

40.01

Acid Broth*

Proteose peptone	5 g
Yeast extract	5 g
Dextrose	5 g
K_2HPO_4	4 g
Distilled H_2O	1 L

Adjust to pH 5.0. Dispense into tubes.

40.02

Anaerobic egg agar*

3 fresh eggs	
Yeast extract	5 g
Tryptone	5 g
Proteose peptone	20 g
NaCl	5 g
Na thioglycollate	1 g
Agar	20 g
Distilled H ₂ O	1 L

Wash eggs with a stiff brush, and drain. Soak them in 70% ethyl alcohol 10-15 minutes, remove them and ignite the adhering alcohol. Crack them aseptically. Separate and discard the whites. Drain the contents of the yolk sacs into a sterile stoppered graduate, and discard the sacs. Add to the yolk material an equal volume of sterile saline. Invert the cylinder several times to mix. Combine the remainder of the ingredients in the water, dissolve, dispense, and sterilize. To 1 L of the melted agar at 45-50°C, add 80 ml of the yolk-saline mixture. Mix and pour plates immediately. Dry plates at room temperature 2-3 days, or at 35°C for 24 hours. Discard contaminated plates. Store sterile plates in the refrigerator.

40.03

Brain heart infusion broth (BHI)

Calf brain, infusion from	200.0 g
Beef heart, infusion from	250.0 g
Proteose peptone or Gelysate	10.0 g
NaCl	5.0 g
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	2.5 g
Dextrose	2.0 g

Dissolve ingredients in 1 L H_2O , heating gently if necessary. Dispense in bottles or tubes for storage, and autoclave 15 minutes at 121°C . Final pH 7.4 ± 0.1 .

40.04

Bromcresol Purple Dextrose Broth (BCP)*

Dextrose	10 g
Beef extract	3 g
Peptone	5 g
Bromcresol purple, 1.6% in alcohol	2 ml
Distilled H ₂ O	1 L

Adjust to pH 7.0. Dispense into tubes.

40.05

Carbohydrate Fermentation Broths

Peptone	10 g
Meat extract	3 g
Sodium chloride	5 g
Carbohydrate	see below
Bromcresol purple	0.04 g
Distilled H ₂ O	1 L

Carbohydrates - 10 g/L: glucose, lactose, sucrose, mannitol.

5 g/L: salicin, inositol, adonitol, maltose,
arabinose, xylose.

The following sugars may be added to the media prior to sterilization: glucose, mannitol, salicin, adonitol, and inositol. The following sugars are prepared in 10% solutions in distilled water, sterilized by filtration, and appropriate amounts added to previously sterilized basal media: lactose, sucrose, maltose, arabinose and xylose.

Dispense all carbohydrates into tubes containing inverted fermentation tubes.

40.06

Chopped Liver Broth*

Fresh beef liver	500 g
Distilled H ₂ O	800 ml
Peptone	10 g
K ₂ HPO ₄	1 g
Soluble starch	1 g

Grind the liver into the water. Heat it to the boiling point, and permit it to simmer 1 hour. Cool, adjust to pH 7, and boil 10 minutes. Filter through cheesecloth, pressing out the excess liquid. To the broth add peptone, K₂HPO₄ and soluble starch. Adjust to pH 7. Bring the volume of broth to 1 L with distilled water. Filter it through coarse filter paper. (At this point the broth and meat can be stored separately in the freezer for future use.) To 18 or 20 mm test tubes add 1/2 to 1 inch of the chopped liver, and 10 to 12 ml of broth. Autoclave for 20 minutes at 121°C.

40.07

Cooked Meat Medium

Beef heart	454 g
Proteose peptone	20 g
Dextrose	2 g
Sodium chloride	5 g

Suspend 12.5 g of commercial dehydrated cooked meat medium in 100 ml of cold distilled water. Mix thoroughly and allow to stand for 15 minutes until all particles are thoroughly wetted. Medium may also be prepared by distributing 1.25 g into test tubes, adding 10 ml of cold distilled water, and mixing thoroughly, letting it stand to insure thorough wetting of all particles.

Sterilize for 15 minutes at 121°C. Final pH 7.2.

40.08

Desoxycholate Citrate Agar (DC)

Meat infusion (pork, beef, or beef heart)	330 g
Peptone	10 g
Lactose	10 g
Sodium citrate	20 g
Sodium desoxycholate	5 g
Ferric ammonium citrate	2 g
Agar	20 g
Neutral red	0.02 g
Distilled H ₂ O	1 L

Heat to boiling to dissolve the medium completely. Do not sterilize in the autoclave. Pour plates and allow the surface to dry for 2 hours with covers partially removed, before inoculation. This is the Difco product. Final pH 7.2.

40.09 Distilled Water (sterile, 200 ml)

Dispense 200 ml portion of distilled water into 500 ml flask or screwcap pint jar; plug or cap loosely. Autoclave 15 minutes at 121°C. Cool before using.

40.10 Distilled Water (sterile, 1000 ml)

Dispense 1000 ml portion of distilled water into 2 L wide-mouth flask or wide-mouth jar; plug or cap loosely. Autoclave 20 minutes at 121°C. Cool before using.

