Add the ingredients to the water and steam to dissolve. Distribute into screw-capped bottles and autoclave at 115°C for 10 minutes. For use, melt in steam and pour into sterile Petri dishes, using 15 mL of medium in each dish.

Lactose Peptone Water

Peptone	10 g
Sodium chloride	5 g
Lactose	10 g
Phenol red (0.4% m/V aqueous solution)	2.5 mL
(or Andrade's indicator)	(10 mL)
Distilled water	1 litre

Dissolve the ingredients in the water and adjust to pH 7.5. Add the phenol red indicator and distribute in 5 mL volumes into tubes containing inverted fermentation (Durham) tubes. Alternatively, adjust pH to between 6.8–7.0 and add the Andrade's indicator. Autoclave at 110°C for 10 minutes. Alternatively, steam (100°C) for 20 minutes on each of three successive days. Test for sterility by incubation at 37°C for 24 hours.

Andrade's Indicator

This is prepared by dissolving 0.5 g of acid fuchsin in 100 mL of distilled water. Add 17 mL of 1M sodium hydroxide solution and leave at room temperature overnight when the solution should appear straw coloured. If any brownish colour appears, add a little more sodium hydroxide solution and allow to stand again. This solution is strongly alkaline, and consequently, media to which it is added should be adjusted previously to a pH of about 6.8.

MEDIA FOR FAECAL STREPTOCOCCI

NOTE Sodium azide is highly toxic if ingested or inhaled and care must be taken when handling it. Solutions containing azide should not be discharged through metal pipework or drains as explosive compounds may be formed. Azides can be decomposed by treatment with an excess of nitrite solution.

Membrane Enterococcus Agar (Slanetz and Bartley 1957)

Tryptose	20 g
Yeast extract	5 g
Glucose	2 g
Dipotassium hydrogen phosphate	4 g
Sodium azide	400 mg
Agar	12 g
2,3,5-triphenyltetrazolium chloride (TTC) (1% m/V aqueous solution)	10 mL
Distilled water	1 litre

Steam the ingredients to dissolve. The pH should be 7.2 without the need for adjustment. Sterilize TTC by filtration, and pour the medium directly into Petri dishes without further sterilization. The medium should not be stored and re-melted, but poured plates may be kept at 4°C for up to 1 month if placed in a sealed container.

Glucose Azide Broth (Mallman and Seligmann 1950)

Double-strength medium:

Beef extract	9 g
Tryptone	30 g
Glucose	15 g
Sodium chloride	15 g
Sodium azide	400 mg
Bromocresol purple (1.5% m/V ethanolic solution)	2 mL
Distilled water	1 litre

Dissolve the ingredients in water by boiling. If necessary, adjust the pH so that after sterilization, the pH is 7.2 ± 0.1. Prepare single strength medium by diluting the double

strength medium with an equal amount of distilled water. Double strength medium (in 10 mL and 50 mL volumes) and single strength medium (in 5 mL volumes) should be dispensed into suitably sized containers. Sterilize by autoclaving at 121°C for 15 minutes.

Kanamycin Aesculin Azide Agar

Tryptone	20 g
Yeast extract	5 g
Sodium chloride	5 g
Sodium citrate	1 g
Aesculin	1 g
Iron(III) ammonium citrate	500 mg
Sodium azide	150 mg
Kanamycin sulphate	20 mg
Agar	12 g
Distilled water	1 litre

Sterilize by autoclaving at 121°C for 15 minutes. Final pH should be 7.0 ± 0.2.

Bile Agar 40% (Cowan 1993)

Ox bile (dehydrated)	40 g*
Serum (sterile)	50 mL
Nutrient agar	1 litre

Melt the nutrient agar, add the ox bile and mix to dissolve. Autoclave at 115°C for 10 minutes. Cool at about 55°C and add the serum aseptically. Mix gently and distribute in bottles, tubes or plates.

*NOTE this is equivalent to 400 mL fresh bile.

Bile Aesculin Azide Agar

Ox bile (dehydrated)	10 g
Tryptone	17 g
Peptone	3 g
Yeast extract	5 g
Sodium chloride	5 g
Sodium azide	150 mg
Iron(III) ammonium citrate	500 mg
Aesculin	1 g
Agar	12 g
Distilled water	1 litre

Steam to dissolve the ingredients and adjust to pH 7.0. Sterilize at 115°C for 10 minutes. Pour into Petri dishes and store at 4°C in sealed containers.

Glucose Phenolphthalein Broth (Clarke 1953)

Basal medium:

Meat extract	10 g
Peptone	10 g
Sodium chloride	5
Distilled water	1 litre

Dissolve the ingredients in the water and adjust the pH to between 7.2–7.4. Sterilize at 115°C for 10 minutes. Prepare a 20% m/V aqueous solution of glucose and sterilize by filtration. Add 50 mL aseptically to 950 mL of the basal medium.

Glycine buffer:

Glycine	600 mg
Sodium chloride	350 mg
Distilled water, freshly boiled	60 mL
0.1M Sodium hydroxide	40 mL

Dissolve the glycine and sodium chloride in the hot water and then add the sodium hydroxide.

Complete medium:

Glucose broth	900 mL
Glycine buffer	100 mL
Phenolphthalein (0.2% m/V aqueous solution)	5 mL

Mix and keep overnight in the refrigerator in a stoppered flask. Sterilize by filtration and distribute aseptically into sterile 5 mL screw-capped bottles leaving as little air space as possible. Incubate at 37°C overnight to check sterility and discard any bottle showing growth or not having a definite pink colour.

Salt Broth (Cowan 1993)
(Nutrient broth with 6.5% m/V sodium chloride)

Meat extract	10 g
Peptone	10 g
Sodium chloride	65 g
Distilled water	1 litre

Dissolve the ingredients in the water, adjust pH to between 7.2–7.4. Distribute in 5 mL volumes and sterilize at 115°C for 10 minutes.

MEDIA FOR SULPHITE-REDUCING CLOSTRIDIA

Tryptose—Sulphite—Cycloserine Agar (Harmon, Kautter and Peeler 1971, Hauschild and Hillsheimer 1974)

Basal medium:

Tryptose	15 g
Soya peptone	5 g
Yeast extract	5 g
Sodium metabisulphite	1 g
Iron(III) ammonium citrate	1 g
Agar	12 g
Distilled water	1 litre

The final pH should be 7.6 ± 0.2. Dispense 100 mL amounts in screw-capped bottles and sterilize by autoclaving at 121°C for 10 minutes. Cool to 50°C for immediate use or store in a cool dark place.

Antibiotic solution:

Prepare a 1% (m/V) solution of D-cycloserine in distilled water, sterilize by filtration and add 4 mL to 100 mL of the cooled molten agar base immediately before pouring plates to give a concentration of 400 $\mu g mL^{-1}$. The solution can be kept at 4°C for at least 2 weeks.

Egg yolk emulsion:

Prepare a 50% egg yolk emulsion by mixing the yolk of one fresh egg with an equal volume of sterile 0.9% saline solution. The yolk can be withdrawn from the yolk sac using a 10 mL syringe fitted with a wide bore needle. Add 4 mL of egg yolk emulsion to each bottle of medium as required. Egg yolk emulsion is commercially available.

Oleandomycin—Polymyxin—Sulphadiazine Perfringens Agar (Handford 1974)

Basal medium:

Tryptone	15 g
Yeast extract	5 g
Soya peptone	5 g
Sodium metabisulphite	1 g
Iron(III) ammonium citrate	1 g
Sulphadiazine (10% m/V solution in molar sodium hydroxide)	1 mL
Agar	12 g
Distilled water to	1 litre

The final pH should be 7.6 ± 0.2. Dispense the medium in 100 mL volumes in screw-capped bottles and sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C for immediate use or store in a cool, dark place.

Antibiotic solutions:

Concentrated stock solutions are prepared as follows:

Oleandomycin: Dissolve 0.5 g of oleandomycin phosphate in 100 mL sterile distilled water.

Polymyxin: Dissolve 500,000 international units (iu) of polymyxin B sulphate in 5 mL of sterile distilled water.

