A Comprehensive guide to

QUALITY ANALYSIS OF FRUIT JUICES AND SOFT DRINK

Analytical Procedures

Edited by

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A Comprehensive guide to-Quality Analysis of Fruit Juices and Soft Drink-Analytical Procedures

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Introduction

Food industry is one of the important segments in our country's industrial front. It is poised for a tremendous growth especially in the light of the thrust and importance given to it by the government. This can result in improved production with emphasis on quality products. The concept of quality products will definitely invite stringent quality control measures to be adopted, both for the existing products and for the development of new products. A food product has to be safe, nutritious and rich in all the vital ingredients required for the maintenance of human body's physiological mechanism. A food item is mostly a complex mixture of many chemical entities. Many times, it can be of natural origin or it can be manufactured to a certain specification. In either case, it should have the required nutrients for human growth and body metabolism. Like any other industry, food industry also stipulates stringent specifications for the control of the quality. Measures of quality control will involve the usage of modern analytical instruments for the analysis of various parameters and quality characteristics of food products. It is in this context that we can explore the possibilities of offering various types of equipments to this industrial sector.

TEST NO: 1 MOISTURE CONTENT IN FRUIT JUICES AND SOFT DRINKS

1.1.0	**Title**	Moisture content in Fruit Juices and Soft Drinks
1.1.1	**Objective**	To estimate the moisture content in Fruit Juices and Soft Drinks
1.1.2	**Scope**	Estimation of moisture content in Fruit Juices and Soft Drinks by air oven method
1.1.3	**Related or relevant procedure**	AOAC official methods of analysis (2005) Ch., 4,P.2, 930.15 (4.1.06).18th Ed., William Horwitz and George W. Latimer Pub. By AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 20877-2417 USA.

1.1.4 Requirements of Procedure

1.1.4.1 Glassware / Apparatus

- Air oven (Automatic Control)
- Weighing dish
- Weighing balance
- Desiccator

1.1.5 Determination

Regulate air oven to $135^0 \pm 2^0$C. Using low, covered Al dishes, ≥50 mm diameter and 40 mm deep, weigh ca 2 g test portion into each dish and shake until contents are evenly distributed. With covers removed, place dishes and dry 2h ± 5 min. Place covers on dishes and transfer to desiccators to cool. Weigh, and calculate loss in weight on drying (LOD) as estimate of H_2O.

1.1.6 Calculation

(%) (w/w) LOD = % (w/w) moisture =

$$100 \times \frac{\text{Wtloss on drying, g}}{\text{wt test portion, g}}$$

% Dry matter = 100 - % LOD

1.1.7 Expression of result

The result is expressed in terms of percentage

TEST NO: 2 ESTIMATION OF PROTEIN IN FRUIT JUICES AND SOFT DRINKS

1.2.0 Title Estimation of Protein

1.2.1 Objective To estimate the protein content in Fruit Juices and Soft Drinks

1.2.2 Scope To estimate the crude protein in Fruit Juices and Soft Drinks by Kjeldahl's method

1.2.3 Related or Relevant Procedure AOAC official methods of analysis (2005) Ch.,4,P.35,2001.11 (4.2.11).18th Ed., William Horwitz and George W. Latimer Pub. By AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 20877-2417 USA.

1.2.4 Requirements of Procedure

1.2.4.1 Glass wares / Apparatus

- Digestion block (Gerhardt - Turbotherm)
- Distillation unit (Gerhardt – Vapodest 20)
- Digestion tubes - 250ml
- Conical Flask - 250ml
- Fume Exhaust Manifold
- Weighing balance
- Pipetting dispenser
- Burette

1.2.4.2 Chemicals/ AR Grade

- Conc. Sulphuric acid
- Potassium Sulfate - N-free
- Copper catalyst
- Sodium Hydroxide
- Methyl red
- Methylene blue
- Boric acid
- Hydrochloric Acid
- Mercuric Oxide

1.2.4.3 Reagents

Sodium Hydroxide Solution:40% (w/w) NaOH

Methyl red indicator: Dissolve 100mg Methyl red in 100ml methanol

Methylene blue indicator: Dissolve 100 mg Methylene blue in 100ml methanol

Mixed indicator: Two parts of Methyl red and one part of Methylene blue are mixed