40.11

Ellner's Sporulation Broth*

Polypeptone (BBL) - Pantone (Difco)	10 g
Yeast extract	3 g
Soluble starch	3 g
MgSO ₄	0.1 g
KH ₂ PO ₄	1.5 g
Na ₂ HPO ₄ ·7H ₂ O	50 g
Distilled H ₂ O	1000 ml

Dissolve salts and other ingredients separately; then combine. Adjust to pH 7.2. Dispense 15 ml in 20 x 150 mm tubes and autoclave at 121°C for 15 minutes.

40.12

Fluid Thioglycollate Medium

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Dextrose	5.5 g
Yeast extract	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate	0.5 g
Resazurin	0.001 g
Distilled H ₂ O	1.0 L

Final pH 7.1 ± 0.1 .

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40.13 Fluid Thioglycollate (Azolectin-Tween Modification)*

Dehydrated prepared fluid thioglycollate medium (or ingredients as in USP)	29.5 g
Azolectin (available from Associated Concentrates Inc., 57-01 32nd Ave., Woodside, N. Y.)	5 g
Tween 20 (available from Atlas Powder Co., Wilmington, Del.)	40 ml
Distilled H ₂ O	1 L

Dissolve the azolectin in 340 ml of cold distilled water.

Dissolve the Tween 20 in 360 ml of distilled water by
holding at the boiling point for several minutes. Dissolve
the dehydrated prepared fluid thioglycollate medium in 300
ml of distilled water. Combine the three solutions.

Adjust to pH 7.7 before sterilization. Autoclave for 20
minutes at 121°C. Final pH 7.1 or 7.2. Dispense according
to USP instructions.

40.14

Gram-Negative Broth (GN)

Polypeptone (BBL) - Pantone (DIFCO)	20 g
Dextrose	1 g
d-Mannitol	2 g
Sodium citrate	5 g
Sodium desoxycholate	0.5 g
Dipotassium phosphate	4 g
Monopotassium phosphate	1.5 g
Sodium chloride	5 g
Distilled H ₂ O	1 L

Dispense into tubes/screwcap jars and autoclave for
15 minutes at 10 lbs. pressure (116°C) or steam steril-
ize for 30 minutes. Avoid overheating.

Final pH 7.0 $\pm$ 0.1.

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IMVC Media

40.15

Koser's Citrate Medium

Sodium ammonium phosphate	1.5 g
Monopotassium phosphate	1.0 g
Magnesium sulfate	0.2 g
Sodium citrate	3.0 g
Distilled H ₂ O	1.0 L

Dispense into tubes. Final pH 6.7 ± 0.1 .

40.16

MR-VP Medium

Polypeptone (BBL) or buffered peptone (DIFCO)	7.0 g
Dextrose	5.0 g
K ₂ HPO ₄	5.0 g
Distilled H ₂ O	1.0 L

Dispense into tubes. Final pH 6.9 ± 0.1 .

40.17

Tryptone Broth

Tryptone (DIFCO) or trypticase (BBL)	10.0 g
Distilled H ₂ O	1.0 L

Dispense into tubes.

40.18

Indole Nitrite Medium

Trypticase	20 g
Na ₂ HPO ₄	2 g
Dextrose	1 g
KNO ₃	1 g
Agar	1 g
Distilled H ₂ O	1000 ml

(Available dehydrated from BBL)

Heat to dissolve agar, adjust to pH 7.2, mix thoroughly, and dispense 11 ml in 16 x 150 mm tubes. Autoclave at 121°C for 15 minutes. (Add 2 g of agar per liter if desired.)

40.19

Iron Milk Medium*

Fresh whole milk

Small nails or steel wool (not stainless)

Heat nails in a Bunsen flame to remove oil or coatings.

Wash steel wool with detergent and rinse. Add pea-sized ball of steel wool or a nail to each 20 x 150 mm tube and fill with 15 ml of milk. Autoclave at 121°C for 5-6 minutes.

40.20

KCN Broth (Caution! KCN is a deadly poison.)Basal broth (DIFCO):

Proteose peptone No. 3	3 g
Disodium phosphate	5.64 g
Monopotassium phosphate	0.225 g
Sodium chloride	5 g
Distilled H ₂ O	1 L

Sterilize in the autoclave in 100 ml portions. Cool and store at 5-8°C. Final pH 7.6 $\pm$ 0.1. Prepare 0.5% KCN with cold (5-8°C) sterile distilled water. Using a sterile syringe or bulb pipet (do not pipet by mouth), add 1.5 ml of cold KCN solution to each 100 ml of cold sterile basal broth. Distribute 1-1.5 ml to sterile 13 x 100 mm test tubes and stopper quickly with No. 2 corks impregnated with paraffin. Prepare corks by boiling in paraffin ca 5 minutes. Store the finished medium in the refrigerator not more than 2 weeks. Final pH 7.6.

40.21 Levine's Eosine Methylene Blue Agar (Levine's EMB)

Peptone	10 g
Lactose	10 g
K_2HPO_4	2 g
Agar	15 g
Eosin Y	0.4 g
Methylene blue	0.065 g
Distilled H_2O	1 L

Heat to boiling with frequent agitation until the ingredients dissolve. Dispense into flasks or bottles for storage.