For each solution dilute 100-fold and add 1 mL of each solution to 100 mL molten basal medium cooled to 50°C to give final concentrations of 0.5 μgmL^{-1} oleandomycin phosphate and 10 $iumL^{-1}$ of polymyxin B sulphate. The concentrated solutions can be kept for up to 2 months at 4°C and the diluted solutions for up to one week at the same temperature.

Shahidi-Ferguson Perfringens Agar (Shahidi and Ferguson 1971)

Basal medium:	
Tryptose	15 g
Yeast extract	5 g
Iron(III) ammonium citrate	1 g
Sodium metabisulphite	1 g
Polymyxin B sulphate	30000 units
Kanamycin sulphate	12 mg
Agar	12 g
Distilled water	900 mL
Egg yolk emulsion (50% in sterile 0.9% sodium chloride)	100 mL

Sterilize the basal medium by autoclaving in 900 mL volumes at 121°C for 10 minutes. Cool to 50°C and add the egg yolk emulsion. The antibiotics may be added to the basal medium before or after sterilization. The final pH should be 7.6.

Overlay agar:

Use the basal medium without the addition of egg yolk emulsion but made up to 1000 mL with water. Dispense in 100 mL amounts and sterilize by autoclaving at 121°C for 10 minutes.

Differential Reinforced Clostridial Medium (Gibbs and Freame 1965)

Basal medium—single-strength:	
Peptone	10.0 g
Meat extract	10.0 g
Sodium acetate (hydrated)	5.0 g
Yeast extract	1.5 g
Soluble starch	1.0 g
Glucose	1.0 g
L(–) Cysteine hydrochloride	500 mg
Distilled water	1 litre

Add the peptone, meat extract, sodium acetate and yeast extract to 800 mL of the water. Dissolve the starch in the remaining 200 mL by first making a cold slurry with a little of the water, boiling the rest and stirring into the paste. Add the glucose and cysteine and dissolve. Adjust the pH to 7.1–7.2, distribute 25 mL volumes in screw-capped bottles and autoclave at 121°C for 15 minutes.

Basal medium—double-strength:

Prepare in the same way using twice the quantities of ingredients; distribute 10 mL volumes in universal screw-capped bottles and 50 mL volumes in 125 mL screw-capped bottles.

Sodium sulphite and iron(III) citrate solutions:

Prepare solutions of 4% (m/V) sodium sulphite (anhydrous) and 7% (m/V) iron(III) citrate in distilled water. Heat the latter to dissolve. Sterilize by filtration. The solutions may be stored at 4°C for up to 14 days.

Final medium:

On the day of use, mix equal volumes of the two solutions. To give final concentrations of 0.04% sodium sulphite and 0.07% iron(III) citrate, add 0.5 mL of the mixture to each 25 mL volume of the single-strength basal medium, freshly steamed and cooled to exclude dissolved oxygen. To each 10 mL and 50 mL volume of double-strength medium, add 0.4 mL and 2.0 mL respectively of the sulphite-iron mixture.

Crossley Milk Medium (Crossley 1941)

Skimmed-milk powder	100 g
Peptone	10 g
Bromocresol purple	100 mg
Distilled water	1 litre

The final pH should be 6.8 ± 0.2. Cream the ingredients with a little distilled water, and gradually dilute to 1 litre with continuous mixing. Dispense in 10 mL volumes and sterilize by autoclaving at 121°C for 5 minutes.

Litmus Milk

To milk prepared from skimmed-milk powder, add sufficient of a 10% (m/V) aqueous solution of litmus to give a blue-purple colour. Distribute in 5 mL volumes in screw-capped bottles and autoclave at 115°C for 10 minutes.

MEDIA FOR COLONY COUNTS

R2A Agar (Reasoner and Geldreich 1985)

Yeast extract	500 mg
Proteose peptone No. 3 or polypeptone	500 mg
Casamino acids	500 mg
Glucose	500 mg
Soluble starch	500 mg
Dipotassium hydrogen phosphate	300 mg
Magnesium sulphate heptahydrate	50 mg
Sodium pyruvate	300 mg
Agar	12 g
Distilled water	1 litre

Adjust pH to 7.2 with solid dipotassium hydrogen phosphate or potassium dihydrogen phosphate before adding agar. Heat to dissolve agar and sterilize at 121°C for 15 minutes.

Yeast Extract Agar

Yeast extract	3 g
Peptone	5 g
Agar	12 g
Distilled water	1 litre

Dissolve the yeast extract and peptone in the water. Adjust the pH to 7.3. Add the agar and steam to dissolve. Distribute in 15 mL amounts in tubes or containers or larger volumes in screw-capped bottles. Autoclave at 115°C for 10 minutes.

Modified King's A Broth (Medium 19, Drake 1966)

Peptone	20.0 g
Ethanol	25 mL
Potassium sulphate, anhydrous	10.0 g
Magnesium chloride, anhydrous	1.4 g
(or magnesium chloride hexahydrate)	(2.9 g)
Cetrimide (cetyltrimethyl ammonium bromide)	0.5 g
Distilled water to	1 litre

Steam to dissolve the ingredients. Distribute in screw-capped bottles and autoclave at 115°C for 10 minutes. Remove the bottles from the autoclave promptly after this sterilization cycle has been completed in order to prevent excessive loss of ethanol from the medium. Alternatively, add filter-sterilized ethanol aseptically to the sterile broth. The final pH should be approximately 7.2; no adjustment is usually necessary.

Asparagine Broth with Ethanol (Medium 10, Drake 1966)

	Single-strength medium	Concentration for use in Repli-dishes
L(–) Asparagine	2 g	3.2 g
L(–) Proline	1 g	1.6 g
Dipotassium hydrogen phosphate, anhydrous	1 g	1.6 g
Magnesium sulphate heptahydrate	500 mg	800 mg
Potassium sulphate	10 g	16.0 g
Ethanol	25 mL	40 mL
Distilled water to	1 litre	1 litre

Steam the ingredients to dissolve. Distribute the single strength medium in 4 mL volumes in screw-capped bijou bottles, and the 40 mL volumes in bottles of 50–100 mL capacity; dispense the concentrated medium in larger bottles for storage. Autoclave at 115°C for 10 minutes. Remove the bottles promptly from the autoclave after this sterilization cycle has been completed in order to prevent excessive loss of ethanol from the medium. Alternatively, add filter-sterilized ethanol aseptically to the sterile medium. The final pH should be approximately 7.2; no adjustment is usually necessary.

Milk Agar with Cetrimide (Brown and Foster 1970)

Yeast extract broth:

Yeast extract	75 mg
Peptone	2500 mg
Sodium chloride	1250 mg
Distilled water	250 mL

Dissolve the ingredients and adjust the pH to between 7.2–7.4. Autoclave at 115°C for 10 minutes.

Final medium:

Skimmed milk powder	100 g
Yeast extract broth	250 mL
Agar	15 g
Cetrimide (Cetyltrimethyl ammonium bromide)	300 mg
Distilled water	750 mL

Add the cetrimide and agar to the yeast extract broth and steam to dissolve. Mix th skimmed milk powder and distilled water. Autoclave both solutions separately at 121° for 5 minutes and remove them promptly from the autoclave after this sterilization cyc has been completed in order to prevent caramelization of the lactose in the milk. Cool t 50–55°C, mix aseptically and pour into Petri dishes. Store the plates at 4°C for not long than 4 weeks in sealed containers to prevent drying,

Thermophile-free skimmed milk powder for microbiological purposes is available commercially.

Pseudomonas Agar (based on Drake 1966, Mead and Adams 1977)

Basal medium:

Acid hydrolysed peptone or casein hydrolysate	10.0 g
Gelatine peptone	16.0 g
Potassium sulphate (anhydrous)	10.0 g
Magnesium chloride (anhydrous)	1.4 g
Agar	11.0 g
Distilled water	1 litre

Add 10 mL glycerol to the above ingredients and steam to dissolve; autoclave at 121°C for 15 minutes. Cool to approximately 50°C and add the following filter-sterilized solutions prepared as 1 % aqueous solutions:

(i) Cephaloridine Add 5 mL to 1 litre of basal medium to give a final concentraion of 50 mgL^{-1}:

(ii) Fucidin Add 1 mL to 1 litre of basal medium to give a final concentraion of 10 mgL^{-1}:

(iii) Cetrimide Add 1 mL to 1 litre of basal medium to give a final concentraion of 10 mgL^{-1}. It may be necessary to warm the solution gently to dissolve the cetrimide prior to filtration.