Boric acid solution: 4% (w/v) Dissolve 4g Boric acid in100ml water

Standard Hydrochloric acid solution: 0.1000M

1.2.5 Determination

1.2.5.1 Digestion: Place weighed test portion (0.7 - 2.2g) in a digestion flask (Gerhardt - Turbotherm). Mix with 0.7g mercuric oxide, 10g of powdered potassium sulphate or anhydrous sodium sulphate, 1g of copper sulphate and 25ml of concentrated Sulphuric acid. Place the digestion tubes in digestion chamber and digest the sample until solution clears (2 hour for test portions containing organic material). Turn the digester off and remove the tubes. Cool and add 200 ml of water

1.2.5.2 Distillation: Place 40% Sodium Hydroxidesolution in the alkali tank of distillation unit (Gerhardt – Vapodest 20). Adjust volume dispensed to 50 ml. Connect the digestion flask to distilling bulb on condenser and with the tip of the condenser immersed in a standard 4% Boric acid solution and add 5 - 7 drops of mixed indicator. Collect the steam of distillate after the distillation completed. Remove the receiving flask. Titrate Boric acid receiving solution with standard 0.1000M HCl to violet end point (just before the solution goes back to pink). Correct for blank determination on reagents.

1.2.6 Calculation

$$N\ (\%) = \frac{\{V_S - V_B\} \times M \times 14.01}{W \times 10}$$

Crude protein, % = % N x F

Where,

V_S = volume (ml)of standardized acid used to titrate a test

V_B = volume (ml)of standardized acid used to titrate reagent blank

M= Molarity of standard HCl

14.01 = atomic weight of N

W= weight(g) of test portion or standard

10= factor to convert mg/g to percent

F= factor to convert N to protein

F factors are 5.70 for wheat, 6.38 for dairy products and 6.25 for other feed materials.

1.2.7 Expression of result The results are expressed in percentage

TEST NO: 3 ESTIMATION OF FAT CONTENT IN FRUIT PRODUCTS AND SOFT DRINKS

1.3.0 Title Estimation of Fat content in Fruit Juices and Soft Drinks

1.3.1 Objective To estimate the fat content in Fruit Juices and Soft Drinks

1.3.2 Scope Estimation of fat content in Fruit Juices and Soft Drinks by soxtherm

1.3.3 Related or Relevant Procedure AOAC official methods of analysis (2005) Ch.,4,p.42-43, 930.15 (4.1.06).18th Ed., William Horwitz and George W. Latimer Pub. By AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 20877-2417 USA.

1.3.4 Requirements of Procedure

1.3.4.1 Apparatus / Glass wares

- Soxtherm - fat extractor
- Oil flask
- Thimble
- Coarse filter paper,
- Air oven
- Weighing balance

1.3.4.2 Reagents/AR

- Hexane (68.7^{0}C).
- Cotton- Defatted. Soak medical grade cotton in diethyl ether or
- Hexanes for 24 h, agitating several times during this period. Remove and air dry.
- Sand
- Celite 545

1.3.5 Determination Weigh 1-5 g test portions containing ca 100-200 mg fat directly into tared cellulose thimbles, according to the following scheme:

Record weight to nearest 0.1 mg (S) and thimble number. Dry thimbles containing test portions at 102^0 ± 2^{0}C for 2 h. If dried test portions will not be extracted immediately, store in desiccators. Both solvent and test materials must be free of moisture to avoid extraction of water- soluble components such as carbohydrates, urea, lactic acid, and glycerol, which will result in false high values.

Extract, following manufacturer's instructions for operation of Soxtherm-fat extractor. Preheat extractor and turn on condenser cooling water. Attach thimbles containing dried test portions to extraction columns. Put sufficient amount of solvent into each extraction cup to cover test portion when thimbles are in boiling position. Place cups under extraction columns and secure in place. Make sure that cups are matched to their corresponding thimble. Lower thimbles into solvent and boil for 6hrs.

Raise thimbles out of solvent and extract in this position for 40 min. then distill as much solvent as possible from cups to reclaim solvent and attain apparent dryness.

Remove extraction cups from extractor and place in operating fume hood to finish evaporating solvent at low temperature.