Autoclave for 15 minutes at $121^{\circ}C$. Final pH 7.1 ± 0.1 .

On remelting the agar, resuspend the precipitate by gentle agitation before pouring plates.

40.22

Liver Veal Egg Yolk Agar (LVEY)*

3 fresh eggs (antibiotic-free)

1 L melted sterile Difco liver veal agar

0.1% mercuric chloride solution

70% ethyl alcohol

Wash eggs with a stiff brush and drain. Soak eggs in 0.1% mercuric chloride solution for 1 hour. Pour off mercuric chloride solution and replace with 70% ethyl alcohol. Soak in 70% ethyl alcohol for 30 minutes. Crack the eggs aseptically and discard the whites. Remove the yolk with a 50 ml Luer-lok syringe. Place in a sterile container and add an equal volume of sterile saline (0.85% NaCl). Mix thoroughly. To each 500 ml of melted liver veal agar at 50°C, add 40 ml of the egg yolk-saline suspension. Mix thoroughly and pour plates. Dry plates at room temperature for 2 days or at 35°C for 24 hours. Discard contaminated plates and store sterile plates in the refrigerator.

40.23

Malonate Broth

Yeast extract	1 g
Ammonium sulfate	2 g
Dipotassium phosphate	0.6 g
Monopotassium phosphate	0.4 g
Sodium chloride	2 g
Sodium malonate	3 g
Glucose	0.25 g
Bromthymol blue	0.025 g
Distilled H ₂ O	1 L

Dissolve by heating if necessary. Dispense 3 ml portions into 13 x 100 mm test tubes and autoclave for 15 minutes at 121°C. Final pH 6.7 $\pm$ 0.1.

40.24

Malt Extract Broth

Malt extract	15 g
Distilled H ₂ O	1 L

Adjust pH to 4.7. Dispense into tubes and autoclave for 15 minutes at 121°C. Subject medium to as little heat as possible.

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40.25

Motility-nitrate Medium

Beef extract	3 g
Bacto peptone	5 g
KNO ₃	1 g
Agar	3 g
Distilled H ₂ O	1000 ml

Heat to dissolve agar. Adjust to pH 7.0, mix thoroughly and dispense 11 ml in 16 x 150 mm tubes. Autoclave 15 minutes at 121°C.

(May use Difco nitrate broth with 3 g of agar added per liter.)

40.26

Motility Test Medium

Beef extract	3 g
Peptone	10 g
NaCl	5 g
Agar	4 g
Distilled H ₂ O	1 L

Heat gently to dissolve. Boil 1-2 minutes. Dispense 20 ml into test tubes and autoclave for 15 minutes at 121°C. Final pH 7.4 ± 0.1.

40.27

Nonfat Dry Milk Solution (reconstituted)

Add 1000 ml of distilled water to 100 g nonfat dry milk. Mix well. Autoclave 20 minutes at 121°C. Cool before using.

40.28

Nutrient Agar

To nutrient broth formula (40.29), add 15 g of agar per liter.

40.29

Nutrient Broth

Suspend 3 g of beef extract, 5 g of peptone (DIFCO) or 5 g of Gelysate, and 3 g of beef extract (BBL) in 1 L of distilled water. Heat with occasional agitation until ingredients dissolve. Autoclave 15 minutes at 121°C. Cool before using.

40.30

Penassay Seed Agar

Peptone	6.0 g
Pancreatic digest of casein	4.0 g
Yeast extract	3.0 g
Beef extract	1.5 g
Dextrose	1.0 g
Agar	15.0 g
Distilled H ₂ O	1.0 L

Boil to dissolve. Dispense in 100 ml portions. Final
pH 6.5-6.6.

40.31

Selenite-Cystine Broth

Tryptone	5 g
Lactose	4 g
Na_2HPO_4	10 g
Sodium selenite	4 g
Cystine	0.01 g
Distilled H_2O	1 L

Dissolve and heat with frequent agitation. Dispense 10 ml portions into sterile 16 x 150 mm test tubes. Do not autoclave. Heat 10 minutes in flowing steam. Final pH 7.0 ± 0.1 . Medium is not sterile. Use same day as prepared.

40.32 Sulfite-Polymyxin-Sulfadiazine (SPS) Agar

Tryptone	15 g
Yeast extract	10 g
Ferric citrate	0.5 g
Agar	15 g
Distilled H₂O	1000 ml

Adjust to pH 7. Autoclave for 15 minutes at 121°C. To each liter of sterile medium add the following amounts of filter-sterilized solutions:

- 5 ml of 10% sodium sulfite ($\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$) solution
- 10 ml of 0.1% polymyxin B sulfate solution
- 10 ml of sodium sulfadiazine solution containing 12 mg/ml.