MEDIUM FOR AEROMONAS

Ampicillin-dextrin agar

Tryptose	5 g
Dextrin	10 g
Yeast extract	2 g
Sodium chloride	3 g
Potassium chloride	2 g
Magnesium sulphate heptahydrate	200 mg
Iron (III) chloride	100 mg
Bromothymol blue	80 mg
Agar	15 g
Sodium desoxycholate	100 mg
Ampicillin	10 mg
Distilled water	1 litre

Dissolve all the ingredients except the agar, ampicillin and desoxycholate in the water. Adjust the pH to 8.0. Add the agar and dissolve by boiling. Autoclave at 121°C for 15 minutes. Cool to about 55°C and add the ampicillin (10 mL of a freshly prepared solution containing 1 $mgmL^{-1}$) and desoxycholate (10 mL of a solution of 10 $mgmL^{-1}$). Mix well and pour plates. Keep plates in a refrigerator and use within 7 days. For marine or estuarine samples 0.05 g of 2,4-diamino-6,7-diisopropyl pteridine phosphate (O/129 phosphate) can be added to each litre of medium before autoclaving.

MEDIA FOR THERMOPHILIC CAMPYLOBACTERS

Preston Enrichment Broth (Bolton and Robertson 1982, Bolton et al 1983)

Basal medium:

Nutrient broth No 2	25 g
Iron(II) sulphate	250 mg
Sodium metabisulphite	250 mg
Sodium pyruvate	250 mg
Distilled water	950 mL

Dissolve and sterilize by autoclaving at 121°C for 15 minutes.

Blood supplement:

Add 50 mL saponin-lysed horse blood to the basal medium to give a final concentration of 5%.

Antibiotic solutions:

The following should be filter-sterilized:

(i) Trimethoprim: Dissolve 0.5 g trimethoprim lactate in 100 mL distilled water. Ac
2 mL of this solution to the basal medium to give a final concentration of 10 mgL-

(ii) Polymyxin: Dissolve 500,000 units of polymyxin B sulphate in 40 mL distill
water. Add 0.4 mL to the basal medium to give a final concentration
5000 units L^{-1};

(iii) Rifampicin: Dissolve 0.2 g of rifampicin in 20 mL methanol. Add 1 mL to the bas
medium to give a final concentration of 10 mgL^{-1};

(iv) Actidione: Dissolve 2 g of actidione in 10 mL of methanol and add 10 mL steri
distilled water. Add 1 mL to the basal medium to give a final concentration of 1
mgL^{-1}.

Preston Selective Agar (Bolton et al 1982)

Basal medium:	
Nutrient broth No. 2	25 g
Agar	12 g
Distilled water	950 mL

Sterilize by autoclaving at 121°C for 15 minutes.

Antibiotic solution and blood supplements:

Allow the basal medium to cool to approximately 50°C and add the antibiotics and blo
supplement as described for the Preston Enrichment Broth.

Blood-Free Selective Agar (Bolton, Hutchinson and Coates 1986)

Nutrient broth No. 2	25 g
Bacteriological charcoal	4 g
Casein hydrolysates	3 g
Sodium desoxycholate	1 g
Iron(II) sulphate	250 mg
Sodium pyruvate	250 mg
Agar	12 g
Distilled water	1 litre

Adjust pH to 7.4 and sterilize by autoclaving at 121°C for 15 minutes. Coo
approximately 50°C and add (i) 1 mL of a filter-sterilized aqueous solution contai
32000 mgL^{-1} of cefoperazone, to give a final concentration of 32 mgL^{-1}; (ii) 1 mL
filter-sterilized aqueous solution containing 10000 mgL^{-1} of amphotericin to give a
concentration of 10 mgL^{-1}.

MEDIA FOR *E. COLI* O157:H7

Modified buffered peptone water (Chapman et al 1993)

Peptone	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	3.5 g
Potassium dihydrogen phosphate	1.5 g
Distilled water	1 litre

Dissolve the ingredients in the water. Distribute in suitable volumes in screw-ca
bottles and autoclave at 121°C for 15 minutes. The final pH should be 7.2 ± 0.1. Usu
no adjustment is necessary.

Antibiotic solution:

The following can be purchased as sterile powders and dissolved in sterile water, or
sterilized.

(i) Vancomycin: Dissolve 80 mg of vancomycin hydrochloride in 10 mL distilled water. The solution may be stored at –20°C and used within 3 months. Add 1 mL of the solution to the basal medium to give a final concentration of 8 mgL^{-1}.

(ii) Cefixime: Dissolve 0.5 g of cefixime in 100 mL ethanol. Store at 4°C and use within 3 months. Dilute 1 mL to 100 mL in ethanol and add 1 mL to the basal medium to give a final concentration of 0.05 mgL^{-1}.

(iii) Cefsulodin: Dissolve 100 mg of cefsulodin (sodium salt) in 10 mL of distilled water. The solution may be stored at –20°C and should be used within 3 months. Add 1 mL of this solution to the basal medium to five a final concentration of 10 mgL^{-1}.

Modified Sorbitol MacConkey Agar (Zadik et al 1993)

Peptone	20.0 g
Sorbitol	10.0 g
Bile salts No 3	1.5 g
Sodium chloride	5.0 g
Neutral red	30 mg
Crystal violet	1 mg
Agar	15.0 g
Distilled water	1 litre

Add the ingredients to the water and steam to dissolve. Distribute in suitable volumes into screw-capped bottles and sterilize by autoclaving at 121°C for 15 minutes. Allow to cool and store in the dark at room temperature. The solutions can be stored for up to 1 month before use. Alternatively, cool to 50°C and add the selective supplements. Pour into sterile 55 or 90 mm Petri dishes and allow to set. Plates may be stored for up to 1 month at 4°C and should be dried in a suitable oven before use.

Selective supplements

The following can be purchased as sterile powders and dissolved in sterile water or filter-sterilized.

(i) Cefixime: Dissolve 0.5 g of cefixime in 100 mL ethanol. Store at 4°C and use within 3 months. Dilute 1 mL to 100 mL in ethanol and add 1 mL to the basal medium to give a final concentration of 0.05 mgL^{-1}.

(ii) Potassium tellurite: Dissolve 25 mg of potassium tellurite in 10 mL distilled water. The solution may be stored at –20°C and should be used within 3 months. Add 1 mL of this solution to the basal medium to give a final concentration of 2.5 mgL^{-1}.

MEDIA FOR SALMONELLAS

Buffered Peptone Water (Edel and Kampelmacher 1973)

Peptone	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	3.5 g
Potassium dihydrogen phosphate	1.5 g
Distilled water	1 litre

Dissolve the ingredients. Distribute in bulk quantities in screw-capped bottles and autoclave at 121 °C for 15 minutes. The final pH should be approximately 7.2. Usually, no adjustment is needed.

Rappaport-Vassiliadis Enrichment Broth (based on Vassiliadis et al 1976, Vassiliadis 1983)

Solution A:

Tryptone	5.0 g
Sodium chloride	8.0 g
Potassium dihydrogen phosphate	1.6 g
Distilled water	1 litre

Dissolve the ingredients in the distilled water by boiling on the day of use.

Solution B:	
Magnesium chloride hexahydrate	40.0 g
Distilled water	100 mL

Solution C:	
Malachite green	0.4 g
Distilled water	100 mL

For use, add 100 mL of Solution B and 10 mL of Solution C to 1 litre of freshly prepare Solution A. Dispense in 10 mL volumes and sterilize by autoclaving at 115°C for 1 minutes. The final pH should be approximately 5.2.

Selenite Broth (Hobbs and Allison 1945a,b)

Tryptone or Polypeptone	5 g
Mannitol	4 g
Disodium hydrogen phosphate	10 g
Sodium hydrogen selenite	4 g
Distilled water	1 litre

The final pH should be approximately 7.0. If the medium is to be used immediately, war gently to dissolve the ingredients. If stored before use then sterilize either by filtration by steaming for no more than 30 minutes.

