Microbial assessment of Ready To Eat foods including traditional dairy products and probiotics

Introduction
Even hygienically produced foods contain micro-organisms, some of which can be food poisoning bacteria. Generally if foods are not further contaminated or mishandled (such as poor storage and temperature abuse) then there is a negligible threat to human health. However it is important that food producers monitor the bacteria in their foodstuffs. This practical uses the PHLS Guidelines for Ready-to-eat (RTE) foods as the criteria of acceptability (Comm Dis Pub Health 3(3):163-7, 2000).

There are a number of foods to be analysed, make a note of which one you are using as the criteria of acceptance vary according to the type of food. Additional tests have been included as PHLS is primarily relevant to food poisoning not food spoilage.

The purpose of this practical is threefold:
1. Test ready to eat foods, including dairy products, according to the PHLS (now HPA) Guidelines for Ready-to-Eat Foods (Communicable Disease and Public Health 3(3):163-167, 2000)
2. Determine the microbial flora of probiotics and similar fermented milk products
3. Assess the hygienic quality of milk.
   Note: Additional tests to those in the PHLS Guidelines have been included.

See PHLS tables for criteria for your particular food.

Additional notes:

The microbiological criteria for cheese is :

\[ Staphylococcus aureus \quad m=10^2, \quad M=10^3, \quad n=5, \quad c=2 \]

\[ Enterobacteriaceae \quad m=10^4, \quad M=10^5, \quad n=5, \quad c=2 \]

What do these sampling plans mean?

One would expect the probiotics to include one or possibly two strains of lactic acid bacteria only. Therefore the results on the MRS agar are crucial to this experiment.
Microbial assessment of ready to eat foods

Part 1: Gram stain of dairy product.
1a. Liquid dairy product: Smear a drop of the dairy product onto a clean glass slide.
1b. Solid dairy product (cheese, etc): Press a small amount of the dairy product between two clean glass slides. Separate and remove excess material with the edge of a slide.
2. De-fat with xylol for one minute, drain and dry in air.
3. Gram stain the preparation and report on the kinds of organisms and relative numbers present. Scan several fields of view as the organisms may not be evenly distributed.

Part 2: Viable count of Ready to eat foods
1. Dilution series preparation
Using sterile equipment weigh approx. 2.5g of product into a stomacher bag. Add 22.5ml saline and stomach for one minute. This is the $10^{-1}$ dilution. Further dilute by pipetting 1ml into 9ml of saline, to $10^{-3}$.

2. Pour plate inoculation
   - **Aerobic Colony Count**
     Pipette 1ml of each dilution (from $10^{-3}$ to $10^{-1}$ to save on pipette usage) into 3 sterile Petri dishes. Add 20ml of molten skim-milk plate count agar. Carefully swirl to mix the samples. Incubate at 30°C.
   - **Enterobacteriaceae**
     Aseptically pipette 1ml of each dilutions into 3 sterile Petri dishes. Add 15ml of molten VRBGA (NOT THE 4ml VOLUMES!) and carefully swirl to mix the sample. WHEN the agar has set gently add 4ml of molten VRBGA onto the surface and allow to solidify before incubating at 37°C.

3. Spread plate inoculation
   Pipette 0.1ml of each dilution onto the surface of three plates of:
   - St. aureus (BP) plates
   - Plate count agar (PCA)
   - Mann Rogosa Sharpe (MRS)
   - B. cereus plates (BC)
   Spread the samples using a sterile glass rod ($10^{-3}$ to $10^{-1}$).

4. Salmonella and Listeria (HAZARD GROUP 2 PATHOGEN!) detection
   Pathogens are often heat-injured in processed foods and must be resuscitated before isolation.
   - For *Salmonella* isolation: 25g of your food was added to 225ml of buffered peptone water (24h, 37°C) and then used to inculcate the RV broth (selective enrichment). You only need to streak for SINGLE colony isolation the RV broth onto the XLD and BGA plates.
   - For *Listeria* isolation: sample enrichment has already been done in Fraser broth, therefore you only need to streak the relevant broth onto the Oxford agar plate for SINGLE colony isolation.