

FOOD POISONING AND FOOD SAFETY

Microbial Contamination of Food

You are divided into speciality (expert) groups to investigate possible sources of microbial contamination of food.

Each bench pair is a speciality 'team', you may have to read and make additional notes from the literature provided. Work as a co-ordinated pair and ensure you bring all relevant information to the follow-up practical as you need to inform the other teams of your experiments and results.

The key sources of microbial contamination are :

- (a) Food ingredients; raw meats, vegetables, seasonings, etc.
- (b) Environment; water, air, and work surfaces
- (c) Personnel

Hence different teams will be specializing on different areas of possible microbial contamination.

Everybody:

Place your fingertips in the surface of two VRBGA plates and two Baird-Parker plates. These will be incubated at 37°C aerobically.

Bench W: Microbial flora of raw meats

Analyse two different raw meats.

Check whether your agar plates need pre-drying.

(a) Weigh 2.5 g of the raw meat provided into a Stomacher bag and add 22.5ml of saline. This is the 10^{-1} dilution. If you were not able to weight exactly 2.5 g then make a note of the value and use it to correct your calculations later.

(b) Homogenise using the Stomacher for 30 seconds.

(c) Allow the large particles to settle (fatty material will float to the surface) and then dilute your sample in 9ml sterile saline to 10^{-8} .

(d) Spread plate inoculation:

Dispense 0.1ml of each dilution (10^{-8} to 10^{-1} to save on pipette usage) onto the surface of dried :

Plate Count Agar (PCA), Malt Extract Agar (MEA), *B. cereus* agar,.

(e) Pour plate inoculation

Pipette 1ml of each dilution into 8 Petri dishes. Add approx. 20ml molten Tryptose sulphite cycloserine agar (TSCA, also known as Perfringens agar) stock vol. 200ml. Carefully swirl to mix the samples.

Pipette 1ml of each dilution into 8 Petri dishes. Add approx. 20ml molten VRBGA (stock vol. 200ml). Carefully swirl to mix the samples. NOTE: this will need a 4ml VRBGA overlay (stock vol. 50ml).

All media will be incubated aerobically at 37°C; except for the TSCA agar which will be incubated anaerobically and the MEA which will be incubated at 25°C.

Bench X: Microbial flora of vegetables

Each pair must analyse a different vegetable.

Check whether your agar plates need pre-drying.

(a) Weigh 2.5 g of the vegetable provided into a Stomacher bag and add 22.5ml of saline. This is the 10^{-1} dilution. If you were not able to weight exactly 2.5 g then make a note of the value and use it to correct your calculations later.

(b) Homogenise using the Stomacher for 30 seconds.

(c) Allow the large particles to settle and dilute your sample in 9ml sterile saline to 10^{-8} .

(d) Spread plate inoculation:

Dispense 0.1ml of each dilution (10^{-8} to 10^{-1} to save on pipette usage) onto the surface of dried :

Plate Count Agar (PCA), Malt Extract Agar (MEA), *B. cereus* agar.

(e) Pour plate inoculation

Pipette 1ml of each dilution into 8 Petri dishes. Add approx. 20ml molten Tryptose sulphite cycloserine agar (TSCA) stock vol. 200ml. Carefully swirl to mix the samples.

Pipette 1ml of each dilution into 8 Petri dishes. Add approx. 20ml molten VRBGA (stock vol. 200ml). Carefully swirl to mix the samples. NOTE: this will need a 4ml VRBGA overlay (stock vol. 50ml).

All media will be incubated aerobically at 37°C; except for the TSCA agar which will be incubated anaerobically and the MEA which will be incubated at 25°C.

Bench Y: Microbial flora of herbs and spices

Each pair must analyse a different herb or spices.

Check whether your agar plates need pre-drying.

(a) Weigh 2.5 g of the herb or spice provided into a Stomacher bag and add 22.5ml of saline. This is the 10^{-1} dilution. If you were not able to weight exactly 2.5 g then make a note of the value and use it to correct your calculations later.

(b) Homogenise using the Stomacher for 30 seconds.

(c) Allow the large particles to settle. Dilute your sample in 9ml sterile saline to 10^{-8} .

(d) Spread plate inoculation:

Dispense 0.1ml of each dilution (10^{-8} to 10^{-1} to save on pipette usage) onto the surface of dried :

Plate Count Agar (PCA), Malt Extract Agar (MEA), *B. cereus* agar.

(e) Pour plate inoculation

Pipette 1ml of each dilution into 8 Petri dishes. Add 20ml molten Tryptose sulphite cycloserine agar (TSCA) stock vol. 200ml. Carefully swirl to mix the samples.

