10.12 Candy, candy coating

Aseptically weigh 100 g of candy into a sterile blending container. Add approximately 300 ml from a liter of autoclaved reconstituted nonfat dry milk solution (100 g of nonfat dry milk + 1000 ml of distilled water, autoclaved 20 minutes at 121°C). Blend for 2 minutes. Aseptically transfer the blended mixture back to the remaining portion (ca 700 ml) of nonfat dry milk solution. If pH is below 6.6, adjust pH to 6.8 ± 0.2 with sterile 1 N NaOH. Add 4 ml of 1% aqueous crystal violet or 2 ml of 1% aqueous brilliant green and mix well. Incubate 24 ± 2 hours at 35°C. Continue examination according to sections 37.X19-37.X28 of Reference Method (10.04).

10.13 Frosting mixes, topping mix (Mixes with large quantity of sugar)

Aseptically weigh 25 g into a sterile, wide-mouth, screwcap pint jar. Add 225 ml of sterile nutrient broth. If pH is below 6.6, adjust to 6.8 ± 0.2 with sterile 1 N NaOH. Loosen jar cap ca 1/4 turn and incubate 24 ± 2 hours at 35°C. Continue examination according to sections 37.X19-37.X28 of Reference Method (10.04).

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11. ISOLATION OF SHIGELLA

11.01 Introduction

Shigellosis is a serious problem in the U. S. Currently the incidence of this disease is well in excess of 7000 cases per year. Food-borne outbreaks usually result from the consumption of contaminated foodstuffs such as potato salads served by food handlers who were previously ill with the disease. The extensive carrier state that is characteristic of this organism causes secondary attacks due to hand-to-mouth contacts. The attack rate per outbreak of shigellosis is 2-1/2 times that of salmonellosis.

Shigella in many respects duplicates the behavior of Salmonella as an infective and contaminating agent. Thus it is likely that the frequency of input of these organisms into foodstuffs is the same as or even greater than that of salmonellae. It is possible that interstate foods may be a vector in the dissemination of this organism.

11.02 Suspect foods

Suspect foods would be those types requiring hand processing or minimal heating before consumption. They could be products

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derived from animal sources, or products freshly delivered to consumer outlets. They are more likely to be products whose pH falls in the range of 5.5-7.5. In general, foods showing a high incidence of coliforms, E. coli, and salmonellae are suspect for shigellae.

11.03 Preview of the isolation and identification of Shigella

Briefly the method calls for inoculation of the foodstuff into enrichment broths consisting of GN and selenite cystine broths. This is followed by streaking onto XLD, DC, and EMB isolation agars. Suspicious colonies from these are inoculated onto TSI agar slants. If the growth from this produces an acid butt and an alkaline slant, the organism is nonmotile and urease-negative; it is inoculated into KCN, malonate, and IMViC media. The organism should be MR-positive and indole positive or negative, but negative in the remaining tests. It is then inoculated into one group of sugar media in which it is inert, producing neither acid nor gas. These consist of lactose, sucrose, salicin, inositol, and adonitol broths. This is followed by a final group of sugar media in which no gas is produced, but acid may or may not be produced. This last group consists of glucose, maltose, arabinose, xylose, and mannitol broths. The organism conforming to these criteria is considered to be a presumptive member of the Shigella group.

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058 of 170
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Any isolate with presumptive membership in the Shigella group is tested against Shigella somatic polyvalent antisera containing poly groups A, B, C, D, and A-D. Regardless of a positive or negative serological response, the organism should be further tested for confirmation of the genus finding, as well as for species and serotype identification.

11.04 Equipment and materials

(a) sterile wide-mouth screwcap jars, pints

(b) 35°C incubator

(c) sterile glass rods

(d) petri plates

(e) blender and sterile jars

(f) Shigella somatic antisera poly groups A, B, C, D, and A-D

11.05 Media and reagents

Brain heart infusion (BHI) (40.03); carbohydrate fermentation broth (40.05); desoxycholate citrate agar (DC) (40.08); Gram-negative broth (GN) (40.14); Koser's citrate medium (40.15); MR-VP medium (40.16); tryptone broth (40.17); KCN broth (40.20); Levine's eosine methylene blue agar (EMB) (40.21); malonate broth (40.23); motility test medium (40.26); nutrient agar (40.28); nutrient broth (40.29); selenite cystine broth (40.31);

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059 of 170

triple sugar iron agar (TSI) (40.33); urea broth (40.37); xylose lysine deoxycholate agar (XLD) (40.39); alpha-naphthol solution (41.01); Butterfield's buffered phosphate diluent (41.03); Kovac's reagent (41.07); methyl red solution (41.09); saline, 0.85% solution (41.10); Voges-Proskauer reagent (V-P) (41.15).

11.06 Enrichment (parallel culture)

(a) Weigh 10 g of the sample into 20 ml of GN broth contained in a screw cap jar and stir with a sterile glass rod until the product is completely dispersed. Add additional GN broth equivalent to a total of 240 ml. Close the jar and shake to suspend the sample. Loosen the cap to permit the escape of gas. Incubate overnight (18 hours or less) at 35°C and streak onto selective agars as soon as possible the next morning. Do not discard the jars but incubate another 24 hours. Streak once again on selective agars.

(b) Selenite cystine broth. Repeat the process, weighing 25 g of the sample into a final total of 225 ml of selenite cystine broth. Incubate for 18 hours or less at 35°C. Streak onto selective agars, regardless of visible growth. Incubate an additional 24 hours and streak selective agars again.

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060 of 170

11.07 Selective agars (parallel culture)

XLD, DC, EMB selective agars are streaked by means of a 3 mm loop from GN and selenite cystine enrichment broths. The selective plates are incubated for 24 ± 2 hours at 35°C. Select representative, well-isolated, suspicious[1/] colonies and subculture onto TSI slants. If no growth is evident on the selective plates, incubate another 24 hours and subculture any suspicious colonies onto TSI.

11.08 TSI

Slant-streak and butt-stab the fished colonies from selective agars onto TSI agar slants. Incubate for 24 ± 2 hours[2/] at 35°C.

On TSI the reaction of Shigella is an alkaline (red) slant and an acid (yellow) butt, with negative gas and negative H_2S production. Such a reaction is suspicious for Shigella. Suspicious TSI cultures are subcultured into urea broth, motility test medium, nutrient agar, and nutrient broth.

1/ Shigella will appear on all selective media as gray or slate gray, entire, glistening, viscid colonies by reflected light, varying in size according to the inhibitory medium. On XLD agar they will appear to be rose colored, surrounded by a rosy halo, when viewed by transmitted light.