The final medium should have a pale straw colour. Overheating produces a red precipita of selenium with a consequent loss of selectivity and the medium should be discarde

Brilliant Green Agar (ICMSF 1978)

Yeast extract	3.0 g
Proteose peptone or polypeptone	10.0 g
Sodium chloride	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
Phenol red (0.2% m/V aqueous solution)	40.0 mL
Brilliant green (0.5% m/V aqueous solution)	2.5 mL
Agar	20.0 g
Distilled water	960 mL

Steam to dissolve the ingredients and dispense in appropriate volumes. Sterilize autoclaving at 121°C for 15 minutes. The final pH after sterilization should be 6.9 ± (

Bismuth Sulphite Agar (Modified from Wilson and Blair 1927)

Wilson and Blair stock agar:	
Peptone	10 g
Beef extract	6 g
Iron(III) citrate	400 mg
Brilliant green (1% m/V aqueous solution)	1 mL
Agar	20 g
Distilled water	1 litre

Steam to dissolve the ingredients and sterilize by autoclaving at 121°C for 15 minu Cool to approximately 50°C and add solution B as described below.

Wilson and Blair solution B:	
Bismuth ammonium citrate	3 g
Sodium sulphite	5 g
Glucose	5 g
Disodium hydrogen phosphate	5 g
Distilled water	40 mL

Dissolve the ingredients by gently bringing to the boil. The colour of the solution should be grey-white. Any blackening of the solution indicates overheating and the solution should be discarded.

Cool rapidly in cold water and add 40 mL to 1 litre of basal medium.

After pouring, the plates should be held for two days in a refrigerator to oxidise prior to use.

Xylose Lysine Desoxycholate Agar (Taylor and Harris 1965)

Basal medium:

Lactose	7.5 g
Sucrose	7.5 g
Xylose	3750 mg
L(−)Lysine hydrochloride	5.0 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red (0.4% m/V aqueous solution)	20 mL
Agar	12.0 g
Distilled water	1 litre

Steam to dissolve ingredients and distribute in screw-capped bottles. Autoclave at 115°C for 10 minutes.

Solution A:

Sodium thiosulphate pentahydrate	34 g
Iron(III) ammonium citrate	4 g
Distilled water	100 mL

Heat gently to dissolve. Pasteurize at 60°C for 1 hour.

Solution B:

Sodium desoxycholate	10 g
Distilled water	100 mL

Dissolve and pasteurize at 60°C for 1 hour.

Final medium:

For use, melt the basal medium, cool to approximately 50°C and add aseptically 2.0 mL of Solution A per 100 mL of basal medium. Mix gently. With a separate pipette, add aseptically 2.5 mL of Solution B per 100 mL of basal medium. Mix and pour into Petri dishes. The final pH should be 7.3.

Lysine Iron Agar (Edwards and Fife 1961)

Peptone	5 g
Yeast extract	3 g
Glucose	1 g
L(−)Lysine	10 g
Iron(III) ammonium citrate	500 mg
Sodium thiosulphate pentahydrate	40 mg
Bromocresol purple	20 mg
Agar	15 g
Distilled water	1 litre

Steam to dissolve ingredients and dispense in 5 mL volumes in small test tubes. Autoclave at 115°C for 10 minutes. Cool in a sloping position to give agar slopes with a deep butt. The final pH should be approximately 6.7. No adjustment is usually necessary.

Triple Sugar Iron Agar (IAMS 1958)

Lab-Lemco (beef extract)	3 g
Yeast extract	3 g
Peptone	20 g
Sodium chloride	5 g
Lactose	10 g
Sucrose	10 g
Glucose	1 g
Iron(III) citrate	300 mg
Sodium thiosulphate pentahydrate	300 mg
Phenol red (0.4% m/V aqueous solution)	6 mL
Agar	15 g
Distilled water to	1 litre

Steam to dissolve ingredients. Add the indicator. Dispense in 5 mL volumes into small test tubes and autoclave at 115°C for 10 minutes. Cool in a sloping position to form a slope with a deep butt. The final pH should be approximately 7.4. No adjustment is usually necessary.

Urea Broth

Broth base:

Peptone	1 g
Glucose	1 g
Disodium hydrogen phosphate	1 g
Potassium dihydrogen phosphate	800 mg
Sodium chloride	5 g
Phenol red (0.4% m/V aqueous solution)	1 mL
Distilled water	1 litre

Dissolve the ingredients and check the pH is approximately 6.8. Dispense in 95 mL volumes in screw-capped bottles and autoclave at 115°C for 10 minutes.

For use, add aseptically 5 mL of 40% (m/V) solution of urea sterilized by filtration to 95 mL Broth Base. Dispense aseptically in 2–3 mL volumes in sterile bijou bottles.

MEDIA FOR SHIGELLAS

Modified Hajna GN Enrichment Broth

Tryptone	20 g
Glucose	1 g
D-Mannitol	2 g
Sodium citrate	5 g
Sodium desoxycholate	500 mg
Dipotassium hydrogen phosphate	4 g
Potassium dihydrogen phosphate	1500 mg
Sodium chloride	5 g
DL serine	1 g
Distilled water	1 litre

The ingredients are heated to dissolve, distributed into universal bottles in 10 mL amounts and steamed (100°C) for 30 minutes. The final pH should be 7.2.

Hektoen Agar (King and Metzger 1968)

Yeast extract	3 g
Proteose peptone	12 g
Lactose	12 g
Sucrose	12 g
Salicin	2 g
Iron(III) ammonium citrate	1500 mg
Acid fuchsin	100 mg
Bromothymol blue	65 mg
Bile salts No 3	9 g
Sodium chloride	5 g
Sodium thiosulphate pentahydrate	5 g
Agar	14 g
Novobiocin	15 µg
Distilled water	1 litre

Suspend the ingredients in 1 litre of distilled water and soak for 10 minutes. Heat gently and allow to boil for a few seconds to dissolve the agar. Do not autoclave. Cool to 60°C and pour plates.

Modified Desoxycholate Citrate Agar

Tryptone	20.0 g
Lactose	10.0 g
Sodium thiosulphate pentahydrate	6.8 g
Iron(III) ammonium citrate	800 mg
Neutral red	30 mg
Sodium desoxycholate	500 mg
DL serine	1.0 g
Tetracycline hydrochloride	32 mg
Agar	14.0 g
Distilled water	1 litre

The ingredients are dissolved by heating gently until boiling. The medium is then cooled to 50°C and the tetracycline hydrochloride (which has been separately sterilized by filtration through a 0.2 µm membrane filter) is added to give a final concentration of 32 mgL^{-1}. The medium is then poured into sterile Petri dishes.

MEDIUM FOR STAPHYLOCOCCI

Modified lipovitellin-salt-mannitol agar (Stengren and Starzyk 1984)

Beef extract	1.0 g
Proteose peptone	10.0 g
D(–)mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	25 mg
Sodium azide	50 mg
Sterile egg yolk	20 mL
Distilled water	1 litre

Dissolve all the ingredients except the egg yolk by boiling. Adjust the pH, if necessary, so that after sterilization the pH is 7.4 ± 0.2. Sterilize by autoclaving at 121°C for 15 minutes, allow to cool to 50°C and then aseptically add the egg yolk. Mix to distribute the egg yolk evenly and distribute in Petri dishes. If the medium is only to be used for membrane filtration the egg yolk may be omitted.

MEDIA FOR VIBRIOS

Alkaline peptone water

Peptone	10 g
Sodium chloride	5 g
Distilled water	1 litre

Adjust pH to 8.6 with molar sodium hydroxide solution. Sterilize by autoclaving at 121°C for 15 minutes.

Thiosulphate Citrate Bile Salt Sucrose Agar (Kobayashi et al 1963)

Yeast extract	5 g
Peptone	10 g
Sodium thiosulphate pentahydrate	10 g
Sodium citrate	10 g
Ox bile	8 g
Sucrose	20 g
Sodium chloride	10 g
Iron(III) citrate	1 g
Bromothymol blue	40 mg
Thymol blue	40 mg
Agar	14 g
Distilled water	1 litre

Suspend the ingredients in distilled water and dissolve by boiling. Do not autoclave. T
final pH should be 8.6 ± 0.2.