40.33

Triple Sugar Iron Agar (TSI)

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	15.0 g
Proteose peptone	5.0 g
Lactose	10.0 g
Saccharose	10.0 g
Dextrose	1.0 g
Ferrous sulfate	0.2 g
Sodium chloride	5.0 g
Sodium thiosulfate	0.3 g
Agar	12.0 g
Phenol red	0.024 g
Distilled H ₂ O	1.0 L

Suspend ingredients in 1 L of H₂O, mix thoroughly, and heat with occasional agitation. Boil ca 1 minute until ingredients dissolve. Fill tubes and autoclave 12 minutes at 121°C. Before media solidifies, slant tubes so that deep butts and adequate slants are formed. Final pH 7.3-7.4 ± 0.1.

40.34 Trypticase Peptone Glucose Yeast Extract Broth with Sterile**Trypsin Added (TPGYT)*****(a) Trypticase peptone glucose yeast extract broth**

Trypticase	50 g
Bacto-peptone	5 g
Yeast extract	20 g
Dextrose	4 g
Sodium thioglycollate	1 g
Distilled H ₂ O	1000 ml

Adjust to pH 7.0. Dispense the medium in volumes appropriate for the use: 15 ml in 20 mm culture tubes or 100 ml in 6 oz. prescription bottles. Autoclave the dispensed medium at 121°C for 6 minutes and 12 minutes, respectively. Refrigerate and discard if not used within 2 weeks.

(b) Prepare a 1.5% aqueous solution of trypsin (Difco 1:250). Sterilize by filtration through a Millipore filter. The trypsin solution should be prepared just before it is added to the TPGY medium.

(c) Boil the TPGY broth for 10-15 minutes (depending on the volume) in an Arnold steamer or in an autoclave set for flowing steam. Cool immediately after steaming. After cooling, add an appropriate amount of the sterilized trypsin solution (b) to the TPGY medium (a) to give a 0.1% final concentration. Mix very carefully, avoiding the addition of air to the medium as much as possible. After the addition of trypsin to the TPGY broth, the medium cannot be stored.

40.35

Trypticase or Tryptic Soy Agar

Trypticase or tryptone	15.0 g
Phytone or soytone	5.0 g
NaCl	5.0 g
Agar	15.0 g

Suspend ingredients in 1 L of H₂O, mixing thoroughly. Heat with frequent agitation, and boil for about 1 minute to dissolve completely. Autoclave 15 minutes at 121°C. Final pH 7.3 ± 0.1 .

40.36

Tryptone-Sulfite-Neomycin (TSN) Agar

Tryptone	15 g
Yeast extract	10 g
Ferric citrate (soluble)	0.5 g
Sodium sulfite	1.0 g
Agar	15 g
Distilled H₂O	1000 ml

Adjust to pH 7.2. Autoclave for 15 minutes at 121°C. To each liter of sterile medium add the following amounts of filter-sterilized solutions:

10 ml of 0.2% polymyxin B sulfate

10 ml of 0.5% neomycin sulfate

Final concentration in the medium: 20 µg/ml polymyxin,
50 µg/ml neomycin.

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40.37

Urea Broth (DIFCO); Urease Test Medium (BBL)

Yeast extract	0.1 g
Monopotassium phosphate	9.1 g
Disodium phosphate	9.5 g
Urea	20.0 g
Phenol red	0.01 g
Distilled H ₂ O	1.0 L

Dissolve ingredients. Filter-sterilize and distribute aseptically 1.5-3 ml portions into 13 x 100 mm test tubes. Do not heat. Final pH 6.8 ± 0.1 .

40.38 Vogel-Johnson (V-J) Agar (Tellurite Glycine Red Agar Base)

Trypticase or tryptone	10.0 g
Yeast extract	5.0 g
d-Mannitol	10.0 g
K_2HPO_4	5.0 g
$LiCl \cdot 6H_2O$	5.0 g
Glycine	10.0 g
Agar	15.0 g
Phenol red	0.025 g

Suspend ingredients in 1 L of H_2O and mix thoroughly. Heat with frequent agitation and boil 1 minute. Dispense in 100 ml portions and autoclave 15 minutes at $121^{\circ}C$. Cool to $45-50^{\circ}C$. To each 100 ml, add 2 ml of 1% K tellurite solution, which has been autoclaved separately 15 minutes at $121^{\circ}C$. Mix gently. Final pH, 7.2 ± 0.2 . Pour 15-18 ml into sterile 100 x 15 mm petri dishes. Temper plates at room temperature for ca 48 hours to reduce surface moisture.

40.39

Xylose Lysine Deoxycholate Agar (XLD)

Yeast extract	3 g
L-Lysine	5 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g
Na deoxycholate	2.5 g
Ferric ammonium citrate	0.8 g
Na thiosulfate	6.8 g
NaCl	5 g
Agar	15 g
Phenol red	0.08 g
Distilled H ₂ O	1 L

Boil to dissolve completely. Do not overheat. Pour into plates as soon as medium has cooled. Overheating may cause precipitation but reactions will be satisfactory although colonies may be slightly smaller. Final pH 7.4.

40.40

Lauryl Sulfate Tryptose Broth

Trypticase or tryptose	20.0 g
NaCl	5.0 g
Lactose	5.0 g
K_2HPO_4	2.75 g
KH_2PO_4	2.75 g
Sodium lauryl sulfate	0.1 g
Distilled H_2O	1 L

Dispense into test tubes containing inverted fermentation tubes (10 x 75 mm) and autoclave at $121^{\circ}C$ for 15 minutes.