2/ Readings of TSI beyond 24 hours are not valid. After this the entire TSI tube turns alkaline in reaction. If for any reason a longer time interval elapses before the tube can be read (weekend), a fresh tube of TSI should be inoculated.

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061 of 170
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11.09 Preliminary tests, inoculated from TSI

(a) Urea broth. Inoculate a urea broth tube from TSI. Incubate 24 ± 2 hours at 35°C. Cultures producing a red color (alkaline reaction) are urease positive. Shigella is urease negative.

(b) Motility test medium. Inoculate a tube of motility test medium from TSI by stabbing the top of the column of medium to a depth of approximately 5 mm. Incubate for 1-2 days at 37°C. A circular growth from the line of stab constitutes a positive test for motility. Shigella is nonmotile.

(c) Nutrient agar. Inoculate a tube of nutrient agar from TSI. Use growth from this medium as a source of inoculum and for test of purity[3/] of culture. Perform a Gram stain and examine microscopically. Shigella is a Gram-negative rod.

(d) Nutrient broth. Inoculate a tube of nutrient broth from TSI. Use growth from this medium as a source of inoculum and for a check on motility, should any doubtful reaction occur in the motility test medium.

3/ The purity of a suspected culture must be assured before further work is done on it. If a culture is suspected of being impure, make a light suspension in 0.85% NaCl. Streak the suspension and work it out well on BHI agar plates by means of a needle. Do not use inhibitory media, because it is desirable that the contaminating organism express its presence by means of a visible colony, so that it can be avoided. The Shigella organism is a Gram-negative rod, 1-3 μ long by 0.4-0.6 μ wide, non-capsulated, non-sporeforming and occurring singly.

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11.10 Further biochemical tests

If the suspicious organism as tested under 11.09 is Gram-negative, nonmotile, and urease negative, inoculate the following test media: KCN broth, malonate broth, tryptone broth, MR-VP broth, and Koser's citrate broth as described below.

(a) KCN broth. Inoculate KCN broth with a 3 mm loopful of a 24 hour nutrient broth culture and incubate for 48 ± 2 hours at 35°C. Positive results are indicated by turbidity in the tube. Shigella is negative.

(b) Malonate broth. Inoculate malonate broth with a 3 mm loopful of a 24 hour nutrient broth culture and incubate for 48 ± 2 hours at 35°C. A positive reaction is indicated by a change in the color of the indicator from green to dark blue. Shigella is negative.

(c) Indole test. Perform the indole test by inoculating a tryptone broth tube from the nutrient agar slant. Incubate the culture for 48 ± 2 hours at 35°C. Test by adding 0.5 ml of Kovac's reagent and shaking the tube gently. A positive test is indicated by the formation of a deep red color. Shigella is either positive or negative.

(d) Methyl red and V-P test. Inoculate a tube of MR-VP medium by means of a needle from the nutrient agar culture. Incubate

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063 of 170

at 35°C for 48 ± 2 hours. To 1 ml of this culture add 0.2 ml of 40% KOH. Shake well; add 0.6 ml of 5% alpha-naphthol. Add a few crystals of creatine and shake well. A red color forming in 2-4 hours constitutes a positive V-P test. Shigella is V-P negative.

Incubate the remainder of MR-VP medium for an additional 48 ± 2 hours. Add a few drops of methyl red solution to the culture. A distinct red color constitutes a positive reaction. A yellow color is a negative reaction. Shigella is MR-positive.

(e) Citrate utilization. Inoculate a tube of Koser's citrate medium by means of a needle from the nutrient agar culture, effecting the barest transfer of cells to the citrate medium by inoculating the side of the tube just below the surface of the medium. Incubate at 35°C for 4 or 5 days. A positive test consists of visible, turbid growth in the medium. Shigella is negative.

11.11 Fermentation reactions in carbohydrate media in which Shigella is inert

Inoculate lactose, sucrose, salicin, inositol, and adonitol broths from the nutrient agar culture. Incubate at 35°C for

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064 of 170

4 or 5 days and examine daily for acid and gas production. *Shigella* produces neither acid nor gas in these sugars.

11.12 **Fermentation reactions in carbohydrate media in which *Shigella* produces no gas**

Inoculate glucose, maltose, arabinose, xylose, and mannitol broths from the nutrient agar culture. Incubate for 4 or 5 days and examine daily for acid and gas production. *Shigella* produces no gas.[4/] *Shigella* may or may not produce acid from these carbohydrate media.

11.13 **Recapitulation of Shigella group characteristics**

Gram stain - Gram-negative rod

motility - nonmotile

urease - negative

H_2S - negative

KCN broth - no growth

malonate - no growth

indole - negative or positive

methyl red - positive

Voges-Proskauer - negative

citrate utilization - negative

lactose - negative acid

[4/] *S. flexneri 6* can produce gas from glucose and mannitol.

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065 of 170

sucrose - negative acid

salicin - negative acid

inositol - negative acid

adonitol - negative acid

glucose - negative gas; ± acid

maltose - negative gas; ± acid

arabinose - negative gas; ± acid

xylose - negative gas; ± acid

mannitol - negative gas; ± acid

Any microorganism that produces H_2S in TSI and is a urease positive, motile form, capable of growing in citrate medium or KCN broth and producing acid in inositol, salicin, or adonitol, does NOT belong to the Shigella group.

Any suspicious organism which conforms to the physiological and biochemical criteria listed above may be presumptively considered a member of the Shigella group. It is extremely hazardous to venture a decision based only on this limited information. Proceed to 11.14.

11.14 Serologic identification of presumptive Shigella - slide agglutination test

Test a 24 hour growth of the suspected Shigella organism from nutrient agar with Shigella somatic antisera poly groups A, B, C, D and Alkalescens-Dispar (A-D). Emulsify the

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066 of 170

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growth in 2 ml of 0.85% saline to make a heavy suspension in a serological test tube. Mark off six 1/2 inch squares with a wax pencil in a petri plate. In the upper left hand corner of each square, place a drop of the cell suspension. In the lower right hand corner place 0.05 ml of the antisera group A in the first box, group B in the second box, etc. In the sixth box place a drop of 0.85% NaCl. This is the autoagglutination control. Mix all cell suspensions with the respective antisera by means of a needle. Now rock the petri plate to enhance agglutination. Agglutination is usually not rapid and may take as long as 3-4 minutes to occur. A 4+ reaction will show a visible floc with the suspending fluid totally clarified.