Pipette 1ml of each dilution into 8 Petri dishes. Add 20ml molten VRBGA, stock vol. 200ml. Carefully swirl to mix the samples. NOTE: this will need a 4ml VRBGA overlay, stock vol. 50ml.

All media will be incubated aerobically at 37°C ; except for the TSCA agar which will be incubated anaerobically and the MEA which will be incubated at 25°C .

Bench Z: Rapid hygiene monitoring using ATP bioluminescence and transference of micro-organisms using cleaning cloths

(a) There are groups using raw meats, vegetables, etc. in the laboratory. You must ascertain whether any organisms are being left on worksurfaces using ATP bioluminescence.

Plan how to sample the 'clean' worksurfaces, i.e. before the meat (etc) is placed on them. ONLY use the ATP bioluminescence swabs and NOT the conventional swabs.

Sample 10 x 10 cm areas of the worksurfaces before and after the meat, etc. has been placed on it.

Sample 10 x 10 areas which have not been in contact with food, etc. for comparison.

(b) At your own bench.

Moisten 4 cotton wool swabs in saline and return to their packaging. Swab a work surface (10x10 cm area) and streak on nutrient agar, *B. cereus*, Pseudomonas agar and VRBGA agar plates (remember the 4ml overlay).

Wipe the surface clean with a cleaning cloth.

Reswab and streak onto predried nutrient agar, *B. cereus*, Pseudomonas agar and VRBGA agar plates.

Put Hycolin onto the surface and allow to act for 2 minutes. Then take another swab sample and streak as before onto nutrient agar, *B. cereus*, Pseudomonas agar and VRBGA plates (4ml overlay required).

Place the cleaning cloth in a Stomacher bag with 20ml saline and 'homogenise' for 30 seconds.

Dilute the Stomacher bag contents to 10^{-8} in 9ml saline.

In the sequence 10^{-8} to 10^{-1} , inoculate predried nutrient agar, *B. cereus*, Pseudomonas agar and VRBGA with 0.1ml of each dilution. Remember the 4ml overlay of the VRBGA and incubate 37°C aerobically.

Microbial Contamination of Food REQUISITION

There are 21 students most of whom will work in pairs, there is one group of three.

Food:

3 raw meats (minced meat, then two from sausages, raw chicken and beef cubes)

3 raw vegetables (**unwashed** potatoes, carrots, lettuce, cucumber)

3 herbs and spices (peppercorns, paprika, garlic, one other - not salted, nor onion)

Equipment:

Stomacher and bags (approx. 20)

Balances

Sterile spoons

Everyone (21) :

2 x VRBGA

2 x Baird Parker agar

Bench W: Microbial flora of raw meats (3 pairs)

3 raw meats

3 x 22.5ml saline

3 x 7 x 9ml saline

3 x 10 x 1ml pipette

3 x 8 PCA

3 x 8 MEA

3 x 8 Oxford

3 x 8 Perfringens agar

3 x 8 B. cereus agar

3 x 200ml VRBGA (plus 4ml overlay molten)

8 Sterile Petri dishes

Pipettes 0.1 and 1ml automatic plus tips

Glass rod spreader plus alcohol.

Bench X: Microbial flora of vegetables (3 pairs)

3 raw vegetables

3 x 22.5ml saline

3 x 7 x 9ml saline

3 x 10 x 1ml pipette

3 x 8 PCA

3 x 8 MEA

3 x 8 Oxford

3 x 8 Perfringens agar

3 x 8 B. cereus agar

3 x 20ml VRBGA (plus 4ml overlay molten)

Pipettes 0.1 and 1ml automatic plus tips

Glass rod spreader plus alcohol.

8 Petri dishes

Bench Y: Microbial flora of herbs and spices (3 pairs)

3 herbs and spices
3 x 22.5ml saline
3 x 7 x 9ml saline
3 x 10 x 1ml pipette
3 x 8 PCA
3 x 8 MEA
3 x 8 Oxford
3 x 8 Perfringens agar
3 x 8 B. cereus agar
3 x 200ml molten VRBGA (plus 4ml overlay molten)
Pipettes 0.1 and 1ml automatic plus tips
Glass rod spreader plus alcohol.
8 Petri dishes

**Bench Z: Rapid hygiene monitoring using ATP bioluminescence and Personnel
(three students = 1 group)**

20 ATP swabs (just check there's enough in the coldroom)
Biotrace luminometer

One (appropriate) cleaning cloth

12 x sterile swabs
50 ml saline for moistening swabs
11 x Nutrient agar plates
11 x Pseudomonas agar plates
11 x VRBGA agar plates (plus 4ml overlay molten)
11 x B. cereus agar plates

8 x 9ml saline

Automatic pipettes 0.1 and 1ml plus tips