Final pH 6.8 ± 0.1 .

40.41

Plate Count Agar

Pancreatic digest of casein, USP	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	15.0 g
Distilled H ₂ O	1.0 L

Suspend ingredients in water. Heat to boiling until all ingredients are dissolved. Autoclave at 121°C for 15 minutes. Final pH 7.0 ± 0.1.

41. REAGENTS, DILUENTS, ETC.

alpha-Naphthol solution.....	41.01
Brilliant green solution (1% aqueous).....	41.02
Butterfield's buffered phosphate diluent.....	41.03
Crystal violet solution (1% aqueous).....	41.04
Desiccated coagulase plasma (rabbit) with EDTA.....	41.05
Gel-phosphate diluent.....	41.06
Kovac's reagent.....	41.07
Methylene blue stain (Loeffler's).....	41.08
Methyl red solution.....	41.09
Saline (0.85% solution).....	41.10
Peptone water diluent.....	41.11
Reagent I.....	41.12
Reagent II.....	41.13
Tergitol Anionic 7 (sodium heptadecyl sulfate).....	41.14
Voges-Proskauer reagent (V-P).....	41.15

41.01 alpha-Naphthol Solution

alpha-Naphthol	5 g
Absolute ethyl alcohol	100 ml

41.02 Brilliant Green Solution (1% aqueous)

Dissolve 1 g of brilliant green dye in sterile distilled water and dilute to 100 ml.

41.03 Butterfield's Buffered Phosphate Diluent

(a) Stock solution. Dissolve 34 g of KH_2PO_4 in 500 ml of distilled water, adjust to pH 7.2 with ca 175 ml of 1 N NaOH, and dilute to 1 L. Store under refrigeration.

(b) Diluent. Dilute 1.25 ml of stock solution (a) to 1 L with distilled water. Prepare dilution blanks using this solution, dispensing a sufficient quantity to allow for losses due to autoclaving.

Autoclave for 15 minutes at 121°C.

41.04 Crystal Violet Solution (1% aqueous)

Dissolve 1 g of crystal violet dye in sterile distilled water and dilute to 100 ml.

41.05 Desiccated Coagulase Plasma (Rabbit) with EDTA

Reconstitute according to directions of manufacturer. If not available, reconstitute "Desiccated coagulase plasma (rabbit)" and add disodium EDTA to make final concentration of 0.1%.

41.06 Gel-Phosphate Diluent

Gelatin	2 g
Na_2HPO_4	4 g
Distilled H_2O	1 L

Adjust to pH 6.2 with 1 N HCl. Autoclave for 20 minutes at 121°C.

41.07

Kovac's Reagent

p-Dimethylaminobenzaldehyde	5 g
Amyl alcohol	75 ml
Concentrated HCl	25 ml

Dissolve the p-dimethylaminobenzaldehyde in the amyl alcohol; then add the HCl. Test the reagent on a known sample of indole to obtain the typical red color before using it on unknowns.

41.08

Methylene Blue Stain (Loeffler's)Solution A

Methylene blue (90% dye content)	0.3 g
Ethyl alcohol (95%)	30 ml

Solution B

Dilute KOH (0.01%)	100 ml
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Mix solutions A and B.

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41.09

Methyl Red Solution

Methyl red	0.1 g
95% ethyl alcohol	300 ml
Distilled H ₂ O	200 ml

Dissolve the methyl red in the alcohol. Add the water.

41.10

0.85% NaCl

Sodium chloride	8.5 g
Distilled H ₂ O	1 L

41.11

Peptone Water Diluent

Bacto peptone	1 g
Distilled H ₂ O	1000 ml

Adjust to pH 6.8. Prepare dilution blanks with this solution, dispensing a sufficient quantity to allow for loss during autoclaving. Autoclave at 121°C for 15 minutes.

41.12

Reagent I

Sulfanilic acid	0.8 g
5 <u>N</u> acetic acid ^{1/}	100.0 ml

41.13

Reagent II

alpha-Naphthylamine or	
dimethyl-alpha-naphthylamine	0.6 ml
5 <u>N</u> acetic acid ^{1/}	100.0 ml

41.14

Tergitol Anionic 7 (Sodium Heptadecyl Sulfate)

Steam for 30 minutes. Cool before using.