MEDIA FOR YERSINIAS

Tris-buffered peptone

Peptone	10.0 g
Tris-(hydroxymethyl) aminomethane	12.1 g
Sodium chloride	5.0 g
Distilled water	1 litre

Dissolve the ingredients in the water. Adjust pH to 8.0 with 10M hydrochloric ac
Sterilize by autoclaving at 121°C for 15 minutes.

Potassium hydroxide/sodium chloride solution

Potassium hydroxide	500 mg
Sodium chloride	500 mg

Dissolve in distilled water and make up to 100 mL.

Cefsulodin Irgasan Novobiocin Agar (Mair and Fox 1986)

Special peptone	20.0 g
Yeast extract	2.0 g
D(–)Mannitol	20.0 g
Sodium pyruvate	2.0 g
Sodium chloride	1.0 g
Magnesium sulphate	10 mg
Sodium desoxycholate	500 mg
Neutral red	30 mg
Crystal violet	1 mg
Agar	12.5 g
Distilled water	1 litre

Antibiotic supplement:

Cefsulodin	7.50 mg
Irgasan	2.00 mg
Novobiocin	1.25 mg

Dissolve 29 g of base in 500 mL of distilled water by bringing slowly to the boil. Ster
by autoclaving at 121°C for 15 minutes and cool to 50°C. Re-constitute antib
supplement by adding 2 mL of sterile distilled water and 1 mL ethanol to the a
ingredients, mix gently and add aseptically to 500 mL molten agar. Pour plates, allo
dry prior to use.

REAGENTS

Kovacs' Reagent (Kovacs 1928)

p-Dimethylaminobenzaldehyde	5 g
Amyl alcohol (analytical grade reagent, free from organic bases)	75 mL
Hydrochloric acid (concentrated)	25 mL

Dissolve the aldehyde in the alcohol. Add the concentrated acid with care. Protect from light and store at 4°C.

NOTE: The reagent should be pale yellow to light brown in colour; some samples of amyl alcohol are unsatisfactory, and give a dark colour with the aldehyde.

Oxidase Reagent

Tetramethyl-p-phenylenediamine hydrochloride	0.1 g
Distilled water	10 mL

This reagent does not keep and it must therefore be freshly prepared for use in small amounts each time that it is needed.

Ringer's Solution (Quarter-strength)

Sodium chloride	2.25 g
Potassium chloride	105 mg
Calcium chloride, anhydrous	120 mg
Sodium bicarbonate	50 mg
Distilled water	1 litre

Dissolve the ingredients and dispense in convenient volumes. Sterilize by autoclaving at 121°C for 15 minutes.

Filter-Aid (Hammarstrom and Ljutove 1954)

Hyflo-supercel	1 g
Distilled water	15 mL

Add the filter-aid to the water in a screw-capped universal bottle and autoclave at 121°C for 15 minutes. Prepare several bottles at the same time and store until required.

Appendix C: Tables of Most Probable Numbers

These tables indicate, from the various combinations of positive and negative reactions for the different volumes examined, the estimated number of bacteria in 100 mL of sample. It is important to realise that this, the most probable number (MPN), is only an estimate based on statistical probability and that the true number may lie within a range of values (see section 6.3.2.3). Approximate 95% confidence intervals, which demonstrate a range of possible counts which could yield the tabulated number of positive tubes, have been published (Swaroop 1951). A computing procedure for estimating these approximate intervals for other dilution series has been published (Hurley and Roscoe 1983). These intervals can be explored more accurately (Tillett and Coleman 1985) but are seldom of practical use when reporting results, because they apply to the accuracy of the method and not the likely range of organisms at the sampling source. The tables in this Appendix give the MPN and the MPR as described in section 6.3.2.3. The ranges of numbers are close contenders for the title MPN and each count has a probability of being the true count which is at least 0.95 times as large as the probability that the MPN is correct. The MPR illustrates situations where the method becomes imprecise, particularly when nearly all the tubes show growth and, in practice, a further dilution should have been made to give a clearer estimate of the MPN.

Tables 10 and 11 give the MPN (and where applicable the MPR) for a 6-tube series containing 1×50 mL and 5×10 mL volumes; and an 11-tube series with 1×50 mL, 5×10 mL and 5×1 mL volumes respectively. Table 12 relates to a 15-tube series with 5×10 mL, 5×1 mL and 5×0.1 mL volumes, but only the likely combinations of positive and negative reactions are listed. For example, multiple positive reactions in the 0.1 mL tubes would not be expected if all the 10 mL and 1 mL tubes are negative and so combinations of 0,0,2 etc are not tabulated. If these unlikely combinations are observed in practice with a frequency greater than about 1 in 100 tests, it is an indication that the statistical assumptions underlying the MPN estimation are not being fulfilled (Swaroop 1951, Woodward 1957, Man 1975). For example, the organisms may not have been uniformly distributed throughout the sample, or toxic substances may have been present.

Calculation of MPN Values

Record the number of positive reactions for each set of tubes and, from the relevant table read the MPN of organisms present in 100 mL of the sample.

Where a series of dilutions of the sample is used, apply the following rules:

(i) Use only three consecutive sets of dilutions for calculating the MPN.

(ii) Select, wherever possible, three consecutive dilutions where the results are neither all positive nor all negative. The most efficient statistical estimate will result when about half the tubes are positive. (See examples a, b and c in Table 9).

Table 9 Examples of the derivation of the MPN from the numbers of positive reactions in a series of dilutions.*

Example in text	Volume of sample (mL)					MPN per 100 mL
	10	1	0.1	0.01	0.001	
a	**5**	**3**	**2**	0		140
b	5	**5**	**3**	**2**	0	1400
c	5	**5**	**2**	**0**	0	500
d	**3**	**1**	**0**	0		9
e	**0**	**1**	**0**	0		2

*Numbers in bold type indicate which results should be used in determining the MPN values.

(iii) If less than three sets of dilutions give positive results, start with the set containing the largest volume of the sample (see example d in Table 9).

(iv) If only one set of the tubes gives a positive reaction, use this dilution and the one higher and one lower (see example e in Table 9).

Table 10 MPN and MPR per 100 mL of sample for a 6-tube series containing 1 × 50 mL and 5 × 10 mL volumes.

Number of tubes giving a positive reaction		MPN per 100 mL	MPR* per 100 mL
1 × 50 mL	5 × 10 mL		
0	0	none found	
0	1	1	
0	2	2	
0	3	3	
0	4	4	4–5
0	5	6	
1	0	1	
1	1	2	
1	2	5	4–5
1	3	9	8–10
1	4	15	13–18
1	5	>18**	–

*MPR gives counts which are at least 95% as probable as the MPN in being the correct number (see sections 6.3.2.3 and 7.6.2)
**There is no discrimination when all tubes show growth; the theoretical MPN is infinity. The true count is likely to exceed 18.

Table 11 MPN and MPR per 100 mL of sample for an 11-tube series of 1 × 50 mL, 5 × 10 mL and 5 × 1 mL volumes.

Number of tubes giving a positive reaction			MPN per 100 mL	MPR* per 100 mL
1 × 50 mL	5 × 10 mL	5 × 1 mL		
0	0	0	none found	
0	0	1	1	
0	1	0	1	
0	1	1	2	
0	2	0	2	
0	2	1	3	
0	3	0	3	
1	0	0	1	
1	0	1	2	
1	1	0	2	
1	1	1	4	
1	1	2	6	
1	2	0	4	4–5
1	2	1	7	6–7
1	2	2	9	9–10
1	3	0	8	7–9
1	3	1	10	10–11
1	3	2	13	12–15
1	3	3	17	15–18
1	4	0	12	11–14
1	4	1	16	15–19
1	4	2	21	19–24
1	4	3	27	24–30
1	4	4	33	30–38
1	5	0	23	20–27

(**Table 11** continued)

Number of tubes giving a positive reaction			MPN per 100 mL	MPR* per 100 mL
1 × 50 mL	5 × 10 mL	5 × 1 mL		
1	5	1	33	29–40
1	5	2	53	44–65
1	5	3	91	75–110
1	5	4	160	134–190
1	5	5	>180**	

*MPR gives counts which are at least 95% as probable as the MPN in being the correct number (see sections 6.3.2.3 and 7.6.2)
**There is no discrimination when all tubes show growth; the theoretical MPN is infinity. The true count is likely to exceed 180.