Dry extraction cups in 102^{o}C ± 2^{o}C oven for 30 min to remove moisture. Excessive drying may oxidize fat and give high results. Cool in desiccators to room temperature and weigh to nearest 0.1 mg (F).

1.3.6 Calculation

$$\%\ \text{Crude fat} = \frac{F - T}{S} \times 100$$

Where F= weight of cup + fat residue, g; T= weight of empty Cup ,g; S= test portion weight, g.

1.3.7 Expression of Result

Result is expressed in terms of percentage

TEST NO: 4 ESTIMATION OF CRUDE FIBER IN FRUIT PRODUCTS AND SOFT DRINKS

1.4.0 Title Estimation of Crude Fiber in Fruit Juices and Soft Drinks

1.4.1 Objective To determine the crude fiber content in Fruit Juices and Soft Drinks

1.4.2 Scope Determination of crude fiber in Fruit Juices and Soft Drinks by using Foss Tectator

1.4.3 Related or Relevant Procedure AOAC official methods of analysis(2005) Ch.,4,P.44,962.09(4.6.01). 18th Ed., William Horwitz and George W. Latimer Pub. By AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 20877-2417 USA.

1.4.4 Requirements of Procedure

1.4.4.1 Apparatus / Glass wares

- Foss Tectator Hot Extraction Unit
- Foss Tectator Cold Extraction Unit
- Crucibles- 50 ml coarse porosity
- Weighing balance
- Volumetric Flask

1.4.4.2 Chemicals/ AR Grade

- Sodium hydroxide
- Sulphuric acid
- Antifoaming agent (n-octanol)
- Alcohol
- Acetone

1.4.4.3 Reagents

- **1.25% Sulphuric acid:**
  1.25g conc. Sulphuric acid in 100ml
- **1.25% Sodium Hydroxide:**
  1.25g Sodium hydroxide in 100ml

1.4.5 Determination Extract 2g ground test portion (w) with ether or n-hexane. If fat is less than 1%, extraction may be omitted. If fat is between 1-5%, Oil extraction will be carried out with Cold Extraction Unit. Take 2g of extracted samples in crucible. Add approximately 1.5 to 2.0g dry weights of celite, 100 ml of boiling 1.25% Sulphuric acid and one drop diluted antifoam (Excess antifoam may give high

results; use only if necessary to control foaming). Determine the blank value by treating ca 2g of celite with acid and alkali as in determination.

Place the crucibles in a hot extraction unit and boil for exactly 30 minutes then filter the solution. Add approximately 100 ml of 1.25% Sodium Hydroxide Solution and boil for 30 minutes and filter the solution. Then the residue is washed with water. Remove the crucible and dry the residue in a hot air oven for 2 hours at130° $\pm$ 2° C. Cool in a desiccator and weigh (W2) Ignite 30 minutes at 600°C $\pm$ 15° C. Cool in a desiccator and weigh (W3)

1.4.6 Calculation

Crude fiber in ground test portion % = C

$$\%\ C = \frac{\text{(loss in weight on ignition-loss in weight ofceramic fiber blank)}}{\text{Weight of test portion}} \times 100$$

$$C = \left[\frac{(W2-W3) + (B2-B3)}{W1}\right] \times 100$$

Where, W2-W3= Loss in weight on ignition

B2 - B3= Loss in weight of ceramic fiber blank

W1 = Weight of the sample

Crude Fiber on desired Moisture basis (%) =

$$C \times \frac{\text{(100 - Percent moisture desired)}}{\text{100 – Percent moisture in ground test sample}}$$

1.4.7 Expression of result

The results are expressed in percentage

TEST NO. 5 ASH CONTENT IN FRUIT JUICES AND SOFT DRINKS

1.5.0 Title Estimation of Ash content in Fruit Juices and Soft Drinks

1.5.1 Objective To estimate the ash content in Fruit Juices and Soft Drinks

1.5.2 Scope Estimation of ash content in Fruit Juices and Soft Drinks

1.5.3 Related or relevant procedure AOAC official methods of analysis(2005) Ch.,4, p.8, 942.05(4.1.10).18th Ed., William Horwitz and George W. Latimer Pub. By AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 20877-2417 USA.

