Department of Microbiology
Water Culture Colony Count Procedure

I. Introduction

Requests for culturing of water samples requires a method of enumeration and possible identification of bacteria. This is accomplished by inoculating a known volume of sample onto a blood or trypticase soy agar plate and calculating colony forming units (CFU) per milliliter of specimen. The inoculation volume is 0.1 ml, spread over the surface of the agar with a sterile loop. Colonies are counted and that number is multiplied by 10 to obtain the CFU per milliliter.

II. Materials

Pipette with sterile tips
Blood agar plate
Sterile loop

III. Procedure

1. Inoculate the center of the appropriate agar plate with 100 ul of specimen using a 100-ul sterile pipette.
2. Streak through the inoculum with a sterile loop and then cross streak the entire plate as is done for a urine culture. Rotate the plate 45 °, re-streak, rotate the plate 45 ° again, and re-streak.
3. Incubate the plate agar side up at 35 ° in non-CO_2 for 48 hours.

IV. Reporting

1. After incubation, count the colonies, and multiply this number by 10 to obtain the CFU/mL.
2. Example for type II water: If the growth shows >100 colonies on the plate, the colony count would be reported as >1000 CFU/mL. "FAIL"
3. If less than 100 colonies on the plate (type II), report as XX CFU/mL. "Pass"
4. See CAP/CLSI limits for type I and type II water below.

Acceptable limits are dependent upon the type of specimen cultured and are determined by the submitting department.

Water samples from the following sources are assayed monthly. Results are provided to each laboratory section on a monthly basis. Each department is responsible for documentation of corrective action when necessary.

V. Definitions of Water Types

Type I water is used for procedures requiring maximum accuracy and precision such as atomic spectrometry, flame photometry, enzymology, blood gas, reference buffer solutions and reconstitution of lyophilized materials used as standards. Type I should always be the water of choice when a minimum level of ionized constituents is essential to test or when solutions are being prepared for trace metal analysis.

Type II water is recommended for most analytical or general laboratory testing such as hematological, serological and microbiological procedures as well as for

chemical methods not specifically stated or proven to require Type I quality. ASTM specifies preparation of Type II by distillation and recommends it whenever freedom from organic impurities is important.

Type III water is satisfactory for some general laboratory tests; for most qualitative analysis such as urinalysis, parasitology and histological procedures; for rinsing of analytical samples; preparation of stock solutions; and for washing or rinsing glassware (final glassware rinsing should be performed with the water type specified for the procedure performed). Distillation, mix bed deionization and reverse osmosis (with high quality feed water) can be used for Type III.

CAP / CLSI

Specifications:	Type I	Type II	Type III
Specific Conductance	<0.1	<0.5	<10.0
Specific Resistance	≥10.0	≥1.0	≥0.1
Colony Count (Colony Forming Units/mL)	≤10.0	$\leq 10^3$	NA
Maximum silicate content	0.05	0.1	1.0

VI. References

Preparation and Testing of Reagent Water in the Clinical Laboratory, 2nd Ed; NCCLS document C3-A2, Vol. 11 No. 13, August 1991
ASTM – The American Society for Testing and Materials
CAP – The College of American Pathologists
NCCLS – The National Committee for Clinical Laboratory Standards

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