A positive agglutination with group A, B, C, or D is presumptive evidence of the presence of Shigella. It is not a positive test for Shigella, nor should any final interpretive decision be made on the basis of this reaction. In the event of a negative agglutination reaction, it is advisable to boil the cell suspension for 30 minutes to destroy any interfering K antigen, which may be responsible and repeat the agglutination test once again with the boiled antigen.

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11.15 Generic confirmation, and specific and serotypic identification

If the organism conforms to the biochemical criteria listed under 11.13 and agglutinates under the conditions of 11.14, or if it conforms to the biochemical conditions 11.13 but does not agglutinate 11.14, the organism should be further tested as to genus, species, and serotype.

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068 of 170

12. DIRECT PLATING METHOD FOR THE DETECTION AND ENUMERATION OF STAPHYLOCOCCUS AUREUS OCCURRING IN FOOD

12.01 Equipment and materials

(a) sterile 100 x 15 mm petri dishes

(b) sterile, bent-glass streaking rods

(c) balance

(d) blender jars

(e) water bath, 37°C

(f) incubator, 35-37°C

12.02 Media and reagents

Trypticase or tryptic soy agar (40.35); Vogel-Johnson agar (40.38); brain heart infusion broth (BHI) (40.03); Butterfield's buffered phosphate diluent (41.03); desiccated coagulase plasma (rabbit) with EDTA (41.05).

12.03 Preparation of sample

Use balance with capacity of at least 2 kg and sensitivity of 0.1 g to aseptically weigh 50 g of unthawed (if frozen) sample into sterile high-speed blender jar. Add 450 ml of diluent, and blend 2 minutes. (If necessary to temper frozen sample to remove 50 g portion, hold not more than 18 hours at 2-5°C.)

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069 of 170

Not more than 15 minutes should elapse from time sample is blended until all dilutions are in appropriate media.

If entire sample consists of less than 50 g, weigh portion equivalent to 1/2 sample and add amount of sterile diluent required to make 1:10 dilution. Total volume in blender jar must completely cover blades.

Prepare all decimal dilutions with 90 ml of sterile diluent plus 10 ml of previous dilution. Shake all dilutions 25 times in 1 footarc.

Pipets must deliver accurately the required volume. Do not use to deliver less than 10% of their total volume. For example, to deliver 1 ml, do not use pipet larger than 10 ml; to deliver 0.1 ml, do not use pipet larger than 1 ml.

12.04 Isolation and enumeration

Deliver 0.5 ml aliquots of decimal dilutions to duplicate plates of Vogel-Johnson agar. Distribute inoculum over surface of plates with sterile, bent-glass, streaking rods. When inoculum is completely absorbed by medium, invert plates and incubate 40-48 hours at 35-37°C. Select plates at the dilution having 20-200 well-distributed colonies. Plates from minimal dilution having less than 20 colonies may be

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070 of 170

utilized. Count colonies (by groups of colonies having the same colonial appearance) not less than 1.0 mm in diameter which have reduced K_2TeO_3. Select at least one colony from each group and inoculate into 13 x 100 mm tube containing 0.2-0.3 ml of sterile brain heart infusion broth. Incubate at least 18 hours at 35-37°C. If a stock culture is desired, inoculate slant of trypticase soy agar and incubate 18-24 hours at 35-37°C. Store stock slants at 4°C.

To remaining brain heart infusion culture add 0.5 ml of reconstituted coagulase plasma containing EDTA and mix thoroughly. Incubate at 37°C and examine for clot formation at hourly intervals from 1 through 6 hours. Water bath incubation is preferable, but if adequate water bath space is not available, air incubation at 37°C is satisfactory. All cultures giving a positive coagulase reaction in 6 hours may be considered as Staph. aureus. Calculate number of Staph. aureus per gram of sample as follows:

Add number of colonies represented by coagulase-positive cultures on duplicate plates of the test dilution; then multiply total number of Staph. aureus colonies by dilution factor to get number of Staph. aureus per gram of sample.

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071 of 170

13. RAPID METHOD FOR THE RECOVERY OF ESCHERICHIA COLI[1/]

13.01 Introduction

This method should be used only on products where the presence of coliforms is not critical for the evaluation of the microbial flora, but it should not be used on those products for which the AOAC method is applicable.

13.02 Equipment and materials

(a) water bath, covered; 44.0°C ± 0.05°C

(b) blender

(c) balance

(d) pipets

(e) test tubes

13.03 Media and reagents

Lauryl sulfate tryptose broth (LST) (40.40); Levine's eosine methylene blue agar (EMB) (40.21); plate count agar (40.41); tryptone broth (40.17); MR-VP medium (40.16); Koser's citrate medium (40.15); Butterfield's buffered phosphate diluent (41.03); Kovac's reagent (41.07); alpha-naphthol solution (41.01); Voges-Proskauer reagent (41.15); methyl red solution (41.09).

[1/] Fishbein, et al. (11).

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072 of 170

13.04 Preparation of sample

Prepare sample as directed in the method for the examination of frozen and/or prepared foods for the presence of coliforms, E. coli, aerobic plate count and coagulase-positive staphylococci. JAOAC 49 (1), 246-250 (1966).

13.05 Inoculation and incubation

Inoculate 3-tube most probable number (MPN) series into lauryl sulfate tryptose broth with 1 ml of inocula of 1:10, 1:100, 1:1000 dilutions, using triplicate tubes at each dilution. Incubate tubes in a covered water bath maintained at 44.0°C ± 0.05°C for 24 hours only.

13.06 Isolation

Streak all positive gassing tubes onto Levine's eosine methylene blue agar (EMB) and proceed as directed in the above referenced method (13.04).

Report results as E. coli MPN/g, RM (Rapid Method).

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073 of 170

14. ISOLATION AND IDENTIFICATION OF VIBRIO PARAHAEMOLYTICUS

14.01 Introduction

Since 1958, Vibrio parahaemolyticus, a food-borne pathogen, has appeared to be a purely Japanese phenomenon. It caused numerous food-borne epidemics in Japan, accounting for well over 50% of all food poisonings or infections. The organism is a strict halophilic, marine vibrio, growing only in certain inland water areas where it successfully implants on marine forms such as fish and shellfish. When these are harvested and prepared for food, by mincing and serving in the raw state (Japanese style), the organism multiplies in great numbers and is the cause of food-borne epidemics. These epidemics occur in the summer season, and in the same prefectures near the Tokyo area, year after year.