Table 12 MPN and MPR per 100 mL of sample for a 15-tube series containing 5 × 10 mL, 5 × 1 mL and 5 × 0.1 mL test volumes.

Number of tubes giving a positive reaction			MPN per 100 mL	MPR* per 100 mL
5 × 10 mL	5 × 1 mL	5 × 0.1 mL		
0	0	0	none found	
0	0	1	2	
0	1	0	2	
1	0	0	2	
1	0	1	4	
1	1	0	4	
1	2	0	5	
2	0	0	4	
2	0	1	5	
2	1	0	5	
2	1	1	7	
2	2	0	7	7–9
2	3	0	11	
3	0	0	7	
3	0	1	9	
3	1	0	9	
3	1	1	13	
3	2	0	13	
3	2	1	16	14–16
3	3	0	16	14–16
4	0	0	11	11–13
4	0	1	14	14–16
4	1	0	16	14–16
4	1	1	20	18–20
4	2	0	20	18–22
4	2	1	25	23–27
4	3	0	25	23–27
4	3	1	31	29–34
4	4	0	32	29–34
4	4	1	38	34–41
5	0	0	22	20–23
5	0	1	29	25–34
5	0	2	41	36–50
5	1	0	31	27–36
5	1	1	43	36–50
5	1	2	60	50–70
5	1	3	85	70–95
5	2	0	50	40–55
5	2	1	70	60–80

(**Table 12** continued)

Number of tubes giving a positive reaction			MPN per 100 mL	MPR* per 100 mL
5 × 10 mL	5 × 1 mL	5 × 0.1 mL		
5	2	2	95	80–110
5	2	3	120	105–135
5	3	0	75	65–90
5	3	1	110	90–125
5	3	2	140	120–160
5	3	3	175	155–200
5	3	4	210	185–240
5	4	0	130	110–150
5	4	1	170	150–200
5	4	2	220	190–250
5	4	3	280	240–320
5	4	4	345	300–390
5	5	0	240	200–280
5	5	1	350	290–420
5	5	2	540	450–600
5	5	3	910	750–1100
5	5	4	1600	1350–1900
5	5	5	>1800**	

*MPR gives counts which are at least 95% as probable as the MPN in being the correct number (see sections 6.3.2.3 and 7.6.2)
**There is no discrimination when all tubes show growth; the theoretical MPN is infinity. The true count is likely to exceed 1800.

Table 13 95% Confidence Intervals (95% CI) for the (unobserved) count from the second half-sample for the observed count from the first half-sample of 0 to 100 (see section 6.2.6)

Observed count in first half-sample	95% CI for unobserved count in second half-sample	Observed count in first half-sample	95% CI for unobserved count in second half-sample
0	0–5	24	12–40
1	0–7	25	13–41
2	0–9	26	13–43
3	0–11	27	14–44
4	0–12	28	15–45
5	0–14	29	16–47
6	1–16	30	16–48
7	1–17	31	17–49
8	2–19	32	18–50
9	2–20	33	19–52
10	3–22	34	19–53
11	3–23	35	20–54
12	4–24	36	21–55
13	5–26	37	22–56
14	5–27	38	22–58
15	6–28	39	23–59
16	6–30	40	24–60
17	7–31	41	25–61
18	8–32	42	26–63
19	8–34	43	26–64
20	9–35	44	27–65
21	10–36	45	28–66
22	10–38	46	29–67
23	11–39	47	29–69

(**Table 13** continued)

Observed count in first half-sample	95% CI for unobserved count in second half-sample	Observed count in first half-sample	95% CI for unobserved count in second half-sample
48	30–70	75	52–102
49	31–71	76	53–103
50	32–72	77	54–104
51	33–73	78	55–105
52	33–75	79	56–106
53	34–76	80	57–107
54	35–77	81	58–108
55	36–78	82	58–110
56	37–79	83	59–111
57	38–80	84	60–112
58	38–82	85	61–113
59	39–83	86	62–114
60	40–84	87	63–115
61	41–85	88	63–117
62	42–86	89	64–118
63	42–88	90	65–119
64	43–89	91	66–120
65	44–90	92	67–121
66	45–91	93	68–122
67	46–92	94	69–123
68	47–93	95	69–125
69	47–95	96	70–126
70	48–96	97	71–127
71	49–97	98	72–128
72	50–98	99	73–129
73	51–99	100	74–130
74	52–100		

Appendix D: List of Abbreviations used in this Report:

Organisations and personnel

AVAB	Automatic Vending Association of Britain
CCDC	Consultant in Communicable Disease Control
CDSC	Communicable Disease Surveillance Centre (PHLS)
CD(S)U	Communicable Diseases (Scotland) Unit
DH	Department of Health
DHSS	Department of Health and Social Security
DoE	Department of the Environment
DPH	Director of Public Health
DT	Department of Transport
DWI	Drinking Water Inspectorate
EC	European Community
EHO	Environmental Health Officer
ICD	Infection Control Doctor
MAFF	Ministry of Agriculture, Fisheries and Food
MOEH	Medical Officer for Environmental Health
NHS	National Health Service
NRA	National Rivers Authority
NWC	National Water Council
PHLS	Public Health Laboratory Service
SCA	Standing Committee of Analysts (DoE)
SHHD	Scottish Home and Health Department
WAA	Water Authorities Association
WHO	World Health Organization
WO	Welsh Office
WOAD	Welsh Office Agriculture Department

Media, reagents etc

ABE	Asparagine Broth and Ethanol
ADA	Ampicillin Dextrin Agar
AODC	Acridine Orange Direct Count
BAA	Bile Aesculin Agar
BiA	Bile Agar
BlA	Blood Agar
BGA	Brilliant Green Agar
BGLBB	Brilliant Green Lactose Bile Broth
BPW	Buffered Peptone Water
BSA	Bismuth Sulphite Agar
CI	Confidence Interval
CINA	Cefsulodin Irgosan Novobiocin Agar
CFU	Colony Forming Unit
CMM	Crossley's Milk Medium
DRCM	Differential Reinforced Clostridial Medium
GAB	Glucose Azide Broth
GPB	Glucose Phenolphthalein Broth
HA	Hektoen Agar
HEB	Hajna GN Enrichment Broth
KAAA	Kanamycin Aesculin Azide Agar
LIAS	Lysine Iron Agar Slope
LMM	Litmus Milk Medium
LPW	Lactose Peptone Water
LTLB	Lauryl Tryptose Lactose Broth
M-5LSMA	Modified Lipovitellin-Salt-Mannitol Agar
MA	MacConkey Agar
MAC	Milk Agar with Cetrimide
MBPW	Modified Buffered Peptone Water

MDCA	Modified Desoxycholate Citrate Agar
MEA	Membrane Enterococcus Agar
MKB	Modified King's A Broth
MLSB	Membrane Lauryl Sulphate Broth
MMGM	Minerals Modified Glutamate Medium
MPN	Most Probable Number
MPR	Most Probable Range
MSMA	Modified Sorbitol MacConkey Agar
NA	Nutrient Agar
NB	Nutrient Broth
OPSPA	Oleandomycin Polymyxin Sulphadiazine Perfringens Agar
PA	*Pseudomonas* Agar
P-A	Presence-Absence
PEB	Preston Enrichment Broth
PSA	Preston Selective Agar
PSO	*Salmonella* Polyvalent O Antiserum
PSH	*Salmonella* Polyvalent H Antiserum
R2A	Reasoner's No 2 Agar
RVB	Rappaport Vassiliadis Broth
SB	Selenite Broth
SFPA	Shahidi-Ferguson Perfringens Agar
TCBSA	Thiosulphate Citrate Bile Salt Sucrose Agar
TSCA	Tryptose Sulphite Cycloserine Agar
TSIAS	Triple Sugar Iron Agar Slope
TTC	2,3,5-triphenyltetrazolium chloride
TW	Tryptone Water
UB	Urea Broth
XLDA	Xylose Lysine Desoxycholate Agar
YEA	Yeast Extract Agar

Appendix E: Legislation and Related References

The Act	The Water Industry Act 1991. In Scotland: The Water (Scotland) Act 1980 as amended by Schedule 22 of the Water Act 1989.
The COSHH regulations	The Control of Substances Hazardous to Health Regulations 1988 (SI 1988/1657).
The Directive	The European Community Directive relating to the quality of water intended for human consumption (Council Directive 80/778/EEC).
The Food Act	The Food Safety Act 1990 (See also the Drinking Water in Containers Regulations 1994 SI 1994/743) and the Natural Mineral Waters Regulations 1985(SI 1985/71 as amended by SI 1990/2487.
The Guidance Document	'Guidance on safeguarding the quality of public water supplies' 1989 HMSO ISBN 0 11 752262 7. In Scotland: 'Guidance on safeguarding the quality of public water supplies in Scotland', Scottish Office Environment Department 1990.
The Health and Safety Act	The Health and Safety at Work etc Act 1974.
The Private Supplies Regulations	The Private Water Supplies Regulations 1991 (SI 1991/2790). In Scotland: The Private Water Supplies (Scotland) Regulations 1992 (Scottish Office Circular 20/1992).
The Regulations	The Water Supply (Water Quality) Regulations 1989 (SI 1989/1147) as amended by SI 1989/1384 and SI 1991/1837. In Scotland: The Water Supply (Water Quality) (Scotland) Regulations 1990 No 119 (S.11) as amended by SI 1991/1333 (S.129).

