes.
e. Do not report results as "To Numerous to Count" (TNTC). Instead, refilter a smaller volume of the original sample.
f. All Samples are to be read within a 22-24 hour period.

STANDARD TOTAL COLIFORM MEMBRANE FILTER PROCEDURE (continued)

Calculation of Coliform Density: For specific rules regarding counting of colonies, refer to Standard Methods 9222 B.,6.

Coliform Verification: For more details regarding Coliform verification consult the Quality Assurance Program and In-house Laboratory Methods Manual.

STANDARD FECAL COLIFORM MEMBRANE FILTER PROCEDURE

(Standard Method 9222 D.)

Procedures: Perform Steps 1-3 from previous procedures section.

Definition: Colonies produced by fecal coliform bacteria on m-FC media are various shades of blue. Pale yellow colonies may be atypical. Nonfecal colonies are gray to cream-colored.

Counting

a. Use the fluorescent magnifying lamp and a hand tally to count colonies. Use the grid marks on the surface of the filter to track position. Count all discreet blue colonies and total the cfu/100 ml. Count each filter twice when numbers exceed 10 per filter.

b. Pale yellow colonies may be verified for gas production in mannitol at 44.5^{o} C.

c. All Samples are to be read within a 22-24 hour period.

Calculation of Fecal Coliform Density Compute the density of sample quantities that produced MF counts within the desired range of 20 to 60 fecal coliform colonies. This density range is more restrictive than the 20 to 80 total coliform range because of larger colony size on m-FC medium. Calculate fecal coliform density as directed in Standard Methods Section 9222 B.,6. Record densities as fecal coliforms per 100 ml.

Coliform Verification: For more details regarding Coliform verification consult the Quality Assurance Program and In-house Laboratory Methods Manual.

ENTEROCOCCUS MEMBRANE FILTER PROCEDURE **(**EPA 1600)

Procedure:

Perform Steps 1-3 from previous procedures section.

Step 4 Cover and invert the dish or tray and incubate for 24 hours at 41.0^{o} C.

Definition: All bacteria that produce a gray colony with a blue halo within 24 hours of incubation are considered enterococcus. These colonies are more visible from the underside of the plate.

Counting: Use the fluorescent magnifying lamp and hand tally to count colonies. Use the grid marks on the filter to track position. Count each filter twice when numbers exceed 10 per filter. All Samples are to be read within a 22-24 hour period.

Calculation of Enterococci Density For specific rules regarding counting of colonies, refer to Standard Methods 9222 B.,6.

RESIDUE, NONFILTERABLE

TOTAL SUSPENDED SOLIDS DRIED AT 103-105°C

Standard Methods 2540 D

The following sample volumes are presented as a guideline for establishing both speed and accuracy of analyses: Influent, 100 ml; Primary, 200-250 ml; Outfall, 250-1000 ml; Aeration Influent, 200-250 ml; Secondary, 500-1000 ml. Industrial Waste samples 100-500 ml.

Interferences

Exclude large floating particles or agglomerates of nonhomogeneous materials from the sample. Limit sample size to that yielding no more than 200 mg residue. For samples high in dissolved solids, such as Receiving Water samples, thoroughly wash the filter to insure removal of the dissolved material.

1. Apparatus

   a. Muffle furnace
   b. Drying oven
   c. Analytical Balance
   d. Desiccator
   e. Whatman 934-AH Glass Fiber Filter Paper
   f. Aluminum or stainless steel planchet

2. Procedure

   a. Preparation of glass fiber filter paper

      1. Insert paper with wrinkled side up in filtration apparatus.
      2. Apply vacuum and wash paper with three successive portions of distilled water and continue suction until all traces of water are removed.
      3. Remove filter from filtration apparatus and place in a porcelain dish and ignite in the muffle furnace at 550°C for at least 15 minutes.
      4. Store in desiccator until needed and weigh immediately before use.

   b. Sample analysis

      1. Assemble filtering apparatus and filter and begin suction.
      2. Wet filter with a small volume of distilled water to seat it.
      3. Filter a measured volume of well mixed sample through the filter.
      4. Wash with three successive 10 ml volumes of distilled water, allowing complete drainage between washings and continue suction for about 3 minutes after filtration is complete.
      5. Carefully remove the filter from the filtration apparatus and transfer to an aluminum planchet for support.
      6. Dry for at least 1 hour at 103° to 105°C in the oven and cool in a desiccator to room temperature before weighing.
      7. Repeat the cycle of drying, cooling, desiccating and weighing until a constant weight is obtained or until the weight loss is less than 4% of the previous weight or 0.5 mg, whichever is less.

NOTE: When performing suspended solids on ocean samples, it is imperative that the filter paper be washed with copious amounts of distilled water after filtering the sample. This will remove all traces of residue from dissolved salts present in the sample. Wash each paper with at least 3, 500ml portions of rinse water before drying.

3. Calculation

$$\text{mg total suspended solids/L} = \frac{(A - B) \times 1000}{\text{ml of sample used}}$$

where: A = weight of filter + dried residue, mg, and
B = weight of filter, mg