In America, V. parahaemolyticus has already been isolated from Puget Sound waters as well as from marine products in the same area. The work has been accomplished in the laboratory of Dr. John Liston in the College of Fisheries at the University of Washington in the last two years. Much of this work has been carried out by Mr. John Baross in the

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074 of 170

form of a graduate study. It is owing to the generous cooperation of Dr. Liston and Mr. Baross that we are able to use their salt water starch agar medium and other formulations, as well as some of their methodology in the following analytical procedure.

14.02 Suspect foods

Mr. Baross has recovered V. parahaemolyticus from oysters, Dungeness crabs, clams, and fish viscera (bottom feeders such as hake and English sole). The organism has also been recovered from marine and estuarine sediments as well as overlying waters. The Pacific oyster, Crassostrea gigas, and the Pacific butter clam may have counts as high as 10,000 vibrios per gram during the summer months. These counts will diminish to less than 10 per gram when the temperature drops to 8°C. This points to the fact that shellfish could well serve as a possible cause of gastroenteritis, particularly when consumed in the raw state. It is well to note that V. parahaemolyticus is not supposed to survive the frozen state, nor is it recoverable from marine environments when temperatures are much above 22°C.

14.03 Preview of the isolation and identification of Vibrio parahaemolyticus

The analytical approach is a parallel scheme employing the

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075 of 170

Japanese methods, as exemplified in some degree by Sakazaki, and an American procedure utilizing the methods of Liston-Baross. The Japanese scheme, because it employs liquid enrichment media, answers the question, "Are any V. parahaemolyticus organisms present in the sample?" It is a qualitative approach. The Liston-Baross method answers the question, "How many V. parahaemolyticus organisms are present in the sample?" It is a quantitative approach. In the broadest sense, each method is both qualitative and quantitative at the same time. In the Japanese method, however, the ratio of the vibrios and competing flora will be altered permanently; in the Liston-Baross method, this ratio of natural types will more likely be fixed by the salt water starch agar plate method.

The Japanese isolation procedure employs a liquid enrichment phase consisting of two liquid broths, glucose-salt-Teepol broth and salt colistin broth. Both broths are inoculated in parallel with the sample. This is followed by streaking onto an isolation agar, thiosulfate-citrate-bile salts-sucrose agar. The isolated colonies from the agar are thereafter characterized biochemically in accordance with the standard classical Japanese data.

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076 of 170

The Liston-Baross method employs no liquid enrichment phase. Instead, the sample is plated directly, by the spread plate technic, onto an isolation agar, salt water starch agar. This medium is cultured anaerobically for 2-3 days. The isolated colonies from this agar are thereafter characterized biochemically in accordance with the standard classical Japanese data.

Because there is no certain serological support for the V. parahaemolyticus isolates at the present time, it is safest to complete the rather lengthy biochemical identification scheme, to assure the correct identification of genus and species. There is no complete agreement among authors as to what constitutes the primary criteria for identification of V. parahaemolyticus. Accordingly, we have listed the biochemical characterization (standard classical Japanese data) under Differential Tests I, II, and III. Differential Test I is a resume of the Liston-Baross approach wherein emphasis is placed on pleomorphism and hemolysis. These are criteria which are not stressed by the Japanese, who tend to rely on tests such as TSI, V-P, halophilism, nitrate reduction, etc. A table is included

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077 of 170

to separate two possible interfering types of nonentero-pathogenic vibrios, V. alginolyticus and V. anguillarum, from the enteropathogen, V. parahaemolyticus.

14.04 Equipment and materials

(a) 35°C incubator

(b) sterile glass rods

(c) blender and sterile jars

(d) Case laboratories anaerobic apparatus and nitrogen gas cylinder or any equivalent anaerobic device

(e) pipets

(f) dilution blanks

(g) sterile wide-mouth screwcap jars, pint size

(h) phase microscope

(i) Millipore filter apparatus

14.05 Media and reagents

Artificial sea water (14.20.01); B-sea water broth (BSWB) (14.20.02); blood agar (14.20.03); carbohydrate media (14.20.04); cholera red broth (14.20.05); glucose salt Teepol broth (GSTB) (14.20.06); Hugh-Liefson glucose broth (HLGB) (14.20.07); Koser's citrate medium (14.20.08); malonate broth (14.20.09); motility agar (14.20.10); MR-VP

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078 of 170
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medium (14.20.11); nitrate broth (14.20.12); nutrient gelatin (14.20.13); phenylalanine agar (14.20.14); salt colistin broth (SCB) (14.20.15); salt trypticase broth (STB) (14.20.16); salt water starch agar (SWSA) (14.20.17); thiosulfate-citrate-bile salts-sucrose agar (TCBS) (14.20.18); triple sugar iron agar (TSI) (14.20.19); trypticase or tryptic soy agar, 3% NaCl (STSI) (14.20.20); tryptone broth (14.20.21); urea broth (14.20.22); alpha-naphthol solution (14.21.01); phenylenediamine solution (14.21.02); dilution water, 3% NaCl (14.21.03); ferric chloride solution (14.21.04); hydrogen peroxide solution (14.21.05); sulfanilic acid solution (14.21.06); alpha-naphthylamine solution (14.21.07); creatine, crystalline; petrolatum, sterile; sulfuric acid, concentrated; zinc powder.

14.06 Enrichment in liquid broth (parallel broth culture) (Japanese method)

Weigh out 50 grams of the shellfish sample into 450 ml of 3% NaCl dilution water in a Waring Blendor jar, and blend for 3 minutes. This represents a 1:10 dilution. From this blended sample,[1/] make 1:100, 1:1000, 1:10,000 dilutions,

[1/] In the examination of marine waters the range 1:10-1:10,000 dilution is tested, and the 1:10 concentration is cultured directly into 2x strength broths.

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079 of 170

using appropriate dilution blanks. Inoculate 10 ml of the 1:10 dilution into GSTB-2x concentration and into SCB-2x concentration. This constitutes the 1:1 dilution. Inoculate the remaining dilutions, 1:10, 1:100, 1:1000, and 1:10,000, into single strength GSTB and into single strength SCB. Incubate the broth tubes overnight (18 hours or less) at 35°C, and thereafter streak a loopful of the culture onto the selective agars. All the prepared dilutions are saved for examination by the Liston-Baross isolation method [14.07 (a)].