41.15

Voges-Proskauer (V-P) Reagent

KOH	40 g
Distilled H ₂ O	100 ml

^{1/}28.75 ml of glacial acetic acid added to 71.25 ml of distilled water

MPN Tables

42.01 MPN per gram of sample planting 1.0, 0.1, and 0.01 ml portions.

No. of Positive Tubes			MPN Per Gram	No. of Positive Tubes			MPN Per Gram
1.0	0.1	0.01		1.0	0.1	0.01	
g	g	g		g	g	g	
0	0	0	0.0	2	0	0	0.91
0	0	1	0.3	2	0	1	1.4
0	0	2	0.6	2	0	2	2.0
0	0	3	0.9	2	0	3	2.6
0	1	0	0.3	2	1	0	1.5
0	1	1	0.61	2	1	1	2.0
0	1	2	0.92	2	1	2	2.7
0	1	3	1.2	2	1	3	3.4
0	2	0	0.62	2	2	0	2.1
0	2	1	0.93	2	2	1	2.8
0	2	2	1.2	2	2	2	3.5
0	2	3	1.6	2	2	3	4.2
0	3	0	0.94	2	3	0	2.9
0	3	1	1.3	2	3	1	3.6
0	3	2	1.6	2	3	2	4.4
0	3	3	1.9	2	3	3	5.3
1	0	0	0.36	3	0	0	2.3
1	0	1	0.72	3	0	1	3.9
1	0	2	1.1	3	0	2	6.4
1	0	3	1.5	3	0	3	9.5
1	1	0	0.73	3	1	0	4.3
1	1	1	1.1	3	1	1	7.5
1	1	2	1.5	3	1	2	12.0
1	1	3	1.9	3	1	3	16.0
1	2	0	1.1	3	2	0	9.3
1	2	1	1.5	3	2	1	15.0
1	2	2	2.0	3	2	2	21.0
1	2	3	2.4	3	2	3	29.0
1	3	0	1.6	3	3	0	24.0
1	3	1	2.0	3	3	1	46.0
1	3	2	2.4	3	3	2	110.0
1	3	3	2.9	3	3	3	>110.0

MPN Tables, Cont.

MPN per gram of sample planting 0.1, 0.01 and 0.001 ml portions

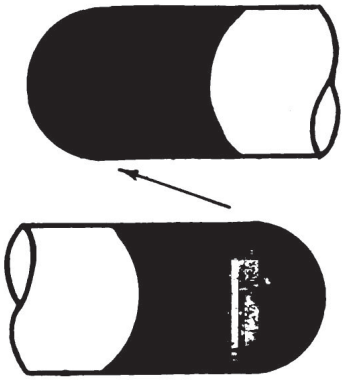
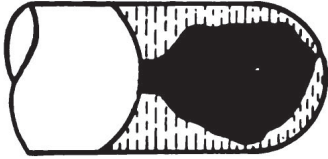
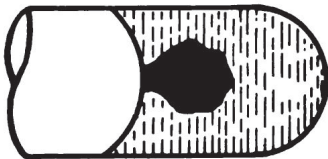
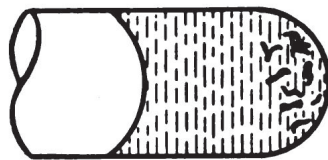
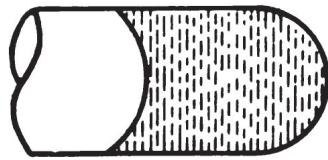
No. of Positive Tubes				No. of Positive Tubes			
MPN Per Gram				MPN Per Gram			
0.1	0.01	0.001		0.1	0.01	0.001	
g	g	g		g	g	g	
0	0	0	0.0	2	0	0	9.1
0	0	1	3.0	2	0	1	14.0
0	0	2	6.0	2	0	2	20.0
0	0	3	9.0	2	0	3	26.0
0	1	0	3.0	2	1	0	15.0
0	1	1	6.1	2	1	1	20.0
0	1	2	9.2	2	1	2	27.0
0	1	3	12.0	2	1	3	34.0
0	2	0	6.2	2	2	0	21.0
0	2	1	9.3	2	2	1	28.0
0	2	2	12.0	2	2	2	35.0
0	2	3	16.0	2	2	3	42.0
0	3	0	9.4	2	3	0	29.0
0	3	1	13.0	2	3	1	36.0
0	3	2	16.0	2	3	2	44.0
0	3	3	19.0	2	3	3	53.0
1	0	0	3.6	3	0	0	23.0
1	0	1	7.2	3	0	1	39.0
1	0	2	11.0	3	0	2	64.0
1	0	3	15.0	3	0	3	95.0
1	1	0	7.3	3	1	0	43.0
1	1	1	11.0	3	1	1	75.0
1	1	2	15.0	3	1	2	120.0
1	1	3	19.0	3	1	3	160.0
1	2	0	11.0	3	2	0	93.0
1	2	1	15.0	3	2	1	150.0
1	2	2	20.0	3	2	2	210.0
1	2	3	24.0	3	2	3	290.0
1	3	0	16.0	3	3	0	240.0
1	3	1	20.0	3	3	1	460.0
1	3	2	24.0	3	3	2	1,100.0
1	3	3	29.0	3	3	3	>1,100.0

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"The clot produced varies from one which is solid and immovable when the tube is inverted, to a loose clot or occasionally only a trace of fibrin. Any degree of clotting is regarded as a positive reaction."

—Blair, J.E. in Dubos' "Bacterial and Mycotic Infections of Man," Lippincott, 1952, p. 372.