Appendix F: Animalcules in mains water

General introduction

Complaints directly attributable to animals may be due either to

(i) the presence of animals in tap water; or

(ii) discoloured water, caused by animal debris.

One of the earliest records of a mains infestation by animalcules was due to the 'wate louse', *Asellus aquaticus*. *Asellus* still causes the greatest number of consumer complaints especially in water derived from lowland surface waters. This type of source will suppor higher crops of algae and a greater density of the eggs and larvae of invertebrates whic may, if not arrested at the treatment works, enter the distribution system. Upland sources in contrast, are usually very low in nutrients and consequently support fewer animalcules Because of this improved quality however, treatment is often minimal, resulting in a greater proportion of those eggs and larvae which are present passing into distribution Groundwaters are generally the least productive, with an associated distinct invertebrat fauna adapted to the permanent darkness of subterranean life.

Passage of eggs and larvae through the water treatment works is likely to be the majo method of entry into the distribution system for most species. Service reservoirs may allow access by aerial insects which then lay their eggs in the water. Ingress into service reservoirs by terrestrial animals such as earthworms, slugs and beetles may occur through cracks, keyholes in covers etc and these types of animals may also enter the mains when being repaired, or when newly laid.

Following entry into the distribution system, however, only a few species are able to colonise and form breeding populations. The most important of these are the water louse *Asellus*, *Gammarus* species and *Crangonyx pseudogracilis* (the 'freshwater shrimps'), *Nais* species (very small, white worms) and some of the small crustaceans such as certain species of the copepod *Cyclops* and the cladoceran *Alona*.

None of the insects are able to complete their life cycle within the mains, with the exception of the unusual, fortunately rare, species of chironomid midges which are able to reproduce parthenogenetically. Some of the chironomid larvae nevertheless live for over two years before pupation, so constant recruitment through a treatment works may allow large numbers to colonise the mains.

Terrestrial animals, clearly, will not survive, but very many aquatic organisms will also be unable to adapt to the conditions in the distribution system; high flows probably being the most common adverse factor. The greatest populations of colonisers are often found in quiescent areas such as at the ends of distribution networks. Most are detritivores, feeding on organic debris; others are carnivorous. A few are filter-feeding organisms, for example the bivalve molluscs *Pisidium* species and these require a constant supply of organic material. Herbivorous leeches may become attached to the walls of the mains by means of their suckers, whilst the tiny Jenkins Spire Shell mollusc, *Potamopyrgus jenkinsi* is able to move amongst the encrustations provided the flow is not too strong. This species is an excellent coloniser, producing numerous live young without the need for a mate, hence an infestation may occur from just one individual.

A wide diversity of animalcules may potentially arrive at consumers' taps—over 150 species have been recorded in water mains. Of these, only a fraction have ever been reported as a consumer complaint, principally because of the small size of most species coupled with a low population density. Those which do cause attention are either large and conspicuous or numerous, small and mobile.

Drowned terrestrial species and larger aquatic animals such as leeches, beetles and certain snails are clearly of concern to a consumer. Such occurrences tend to be occasional in frequency, because of the scarcity of likely points of entry for the organisms. *Asellus* and the gammarids (*Gammarus* and *Crangonyx*) are also large but may be present in greater numbers. Other organisms commonly causing complaint are the mobile worms of the *Nais* genus and *Cyclops* copepods.

Although their presence may indicate a water supply problem, there is no known health risk associated with the animalcules commonly found in mains in the United Kingdom, though in tropical countries intermediate stages of parasites (for example of *Dracunculus medinensis* in *Cyclops*) may be a problem.

It is possible that nematode worms may host pathogenic bacteria in their gut, where they may remain unaffected by chlorine, but there is no evidence to suggest that this presents a public health risk. However, the presence of any of these animalcules may reflect problems with filters at the treatment works or breaches in the integrity of the distribution system, and their presence must lead to an immediate assessment of the microbiological quality of the supply.

Individual animalcules

Asellus species (usually *A. aquaticus*)

These are large (up to 14 mm) isopod crustaceans, dorso-ventrally compressed and superficially resembling the terrestrial woodlouse. Most *Asellus* gain access in the form of eggs via the treatment works and they readily infest mains, clinging to the walls in search of detrital material for food. They are able to breed, twice annually, in the pipework, producing up to 100 young in each brood. Substantial populations may be built up for they are able to withstand both a moderately high flowrate, provided they have a foothold, and low flow conditions, where they are more able than most to survive locally low dissolved oxygen concentrations which may occur in dead-legs.

The appearance of *Asellus* at consumers' taps is often the result of a change in flowrate, causing a dislodgement of the animal from the walls. Such an interruption may occur during the repair of a main, a mains flushing, intermittent service reservoir replenishment etc as well as the more localised flow changes associated with natural draw-off by consumers. There may be a significant population crash following a sudden reduction in supply of an otherwise continual resource, for example a prolonged algal bloom. Dead animals will be passively taken downstream as they have no predators in this environment. The partially decomposed animal fragments are thus able to pass through consumers' taps.

Gammarus and *Crangonyx*

These crustaceans are laterally compressed with curved backs, and are able to swim in moderate currents as well as lying in quiescent crevices in the walls of the mains. *Gammarus pulex* may reach 24 mm, although the more common *Crangonyx pseudogracilis* is only 10 mm in length. They breed in the mains, producing fewer young than *Asellus* but having more broods. Both feed on suspended particles and detritus, hence, as with *Asellus*, may reach high populations if large quantities of algae are allowed to pass through the treatment works.

Cyclops and other water fleas

The copepod water fleas of the *Cyclops* genus are very small, between 0.5 and 3 mm in length, depending on the species and sex of individuals. Of those that breed in the mains, the majority are carnivorous, feeding on a variety of organisms including smaller, herbivorous *Cyclops* species. They produce large numbers of young, and are noticed by a consumer because of their short, jerky movements.

Infestations may also be caused by the adults themselves passing through the treatment works in high numbers. This applies also for certain other copepods, for example the harpacticoids, and the cladocerans *Chydorus* and *Alona*, though other cladocera such as *Bosmina* and *Daphnia* are usually retained by treatment as are the planktonic, herbivorous copepods such as *Diaptomus*.

Nais

Naids are very thin, white worms, which may reach 25 mm in length, noticeable because of the continuous, coiling motion of their bodies. In warm temperatures, asexual reproduction occurs rapidly, with chains of individuals being formed. Naid worms are more susceptible than most other groups to chlorination.

Chironomid larvae

Many flying insects have aquatic pre-adult stages, and the non-biting midges (*Chironomidae*) are typical. The eggs are laid in gelatinous strings from which hatch tiny larvae. These pass through several moults before attaining full larval size, which may exceed 25 mm in length, depending on species, with a whitish, greenish or bright red coloration. Since the larval stage of chironomids may last two years or more, if large numbers of the early stages pass through the treatment works, these may concentrate in particular areas and build up numbers locally. Most species build a protective tube of organic and inorganic material, which adheres to the pipe wall.