14.07 Isolation phase (parallel Liston-Baross and Japanese methods)

(a) Liston-Baross method. From each of the prepared dilutions described in 14.06, pipet a 0.1 ml portion onto SWSA plates and streak the material, using the spread plate technic. Incubate the plates anaerobically for 48-72 hours at 35°C. Anaerobiosis may be obtained by using the Case anaerobic apparatus, evacuating to negative 20 lb, and flushing with nitrogen gas for a total of four times. Other equivalent technics may be employed to establish anaerobic conditions. On this medium Vibrio parahaemolyticus will appear as white, non-spreading colonies, which will be amylase positive (indicated by the presence of a halo around the colony).

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080 of 170

Subculture such colonies onto the following media: TSI, B-sea water broth (BSWB), trypticase or tryptic soy agar, blood agar, and motility agar (14.09).

Second Day

(b) Japanese method. Streak a 3 mm loopful of the 18 hour culture from the GSTB and the SCB broths in Section 14.06 onto thiosulfate-citrate-bile salts-sucrose agar (TCBS). Incubate the TCBS agar at 35°C for 18 hours. On this medium, V. parahaemolyticus colonies are round, 2-3 mm in diameter, with a green or blue center. V. alginolyticus, a non-desirable type, will appear larger and yellow. Coliforms, Proteus, and enterococci may also grow on the medium but these will be very small and translucent colonies, easily distinguished from V. parahaemolyticus. Subculture suspicious colonies from TCBS agar onto the following media: TSI, B-sea water broth (BSWB), trypticase soy agar, blood agar, and motility agar (14.08).

Third Day

14.08 Differential Test I

In the isolation test (14.06), the suspicious colonies on SWSA will have had 2 days' incubation. In 14.07 (b), the suspicious colonies on TCBS agar will have had 18 hours of incubation. Subculture the suspicious colonies from both

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081 of 170

isolation agars as follows:

(a) TSI. Streak the slant and stab the butt. Incubate the medium overnight at 35°C. V. parahaemolyticus produced an acid butt and an alkaline slant. It produces no gas and is a negative H_2S producer.

(b) BSWB. Inoculate and incubate this broth at 35°C overnight. This culture serves as inoculum for other tests, as well as for motility studies, the Gram stain, and pleomorphism observation under the phase microscope. V. parahaemolyticus is a motile, Gram-negative, pleomorphic organism exhibiting short and long rods, as well as spheres and spirals.

(c) Salt trypticase soy agar (STSA). Inoculate a slant of this medium and incubate at 35°C for 24 hours. This will serve as inoculum and holding medium.

(d) Blood agar. Streak a blood agar plate and incubate at 35°C for 24 hours. V. parahaemolyticus produces a positive beta hemolysis reaction (clear transparent zone). This test should be performed on freshly isolated organisms, as this property is lost with repeated subculture of the organism.

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082 of 170

(e) Motility agar. Inoculate a tube of motility agar by stabbing the column of the medium to a depth of approximately 5 mm. Incubate for 24 hours at 35°C. A circular growth from the line of stab constitutes a positive test. This test for motility may be used in lieu of a motility test (hanging drop) from BSWB.

Fourth Day

14.09 Differential Test II

Organisms which are found to be motile, hemolytic, pleomorphic, Gram-negative bacteria, which produce an acid butt and alkaline slant on TSI and are negative for H_2S production, are tested further as follows:

(a) Salt trypticase broth (STB), halophilism. Inoculate four tubes of STB base containing 0, 3%, 7%, and 10% NaCl concentrations from STSA slants and incubate at 35°C for 24 hours. V. parahaemolyticus will grow well in 3% and 7% NaCl concentrations in this medium but will not grow in 0 and 10% NaCl concentrations.

(b) Nitrate broth. Inoculate a tube of nitrate broth from STSA slants. Incubate for 24 hours at 35°C. Prior to testing for reduction of nitrate to nitrite, mix equal amounts of sulfanilic acid solution and alpha-naphthylamine

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083 of 170

solutions, and add 0.1 ml of this mixture to the culture tube. If nitrate has been reduced to nitrite, a red color will develop. V. parahaemolyticus reduces nitrate to nitrite.

It is wise to confirm negative tests by the addition of a pinch of zinc dust. The production of a red color indicates the presence of unreduced nitrate. In the case of equivocal results, cultures may be incubated up to 4 days.

(c) Nutrient gelatin. Inoculate a tube of nutrient gelatin from an STSA slant by means of a needle and incubate at 35°C from 1-7 days. To test for proteolysis, cool the medium below 20°C; if the medium does not resolidify, the gelatin has liquefied. V. parahaemolyticus is a rapid gelatin liquefier.

(d) Hugh-Liefson glucose broth (HLGB). Using an STSA inoculum, stab 2 tubes of Hugh-Liefson glucose broth with a needle. Overlay one tube with melted sterile petrolatum to a depth of about 1 inch. Incubate at 35°C for 2 days, or longer if necessary. If the organism changes the color in both tubes from purple to yellow, it is a carbohydrate fermenter. But if the yellow color change occurs only in the open tube, it is a carbohydrate oxidizer. V. parahaemolyticus is a positive glucose fermenter, producing no gas.

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(e) Cytochrome oxidase test. This test may be performed on any discard plate or slant with growth on it such as blood agar or STSA slant. Two or three drops of the alpha-naphthol solution are allowed to run down the slant or over the colony on a plate. This is followed by an equal amount of the "phenylenediamine" solution. A positive reaction is shown by the rapid development of a dark blue color within 2 minutes. V. parahaemolyticus is positive by this test.

(f) Indole test (tryptone broth). To perform the indole test, inoculate tryptone broth from an STSA slant. Incubate the culture at 35°C for 24 hours. Test for indole production by adding 0.5 ml of Kovac's reagent to the tube and shaking gently. A positive test is indicated by the formation of a deep red color. V. parahaemolyticus is indole positive.

(g) Methyl red test (MR-VP medium). To perform the methyl red test, inoculate MR-VP medium from an STSA slant. Incubate at 35°C for 4 days. Add a few drops of methyl red solution to 5 ml of the culture. A distinct red color (acid reaction) constitutes a positive reaction; a yellow color is negative. V. parahaemolyticus is methyl red-positive.

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085 of 170

(h) Voges-Proskauer test (MR-VP medium). Inoculate a tube of MR-VP medium from an STSA slant and incubate at 35°C for 48 hours. To 1 ml of this culture add 0.2 ml of 40% KOH solution, shake well, and add 0.6 ml of 5% alpha-naphthol solution. Add a few crystals of creatine and shake well. A red color forming within 2-4 hours constitutes a positive V-P test. V. parahaemolyticus is V-P negative.