42.02 TYPES OF COAGULASE TEST REACTIONS

<u>POSITIVE</u>	
4+	
3+	
2+	
1+	
<u>NEGATIVE</u>	
NEGATIVE	NO EVIDENCE OF FIBRIN FORMATION
1+ POSITIVE	SMALL UNORGANIZED CLOTS
2+ POSITIVE	SMALL ORGANIZED CLOT
3+ POSITIVE	LARGE ORGANIZED CLOT
4+ POSITIVE	ENTIRE CONTENT OF TUBE COAGULATES AND IS NOT DISPLACED WHEN TUBE IS INVERTED

—From Turner, F.J. & Schwartz, B.S. The Use of a Lyophilized Human Plasma, Standardized for Blood Clotting Factors, in the Coagulase Test. Presented before the Society of American Bacteriologists, Houston, May 3, 1956

42.03 pH Range of a Few Selected Commercially Canned Foods

<u>Kind of Food</u>	<u>pH Range, approximate</u>
Apples, whole	3.4 - 3.5
Apples, juice	3.3 - 3.5
Asparagus, green	5.0 - 5.8
Beans	
baked	4.8 - 5.5
green	4.9 - 5.5
lima	5.4 - 6.3
soy	6.0 - 6.6
Beans, with pork	5.1 - 5.8
Beef, corned, hash	5.5 - 6.0
Beets, whole	4.9 - 5.8
Blackberries	3.0 - 4.2
Blueberries	3.2 - 3.6
Boysenberries	3.0 - 3.3
Bread	
white	5.0 - 6.0
date and nut	5.1 - 5.6
Broccoli	5.2 - 6.0
Carrots, chopped	5.3 - 5.6
Carrot juice	5.2 - 5.8
Cheese	
Parmesan	5.2 - 5.3
Roquefort	4.7 - 4.8
Cherry juice	3.4 - 3.6
Chicken	6.2 - 6.4
Chicken with noodles	6.2 - 6.7
Chop suey	5.4 - 5.6
Cider	2.9 - 3.3
Clams	5.9 - 7.1
Cod fish	6.0 - 6.1
Corn	
on-the-cob	6.1 - 6.8
cream style	5.9 - 6.5
whole grain	
brine packed	5.8 - 6.5
vacuum packed	6.0 - 6.4
Crab apples, spiced	3.3 - 3.7
Cranberry	
juice	2.5 - 2.7
sauce	2.3 - 2.3
Currant juice	3.0 - 3.0

<u>Kind of Food</u>	<u>pH Range, approximate</u>
Dates	6.2 - 6.4
Duck	6.0 - 6.1
Figs	4.9 - 5.0
Frankfurters	6.2 - 6.2
Fruit cocktail	3.6 - 4.0
Gooseberries	2.8 - 3.1
Grapefruit	
juice	2.9 - 3.4
pulp	3.4 - 3.4
sections	3.0 - 3.5
Grapes	3.5 - 4.5
Ham, spiced	6.0 - 6.3
Hominy, lye	6.9 - 7.9
Huckleberries	2.8 - 2.9
Jam, fruit	3.5 - 4.0
Jellies, fruit	3.0 - 3.5
Lemons	2.2 - 2.4
juice	2.2 - 2.6
Lime juice	2.2 - 2.4
Loganberries	2.7 - 3.5
Mackerel	5.9 - 6.2
Milk	
cow	6.4 - 6.8
evaporated	5.9 - 6.3
Molasses	5.0 - 5.4
Mushrooms	6.0 - 6.5
Olives, ripe	5.9 - 7.3
Orange juice	3.0 - 4.0
Oysters	6.3 - 6.7
Peaches	3.4 - 4.2
Pears (Bartlett)	3.8 - 4.6
Peas	5.6 - 6.5
Pickles	
dill	2.6 - 3.8
sour	3.0 - 3.5
sweet	2.5 - 3.0
Pimento	4.3 - 4.9
Pineapple	
crushed	3.2 - 4.0
sliced	3.5 - 4.1
juice	3.4 - 3.7
Plums	2.8 - 3.0

<u>Kind of Food</u>	<u>pH Range, approximate</u>
Potatoes	
white	5.4 - 5.9
mashed	5.1 - 5.1
Potato salad	3.9 - 4.6
Prune juice	3.7 - 4.3
Pumpkin	5.2 - 5.5
Raspberries	2.9 - 3.7
Rhubarb	2.9 - 3.3
Salmon	6.1 - 6.5
Sardines	5.7 - 6.6
Sauerkraut	3.1 - 3.7
juice	3.3 - 3.4
Shrimp	6.8 - 7.0
Soups	
bean	5.7 - 5.8
beef broth	6.0 - 6.2
chicken noodle	5.5 - 6.5
clam chowder	5.6 - 5.9
duck	5.0 - 5.7
mushroom	6.3 - 6.7
noodle	5.6 - 5.8
oyster	6.5 - 6.9
pea	5.7 - 6.2
tomato	4.2 - 5.2
turtle	5.2 - 5.3
vegetable	4.7 - 5.6
Spinach	4.8 - 5.8
Squash	5.0 - 5.3
Strawberries	3.0 - 3.9
Sweet potatoes	5.3 - 5.6
Tomatoes	4.1 - 4.4
juice	3.9 - 4.4
Tuna	5.9 - 6.1
Turnip greens	5.4 - 5.6
Vegetable	
juice	3.9 - 4.3
mixed	5.4 - 5.6
Vinegar	2.4 - 3.4
Youngberries	3.0 - 3.7

