

POUR PLATE ASSAY

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7/5/89, 28July 93, 21July95, 21July96, 18Aug97, 17July00, 28June02, 18July03, 10Aug05, 23July09, 22July10

http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Meat_Milk/MEAT_count.htm

RELATED PROTOCOLS: *Pour Plate Technique for Bacterial Enumeration*
 Dilutions
 Bacterial Contamination of Milk, Pour Plate Assay

The legal limit for the consumption of ground beef is 50 million bacteria/gm! Since the number of bacteria in a given sample may vary by several orders of magnitude, samples of these foodstuffs must be diluted in order to achieve the desired range of 30-300 CFU per plate. Typically, a gram of meat is suspended in 9 mL of water, and diluted to produce several dilutions from undiluted to 10^3 (or higher) for pour plating (greater dilution is necessary for highly contaminated samples). The following procedure is for that purpose:

SUPPLIES:

Ground meat to be tested
 (Record the source and expiration date.)
 4 sterile capped 16x150mm test tubes
 15 mL tubes of 45° C melted Plate Count Agar
 Sterile dH₂O in 3 repipets:
 3 mL, 7 mL and 9 mL
 3 sterile petri dishes
 paper towel

EQUIPMENT:

flame
 vortex
 balance
 stainless steel spatula
 16x150 mm test tube with 95% EtOH
 3 2 mL glass pipets
 1.0 mL displacement pipetter, sterile 1.0 mL tips
 45° C Hot Block, or water bath
 (deep enough to = agar depth.)
 colony counter with magnifying glass

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PREP OF MEAT SAMPLE, DILUTIONS, AND ALIQUOT ADDITION TO PLATES:

- Label the bottoms of three empty plates:** a) initials, b) date, c) specimen, d) aliquot volume (1.0 mL each) and e) dilution factor (10^1 , 10^2 or 10^3).
- Prepare meat dilution blanks** by labeling three 16x150mm sterile tubes with 1, 2 or 3 for the exponent of the dilution factors 10^1 , 10^2 or 10^3 . Repipet **9.0 mL sterile dH₂O** into each.
- Weigh out 1.00 g ground beef** sterily into an empty fourth sterile capped 16x150mm test tube. Add **3.0 mL** sterile dH₂O, vortex with a sterile spatula inserted to suspend well, then add **7.0 mL** more sterile dH₂O, vortex again to mix well.
- Prepare **serial dilutions** of the specimen in steps of 10^1 , vortex after each dilution to mix completely: (Note: you deliver 1 mL into the appropriate plate *and also* into the next dil tube.)
 - Use an inverted 2.0 mL pipet** to deliver 1.0 mL of meat suspension into the first dilution tube = 10^1 . (Inverted to prevent chunks from stopping up: draw meat suspension up to the 1.0 mark, deliver down to the 0.0 mark.)
 - Use a 2.0 mL pipet** to deliver first 1.0 mL of 10^1 dilution into the appropriately marked empty plate, then deliver the rest (1.0 mL) into the 10^2 dilution tube, vortex
 - Use a fresh 2.0 mL pipet** to deliver first 1.0 ml of 10^2 into the appropriately marked empty plate, then deliver the rest (1.0 mL) into 10^3 dilution tube, vortex.
 - Use 1.0 mL pipet**, deliver 1.0 mL of 10^3 dilution into marked empty plate.
- * . [Greater dilution factors may be achieved by using 0.1 mL into 9.9 mL in one or more of the serial dilutions, or plating 0.1 mL into the final petri dish.]

ADD MELTED AGAR TO MAKE POUR PLATE:

- Add 15 mL 45°C melted agar to each plate in turn, swirl to mix completely. Plunge the emptied tube immediately into warm water before agar solidifies to ease cleansing.
- When solid, invert plate and incubate 35°C for 48 hr.
- Count the colonies on the plates and calculate CFU per gram:

CFU/plate x dil'n factor x aliquot factor (1) x meat suspension factor (10 mL/g) = CFU/g meat

- Enter your results into the class table (your initials, the meat source, its expiration date, CFU/g)