(i) Carbohydrate fermentation-cellibiose and sucrose broths. Inoculate a tube of cellibiose and sucrose broths from an STSA slant. Incubate at 35°C for 4 or 5 days. A negative reaction will leave the culture tube with its original purple color unchanged. Acid production will cause a change in color from purple to yellow. V. parahaemolyticus will not produce an acid reaction in cellibiose within the first 24 hours. Sakazaki states that it is likewise negative in sucrose. However, Liston and Baross report that there may be an element of uncertainty with regard to the sucrose reaction; it may be negative or may produce acid slowly.

14.10 Differential Test III

(a) Citrate utilization. Inoculate a tube of Koser's citrate medium from an STSA slant culture, effecting the

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barest transfer of cells to the citrate medium by inoculating the side of the tube just below the surface of the medium. Incubate at 35°C for 4 or 5 days. A positive test consists of visible, turbid growth in the medium. V. parahaemolyticus is positive.

(b) Phenylalanine deaminase. Inoculate a tube of phenylalanine agar with a fairly heavy inoculum from an STSA slant culture. Incubate at 35°C for 24 hours. Following incubation, allow 4 or 5 drops of ferric chloride reagent to run down the growth on the slant. A positive reaction is denoted by the development of a green color in the syneresis fluid and in the agar slant, indicating that phenylalanine has been deaminated to phenylpyruvic acid. V. parahaemolyticus gives a negative reaction in this test.

(c) Urease test. Inoculate a tube of urea broth from an STSA slant. Incubate at 35°C for 24 hours. Cultures producing a red color (alkaline reaction) are positive for urease production. V. parahaemolyticus is urease-negative.

(d) Malonate utilization. Inoculate a tube of malonate broth by means of a 3 mm loopful from a 24-hour culture of BSWB. Incubate at 35°C for 48 hours. A positive reaction is indicated by a change in the color of the indicator from

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087 of 170
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green to dark blue. V. parahaemolyticus gives a negative reaction in this test.

(e) Catalase test. Pour a few drops of hydrogen peroxide solution on the agar slope surface of a 24-hour culture of a tube of STSA. If catalase is present, bubbles of oxygen will be released from the surface of the growth. V. parahaemolyticus is catalase-positive.

(f) Cholera-red reaction. Inoculate a tube of cholera-red medium from an STSA slant. Incubate at 35°C for 24 hours. The development of a pink to violet color upon the addition of 2-3 drops of concentrated sulfuric acid is a positive test. (This test is based on the simultaneous production of indole from tryptophane and nitrite from nitrate by the test organism. The addition of sulfuric acid to the culture results in the production of a nitroso-indole, which yields a pink to violet color.) V. parahaemolyticus gives a variable response with this test.

(g) Additional carbohydrate fermentations. Media inoculations and incubation are as previously listed under 14.09 - Differential Test II - (i). The following sugar broths yield a positive fermentation reaction with V. parahaemolyticus: maltose, mannitol, and trehalose. The following sugar broths yield a negative fermentation reaction with V. parahaemolyticus: lactose, xylose, adonitol, dulcitol, inositol, sorbitol, and salicin.

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088 of 170
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V. parahaemolyticus yields a variable fermentation response in arabinose broth.

14.11 Recapitulation of the identifying characters of V. parahaemolyticus.

Gram stain	Gram-negative
pleomorphism	positive
motility	motile
blood agar	B-hemolysis
TSI	acid butt, alkaline slant
salt trypticase broth--3%, 7%	positive
salt trypticase broth--0, 10%	negative
indole	positive
methyl red	positive
V-P	negative
citrate utilization	positive
H_2S production	negative
phenylalanine deaminase	negative
nitrate	reduced
urease	negative
gelatin	liquefied
malonate	negative
catalase	positive
cytochrome oxidase	positive
cholera-red	variable

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089 of 170

Hugh-Liefson glucose	positive fermentation without gas
cellibiose	negative fermentation within 24 hours
sucrose	not fermented or very slowly
arabinose	variable
maltose	fermented
mannitol	fermented
trehalose	fermented
lactose	not fermented
rhamnose	not fermented
xylose	not fermented
adonitol	not fermented
inositol	not fermented
sorbitol	not fermented
salicin	not fermented

14.12 At times it may be necessary to distinguish the two non-enteropathogenic types, V. alginolyticus and V. anguillarum, from the enteropathogenic V. parahaemolyticus. The following table indicates how the resolution of the 3 vibrios may be effected.

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Characteristic	V. parahaemolyticus	V. alginolyticus	V. anguillarum
7% NaCl trypticase broth	+	+	-
10% NaCl trypticase broth	-	+	-
Voges-Proskauer reaction	-	+	-
Sucrose fermentation	-	+	±
Cellibiose fermentation (24 hours)	-	-	+

14.13 Serology

Serological identification of the various serotypes of V. parahaemolyticus is in such a state of uncertainty as to preclude its role as a diagnostic tool at the present time.

14.14 Interpretation of positive findings of V. parahaemolyticus

V. parahaemolyticus is not an infective agent for man. A person ill with this organism will not infect a healthy individual. Furthermore, the Japanese have found that large numbers of the organism must be present before the Shigella-like syndromes occur after ingestion of the food. Thus, the kind of food,

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091 of 170

the numbers of V. parahaemolyticus present, the type of market and consumer handling, the epidemiological evidence, and the ecological data must all be considered when interpreting the health hazard potential of a food sample.

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092 of 170
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14.20 MEDIA

Artificial sea water.....14.20.01
B-sea water broth (BSWB).....14.20.02
Blood agar.....14.20.03
Carbohydrate media.....14.20.04
Cholera red broth.....14.20.05
Glucose salt Teepol broth (GSTB).....14.20.06
Hugh-Liefson glucose broth (HLGB).....14.20.07
Koser's citrate medium.....14.20.08
Malonate broth.....14.20.09
Motility agar.....14.20.10
MR-VP medium.....14.20.11
Nitrate broth.....14.20.12
Nutrient gelatin.....14.20.13
Phenylalanine agar.....14.20.14
Salt colistin broth (SCB).....14.20.15
Salt trypticase broth (STB).....14.20.16
Salt water starch agar (SWSA).....14.20.17
Thiosulfate-citrate-bile salts-sucrose agar (TCBS)..14.20.18
Triple sugar iron agar (TSI).....14.20.19
Trypticase or tryptic soy agar, 3% NaCl (STSI).....14.20.20
Tryptone broth.....14.20.21
Urea broth.....14.20.22