Animal fragments in discoloured water

The majority of complaints of discoloured water are not due to animalcules but to vegetable material which persists throughout the treatment process or else to inorganic fragments such as manganese or iron. However, the most common complaint of 'black bits' caused by animalcules is due to *Asellus* frass, which if present in large quantities is highly indicative of an infestation. The pellets are of a very characteristic shape, thus easily identified. The cast exoskeletons of certain crustaceans, especially *Alona* and *Chydora* species (both 'water fleas') may become discoloured with iron which makes them very much more visible. High numbers of these may cause a 'dirty water' complaint. Occasionally, large numbers of dead diatoms (with brown, silicaceous shells) have been responsible for discoloured water—the cause often a stirring up of a deposit such as in a service reservoir. The algae, having passed through the treatment works, cannot survive in the absence of light, and being particularly dense, settle out readily in the reservoir.

Summary

Consumer complaints related to animalcules are due either to whole, visible animals, animal fragments or 'dirty' water. In general, lowland surface waters produce the greatest numbers of animalcules. Passage through a treatment works is the most likely point of entry into the mains but other key points are service reservoirs and during mains repair work. Once in the mains system, the animals colonise deposits and interior surfaces of pipes. Alternatively, they may be free-swimming in the water itself. Only a limited number of organisms are able to breed successfully in the distribution system; these include *Asellus*, the freshwater shrimps, naid worms and some *Cyclops* and *Alona* species. Chironomid midge larvae, whilst not breeding in the mains may nevertheless populate the system in large numbers through constant recruitment through the treatment works. Chironomids are ubiquitous in surface waters.

Heavy infestations of animals generally occur in 'dead ends' of mains, where the water flow within the mains is very low, or in service reservoirs where there is a slow water turnover. In these situations, the build-up of organic matter from droppings and dead animals can lead to an increase in chlorine demand, a deterioration in microbiological quality of the water and cause taste and odour complaints.

In attempting to identify the source of infestation/point of entry of the animalcule it may often be necessary to use microscopy to identify the species precisely. Otherwise, only generalisation is possible.

Measures to control infestations include the application of approved disinfestation agents, systematic flushing, and swabbing or air-scouring the affected areas.

As mentioned above, the presence of these animals in a water supply may indicate a failure at the treatment works or a breach in the integrity of the distribution system, and the detection of animals in a potable water must always lead to an immediate assessment of microbiological quality. However, there are no known health risks associated with these animalcules.

Appendix G: Other possible faecal indicator organisms

The *Bacteroides fragilis* group

This group consists of five separate species—*B. fragilis*, *B. vulgatus*, *B. thetaiotaomicron*, *B. ovatus* and *B. diastonis* (Cato and Johnson 1976). They are Gram-negative, obligately anaerobic, non-motile, non-sporing bacteria which have been found in the faeces of humans and domestic and farm animals (Allsop and Stickler 1984). They outnumber *E. coli* in the faeces of humans, cats and dogs but the converse has been found for farm animals and seagulls. While they have potential as indicators of recent human faecal pollution they do not appear to have any advantage over *E. coli* for, although their resistance to chlorine is similar, their survival in water is relatively poor (Allsop and Stickler 1984).

Isolation and enumeration are by membrane filtration using Wilkins and Chalgren's agar containing gentamicin and penicillin G with anaerobic incubation at 30°C for 4 hours followed by 44 hours at 37°C (Allsop and Stickler 1984).

***Bifidobacterium* species**

The genus *Bifidobacterium* comprises many species all of which are Gram-positive, obligately anaerobic, catalase-negative bacteria which attack sugars fermentatively. They are rod shaped but highly variable in appearance showing Y- or V-shaped bifurcated forms. Bifidobacteria have been found in the faeces of humans, pigs, cattle and sheep but not in horses, cats, dogs, poultry, goats or beavers (Mara and Oragui 1983, Resnick and Levin 1981). Sorbitol-fermenting strains (*B. adolescentis* and *B. breve*) have been found only in humans and could have potential as indicators of recent human faecal pollution of water. However, the use of this group as indicators of faecal pollution is limited by poor survival and sensitivity to chlorine relative to *E. coli* (Resnick and Levin 1981).

Isolation and enumeration of bifidobacteria are by membrane filtration using YN-17 medium and sorbitol-fermenting strains by using Human Bifid Sorbitol Agar (Mara and Oragui 1983). Media are incubated anaerobically at 37°C for 48 hours.

Candida albicans

Candida albicans is a typical yeast in culture. Cells are generally ovoid but not always of a characteristic shape. Asexual reproduction is by bi-polar budding, multiple buds may be formed at either or both poles; no unique pigment is produced and carbohydrate media are fermented.

C. albicans has been found in 14–31% of human faeces as compared to 87–100% for total coliforms including *E. coli* (Geldreich 1977). This relatively low incidence and the low numbers found in polluted waters when compared with faecal coliforms do not commend its use as an indicator of faecal pollution in treated water.

Rhodococcus coprophilus

R. coprophilus is an aerobic nocardia-like actinomycete which is excreted by farm animals but not humans. It can survive in water for long periods and could have potential for the detection of faecal pollution by farm animals, but isolation is slow (17–18 days) and difficult in unskilled hands. It is considered to be of value only in untreated waters as little is known about its resistance to chlorine (Rowbotham and Cross 1977, Mara and Oragui 1981). Isolation and enumeration are carried out using modified M3 agar (Mara and Oragui 1981).

Coliphages

Coliphages have been proposed as indicators of water pollution and as possible models for enteroviruses during treatment of drinking water (Stetler 1984, IAWPRC 1991). It has also been reported that coliphages and enteroviruses are removed or inactivated at similar rates during treatment processes, and that certain coliphages are at least as resistant to both chlorination and environmental stresses as enteroviruses (Kott et al 1974, Kott, Ben-Ari and Vinokur 1978, Simkova and Cervenka 1981, Havelaar and Hogeboom 1984). Stetler (1984) reported that enterovirus isolates correlated better with coliphage counts than with total coliforms, faecal coliforms, faecal streptococci or standard plate count organisms in

analytical data from raw water. These are important considerations, as it is reported th human enteric viruses may survive water treatment processes, especially chlorinatio better than pathogenic or indicator bacteria (Berg et al 1978). Both coliphages an enteroviruses have been found in chlorinated water (Stetler 1984).

Coliphages appear to be widely and consistently distributed in polluted surface water sewage and effluents. However, there is some uncertainty as to the ubiquity of coliphage as faecal rather than sewage indicators (Parry 1980) and it has been suggested that som types of coliphage are carried by only a small proportion of the human populatio (Havelaar, Furuse and Hogeboom 1986). If this is so, then coliphages would not be goo indicators of faecal pollution but might still have real value in the assessment of treatmer efficacy in removing or inactivating viruses, since they are routinely present in sewage contaminated raw waters.

The term 'coliphages' describes a range of bacterial viruses which can be divided into tw main groups. These are the 'somatic' phages which gain entry to the bacterial cell throug its cell wall, and the 'F-specific' or 'male-specific' phages which attach to the sex pili o the *E. coli* host. Both somatic and F-specific RNA bacteriophages can multiply in a appropriate host in sewage, but F-specific RNA bacteriophages are thought less likely t multiply in unpolluted waters. F-specific coliphages have similar resistance to som important human viruses for environmental stresses, including disinfection. Conse quently, it has been argued that the presence of F-specific RNA bacteriophages is a bette index of sewage pollution and the possible presence of human viruses than somati coliphages (IAWPRC 1991). However, because RNA coliphages are frequently absen from faeces, their absence from water does not necessarily indicate the absence of huma viruses (IAWPRC 1991).

The most sensitive approach to the isolation of somatic phages appears to be to select host strain of *E. coli* which is sensitive to the widest range of phage types. *E. coli* CN seems to be particularly suitable (Grabow and Coubrough 1986, Havelaar, Furuse an Hogeboom 1986).

Coliphage isolation techniques are straightforward, inexpensive and rapid, requirin overnight incubation only, and coliphage plaques may often be counted after as little a eight hours (Grabow and Coubrough 1986). Coliphage isolation may therefore prove to b a valuable monitoring tool for evaluating the effectiveness of treatment processes especially with respect to removal or inactivation of enteroviruses. Lowland river source are particularly likely candidates for this type of monitoring.

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