<u>Kind of Food</u>	<u>pH Range, approximate</u>
Other miscellaneous products:	
Beers	4.0 - 5.0
Ginger ale	2.0 - 4.0
Human	
blood plasma	7.3 - 7.5
duodenal content	4.8 - 8.2
feces	4.6 - 8.4
gastric contents	1.0 - 3.0
milk	6.6 - 7.6
saliva	6.0 - 7.6
spinal fluid	7.3 - 7.5
urine	4.8 - 8.4
Magnesia, milk of	10.0 - 10.5
Water	
distilled, CO ₂	6.8 - 7.0
mineral	6.2 - 9.4
sea	8.0 - 8.4
Wines	2.3 - 3.8

42.04 List of Official Methods

(a) Frozen and/or Prepared Foods, and Tree Nut Meats.

Determination of aerobic plate count, coliform group, Escherichia coli and coagulase-positive staphylococci. JAOAC 49 (1), 246-250 (1966); and revisions, JAOAC 51 (2), 505-506 (1968).

(b) Detection and Identification of Salmonella in Dried Whole Eggs, Dried Egg Yolk, and Dried Egg White. JAOAC 50 (1), 231-239 (1967); and revisions JAOAC 51 (2), 505-506 (1968).

(c) Examination of Eggs and Egg Products. Determination of aerobic plate count and direct microscopic count. AOAC (10th edition, 1965) 37.003-37.006, 37.010; and latest revisions.

(d) Shellfish (clams, oysters, mussels, etc.). "Recommended Methods for the Bacteriological Examination of Sea Water and Shellfish." APHA, 1962.

(e) Water and Beverages. Latest edition of "Standard Methods for the Examination of Water and Wastewater." APHA

42.05 List of Other Recommended Methods

(a) Penicillin Contamination in Drugs. "Procedure for Detecting and Measuring Penicillin Contamination in Drugs." Department of Health, Education, and Welfare, Food and Drug Administration, Bureau of Scientific Standards and Evaluation, Division of Antibiotics and Insulin Certification, Washington, D. C. 20204 (1965); addendum August 1967.

(b) Antibiotic Residues in Milk, Dairy Products and Animal Tissue. "Antibiotic Residues in Milk, Dairy Products, and Animal Tissues: Methods, Reports, and Protocols" (Revised October 1968). National Center for Antibiotic and Insulin Analysis, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C. 20204.

42.06

FOUR-PLACE LOGARITHMS

N											Proportional Parts								
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	*4	8	12	17	21	25	29	33	37
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4	8	11	15	19	23	26	30	34
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3	7	10	14	17	21	24	28	31
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3	6	10	13	16	19	23	26	29
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014	*3	6	8	11	14	17	20	22	25
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3	5	8	11	13	16	18	21	24
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2	5	7	10	12	15	17	20	22
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2	4	7	9	11	13	16	18	20
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1	2	4	5	6	7	9	10	11
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1	2	4	5	6	7	8	10	11
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1	2	3	5	6	7	8	9	10
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1	2	3	4	5	7	8	9	10
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1	2	3	4	5	6	8	9	10
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1	2	3	4	5	6	7	8	9
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1	2	3	4	5	6	7	8	9
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1	2	3	4	5	6	7	8	9
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1	2	3	4	5	6	7	7	8
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	5	5	6	7	8
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1	2	3	4	4	5	6	7	8
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1	2	3	4	4	5	6	7	8
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1	2	3	3	4	5	6	7	8
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1	2	3	3	4	5	6	7	8
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	2	3	4	5	6	7	7
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1	2	2	3	4	5	6	6	7
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	2	3	4	5	6	6	7
N	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9

* Interpolation in this section of the table is inaccurate.

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National Beef Ex. 1060

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FOUR-PLACE LOGARITHMS (Continued)

N											Proportional Parts									
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7	
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7	
57	7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	1	2	2	3	4	5	5	6	7	
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7	
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7	
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6	
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6	
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6	
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6	
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6	
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	5	5	6	
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	5	5	6	
67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8319	1	1	2	3	3	4	5	5	6	
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6	
69	8388	8395	8401	8407	8414	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6	
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6	
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5	
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5	
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5	
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5	
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5	
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5	
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5	
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5	
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9025	1	1	2	2	3	3	4	4	5	
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5	
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5	
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5	
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5	
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5	
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5	
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5	
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	0	1	1	2	2	3	3	4	4	
88	9445	9450	9455	9460	9465	9469	9474	9479	9484	9489	0	1	1	2	2	3	3	4	4	
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0	1	1	2	2	3	3	4	4	
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4	
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4	
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4	
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4	
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	0	1	1	2	2	3	3	4	4	
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	0	1	1	2	2	3	3	4	4	
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4	
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4	
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0	1	1	2	2	3	3	4	4	
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0	1	1	2	2	3	3	4	4	
N	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	

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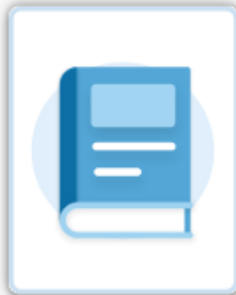
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