14.21 REAGENTS

Cytochrome oxidase reagents
  a. alpha-Naphthol solution.....14.21.01
  b. Phenylenediamine solution.....14.21.02
Dilution water, 3% NaCl.....14.21.03
Ferric chloride solution.....14.21.04
Hydrogen peroxide solution.....14.21.05
Nitrite test reagents
  a. Sulfanilic acid solution.....14.21.06
  b. alpha-Naphthylamine solution.....14.21.07

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093 of 170
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14.20.01 **Artificial Sea Water (Sakazaki)**

NaCl	23.4 g
KCl	66.4 g
Na_2SO_4	3.9 g
$NaHCO_3$	19.3 g
$MgCl_2$	4.9 g
Distilled H_2O	500 ml

Sterilize medium separately at 121°C for 15 minutes and adjust pH to 7.0. Most of the media described in this section are prepared in a 4% NaCl solution, which is a substitute for natural aged and filtered sea water. To use the artificial sea water formulation in a particular medium, it is only necessary to compound the particular medium in 500 ml of distilled water, exclude the 4% NaCl, and add 500 ml of the artificial sea water.

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094 of 170

14.20.02 **B-Sea Water Broth (Liston-Baross) (BSWB)**

Peptone (Difco)	4 g
Phytone (BBL)	4 g
Yeast extract (Difco)	1 g
NaCl	40 g
Distilled H_2O	1 L

Aged, filtered sea water, if available, is preferable to the 4% NaCl solution which has been substituted for it. Final pH 7.5. Sterilize at 121°C for 15 minutes.

14.20.03 **Blood Agar**

Bacto blood agar base	40 g
NaCl	20 g
Distilled H_2O	1 L

Dispense the salt blood agar base in flasks of known volume and autoclave at 15 lbs. pressure (121°C) for 15 minutes. Adjust the pH to 7.0. Cool to 45-50°C and, while it is in the liquid state, add 5% sterile defibrinated blood aseptically, thoroughly mix, and pour into the petri plates.

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14.20.04 **Carbohydrate Media**

BAM formula (40.05) is changed to include 30 g of NaCl per liter of the medium. Sterilize at 121°C for 10 minutes. Final pH 7.0.

All other indications and contraindications of (40.05) should be observed. If there is any doubt about the stability of the carbohydrate to the sterilization process, it is best to Millipore-filter sterilize a concentrated solution of it and add appropriate amounts to the previously sterilized basal medium.

14.20.05 **Cholera Red Broth**

Peptone	10 g
NaCl	25 g
$NaNO_3$	0.01 g
Distilled H_2O	1 L

Adjust to pH 7.2, dispense in 5 ml portions, and sterilize at 121°C for 15 minutes.

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096 of 170
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14.20.06 **Glucose Salt Teepol Broth (GSTB)**

	Single Strength	Double Strength
Beef extract	3 g	6 g
Peptone	10 g	20 g
NaCl	30 g	60 g
Glucose	5 g	10 g
Methyl violet	0.002 g	0.004 g
Teepol[1/]	4 ml	8 ml
Distilled H_2O	1 L	1 L

Dispense the single strength broth in 10 ml portions. Dispense the double strength broth in 20 ml portions in tubes large enough to accommodate a 10 ml charge of sample. If larger quantities of sample are to be examined (25 g), use screwcap jars containing 225 ml of single strength broth. Sterilize at 121°C for 15 minutes. Final pH 9.4.

[1/] Teepol is available through Mr. George Moore, Shell Chemical Company, 110 West 51st Street, New York, N. Y. 10020. One gallon containing 9 pounds costs $4.65.

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14.20.07 Hugh-Liefson Glucose Broth (Liston-Baross) (HLGB)

Peptone	2 g
Yeast extract (Difco)	0.5 g
NaCl	38.0 g
Glucose	10 g
Bromcresol purple	0.015 g
Agar	3 g
Distilled H_2O	1 L

Adjust the pH to 7.4 before autoclaving at 121°C for 15 minutes. By excluding glucose and adding 10 ml of a 10% solution of sterile sucrose, cellibiose, or lactose to the above basal medium, these carbohydrates may be tested by the Hugh-Liefson procedure, in consonance with the data of some Japanese workers.

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14.20.08 **Koser's Citrate Medium**

To BAM formula (40.15) add 30 g of NaCl per liter of the medium. Sterilize at 121°C for 15 minutes. Final pH 6.7.

14.20.09 **Malonate Broth**

To BAM formula (40.23) add 30 g of NaCl per liter of the medium. Sterilize at 121°C for 15 minutes. Final pH 6.7.

14.20.10 **Motility Agar**

To BAM formula (40.26) add an additional 15 g of NaCl per liter of medium. Sterilize at 121°C for 15 minutes. Final pH 7.2.

14.20.11 **MR-VP Medium**

To BAM formula (40.16) add 30 g of NaCl per liter of the medium. Sterilize at 121°C for 15 minutes. If the commercial dehydrated medium is employed, do not adjust pH.

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099 of 170

14.20.12 **Nitrate Broth**

Meat extract	3 g
Peptone	5 g
KNO_3 (nitrite free)	1 g
NaCl	30 g
Distilled H_2O	1 L

Sterilize at 121°C for 15 minutes. Final pH 7.0.

14.20.13 **Nutrient Gelatin**

Beef extract	3 g
Peptone	5 g
Gelatin	120 g
NaCl	30 g
Distilled H_2O	1 L

Sterilize at 121°C for 12 minutes. Final pH 7.0.

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14.20.14 **Phenylalanine Agar**

Yeast extract	2 g
DL-phenylalanine	
or	
L-phenylalanine	1 g
Na_2HPO_4	1 g
NaCl	25 g
Agar	12 g
Distilled H_2O	1 L

Tube and sterilize at 121°C for 10 minutes and allow to solidify in a long slanted position.

Final pH 7.3.

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14.20.15 Salt Colistin Broth[2/] (SCB)

	Single Strength	Double Strength
Yeast extract	3 g	6 g
Peptone	10 g	20 g
NaCl	20 g	40 g
Colistin methansulfonate	500 µg/ml	1000 µg/ml
Distilled H_2O	1 L	1 L

Final pH 7.4. This medium should not be heated.

The volume relationships are identical with those listed under 14.20.06 .

[2/] SCB is available in dehydrated form from Eiken (Nihon Eiyo Kagaku) Co. Ltd., 8-33, 1 Hongo, Bunkyo-Ku, Tokyo, Japan.

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102 of 170

14.20.16 Salt Trypticase Broth (Liston-Baross) (STB)

Trypticase or tryptone	10 g
Yeast extract (Difco)	2 g
Distilled H_2O	1 L

Add 0, 30, 70, 100 g of NaCl per liter to the above medium to make, respectively, 0, 3, 7, and 10% NaCl trypticase broth for salt tolerance tests (halophilism).

Sterilize at 121°C for 15 minutes. Final pH 7.5.

14.20.17 Salt Water Starch Agar (Liston-Baross) (SWSA)

Peptone	3 g
Yeast extract	1 g
Soluble potato starch	5 g
Agar	15 g
NaCl	40 g
Distilled H_2O	1 L

Final pH 7.5-7.8. Sterilize at 121°C for 15 minutes. Where an incidence of Bacilli is anticipated, the addition of 2 units of Penicillin G per ml of medium is recommended.

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14.20.18 Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS)

Yeast extract	5 g
Peptone	10 g
Sucrose	20 g
Sodium thiosulfate (5 H_2O)	10 g
Sodium citrate (2 H_2O)	10 g
Sodium cholate	3 g
Ox gall	5 g
Sodium chloride	10 g
Ferric citrate	1 g
Bromthymol blue	0.04 g
Thymol blue	0.04 g
Agar	15 g
Distilled H_2O	1 L

Final pH 8.6. This medium should not be autoclaved. It is available commercially in dehydrated form from BBL , Difco, and Eiken.

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104 of 170
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14.20.19 Triple Sugar Iron Agar (TSI)

To BAM formula (40.33) add 25 g NaCl per liter of medium. Sterilize at 121°C for 15 minutes. Final pH 7.4.

14.20.20 Trypticase or Tryptic Soy Agar (TSA) 3% Salt

Trypticase or tryptone	15 g
Phytone or soytone	5 g
NaCl	30 g
Agar	15 g
Distilled H_2O	1 L

Sterilize at 121°C for 15 minutes. Final pH 7.3.

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105 of 170
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14.20.21 **Tryptone Broth**

To BAM formula (40.17) add 30 g of NaCl per liter of the medium. Sterilize at 121°C for 15 minutes. Final pH 7.0.

14.20.22 **Urea Broth**

To BAM formula (40.37) add 30 g of NaCl per liter of the medium. Filter-sterilize and aseptically distribute the medium into tubes. Final pH 6.8.

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106 of 170

Cytochrome Oxidase Reagents

14.21.01 alpha-Naphthol Solution

alpha-Naphthol	1 g
Ethyl alcohol	100 ml

14.21.02 "Phenylenediamine" Solution

N,N-dimethyl-p-phenylene-diamine dihydrochloride	1 g
Distilled H_2O	100 ml

14.21.03 3% NaCl Dilution Water

NaCl	30 g
Distilled H_2O	1 L

This formula is employed for both sample dilution and blanks. Sterilize at 121°C for 15 minutes. Final pH 7.0.

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Nitrite Test Reagents

14.21.04 Sulfanilic Acid Solution

Sulfanilic acid	0.8 g
5 N acetic acid	100 ml

14.21.05 alpha-Naphthylamine Solution

alpha-Naphthylamine	0.5 g
5 N acetic acid	100 ml

To make 5 N acetic acid, add 28.75 ml of glacial acetic acid to 71.25 ml of distilled water.

14.21.06 Hydrogen Peroxide Solution

30% H_2O_2	33 ml
Distilled H_2O	67 ml

14.21.07 Ferric Chloride Solution

$FeCl_3$	10 g
Distilled H_2O	100 ml

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108 of 170

40. MEDIA

Acid broth.....40.01
Anaerobic egg agar.....40.02
Brain heart infusion broth (BHI).....40.03
Bromcresol purple dextrose broth (BCP).....40.04
Carbohydrate fermentation broths.....40.05
Chopped liver broth.....40.06
Cooked meat medium.....40.07
Desoxycholate citrate agar (DC).....40.08
Distilled water (sterile, 200 ml).....40.09
Distilled water (sterile, 1000 ml).....40.10
Ellner's sporulation broth.....40.11
Fluid thioglycollate medium.....40.12
Fluid thioglycollate medium (azolectin-Tween modification).....40.13
Gram-negative broth (GN).....40.14
IMVC media
    Koser's citrate medium.....40.15
    MR-VP medium.....40.16
    Tryptone broth.....40.17
Indole nitrite medium.....40.18
Iron milk medium.....40.19
KCN broth.....40.20
Lauryl sulfate tryptose broth (LST).....40.40
Levine's eosine methylene blue agar (EMB).....40.21
Liver veal egg yolk agar (LVEY).....40.22
Malonate broth.....40.23
Malt extract broth.....40.24
Motility-nitrate medium.....40.25
Motility test medium.....40.26
Nonfat dry milk solution (reconstituted).....40.27
Nutrient agar.....40.28
Nutrient broth.....40.29
Penassay seed agar.....40.30
Plate count agar.....40.41
Selenite cystine broth.....40.31
Sulfite-polymyxin-sulfadiazine agar (SPS).....40.32
Triple sugar iron agar (TSI).....40.33
Trypticase peptone glucose yeast extract broth with
  sterile trypsin added (TPGYT).....40.34
Trypticase or tryptic soy agar.....40.35
Tryptone-sulfite-neomycin agar (TSN).....40.36
Urea broth.....40.37
Vogel-Johnson agar.....40.38
Xylose lysine deoxycholate agar (XLD).....40.39

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General Instructions

Ingredients and reagents used to prepare the following media may be the product of any manufacturer if comparative tests have shown that satisfactory results are obtained. Carbohydrates must be chemically pure and suitable for biological use; inorganic chemicals must be ACS reagent grade; and dyes must be certified by "Biological Stain Commission" for use in media.

For convenience, dehydrated media of any brand equivalent to the formulation may be used unless instructions indicate otherwise. Test each lot of prepared medium for sterility and growth-promoting qualities of suitable organisms (e.g., inoculate media containing lactose with coliforms; Staphylococcus media with Staphylococcus, etc.).

Determine hydrogen ion concentrations (pH), using an electronic pH meter which is standardized against known buffers, prepared according to AOAC (latest edition). Adjust pH when necessary by adding sufficient 1 N sodium hydroxide or 1 N hydrochloric acid.

Unless otherwise indicated, sterilize media by steam under pressure at 121°C (15 lbs.) for 15 minutes.

An asterisk (*) beside the medium name means that the dehydrated prepared medium is not available commercially.

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