1.5.4 Requirements of Procedure

1.5.4.1 Glassware / Apparatus

- Muffle furnace
- Desiccator
- Porcelain crucible (Silica Crucible)
- Weighing balance

1.5.5 Determination Weigh 2 g test portion into porcelain crucible and place in temperature controlled furnace preheated to 600^{0}C. Hold at this temperature 2 h. Transfer crucible directly to desiccator, cool, and weigh immediately, reporting percent ash to first decimal place.

1.5.6 Calculation The total ash of the cereals is calculated by using the following formula.

$$\% \text{ (w/w) Ash} = \frac{A-B}{A} \times 100$$

A- Weight of test portion, g

B- Weight loss on ashing, g

1.5.7 Expression of result Result is expressed in terms of percentage.

TEST NO.6 ANALYSIS OF TOTAL CARBOHYDRATE BY ANTHRONE METHOD

1.6.0 OBJECTIVE : To estimate the Carbohydrate content

1.6.1 PRINCIPLE

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm.

1.6.2 MATERIALS

- 2.5 N HCl (91.15ml make upto 1000ml distilled water)
- Anthrone reagent: Dissolve 200 mg anthrone in 100 mL of ice-cold 95% H_2SO4.
- Prepare fresh before use.
- Standard glucose: Stock—Dissolve 100 mg in 100 mL water. Working standard—10 mL of stock diluted to 100 mL with distilled water.
- Test tubes
- Colorimeter
- Cuvette
- Tissue paper
- Wash bottle
- Aluminium foil
- Waterbath
- Reagent bottle – 250 ml
- Beaker- 100 ml

1.6.3 EXTRACTION OF CARBOHYDRATE

1. Grind the sample with Phosphate buffer and centrifuge the homogenize at maximum speed for 5 minutes
2. Collect the supernatant.
3. To the supernatant, add few drops of 100% Acetone to remove the interfering pigments by allowing undisturbed.
4. Discard the colored layer and retain the aqueous layer.

1.6.4 METHODOLOGY

1. Weigh 100 mg of the sample into a boiling tube.
2. Hydrolyse by keeping it in a boiling water bath for three hours with 5 mL of 2.5 N HCl and cool to room temperature.
3. Neutralise it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100 mL and centrifuge.
5. Collect the supernatant and take 0.5 and 1 mL aliquots for analysis.
6. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard. '0' serve as blank.
7. Make up the volume to 1 mL in all the tubes including the sample tubes by adding distilled water.
8. Then add 4 mL of anthrone reagent.
9. Heat for eight minutes in a boiling water bath.
10. Cool rapidly and read the green to dark green colour at 630 nm.
11. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
12. From the graph calculate the amount of carbohydrate present in the sample tube.

1.6.5 Table

Description	**Blank**	**S1**	**S2**	**S3**	**S4**	**S5**	**Test**
Standard/ml	0	0.2	0.4	0.6	0.8	1	0
Sample/ml	0	0	0	0	0	0	0.5
Distilled water/ml	1	0.8	0.6	0.4	0.2	0	0.5
Anthrone Reagent/ml	4	4	4	4	4	4	4
	Incubate the tubes in boiling water for 10 minutes						
Absorbance	625 nm						

1.6.6 CALCULATION

Amount of carbohydrate present in 100 mg of the sample = mg of glucose / Volume of test sample x 100

NOTE:

Cool the contents of all the tubes on ice before adding ice-cold anthrone reagent.

TEST NO. 7 DETERMINATION OF VITAMIN C (ASCORBIC ACID):

The ascorbic acid content in fruits and vegetables can be estimated by macerating the sample with stabilizing agents such as 20 % metaphosphoric acid.

Principle: 2, 6 -dichlorophenol indophenol is reduced to a colorless form by ascorbic acid. The reaction is specific for ascorbic acid at pH 1 to 3.5. The dye is blue in alkaline solution and pink in acid.

Reagents:

1. 4% Oxalic acid.

2. **2, 6-dichlorophenol Indophenol ($C_{12}H_6C_2N\ NaO2.\ 2H_2O$) dye solution:**

52 mg of 2,6-dichlorophenol Indophenol is weighed and taken in a beaker. 42mg Sodium Bicarbonate (Na_2HCO_3) is added to it. It is then dissolved in little amount of distilled water and the volume is made up to 200ml with distilled water.

3. Standard Ascorbic acid ($C_6H_8O_6$) solution: 100 mg ascorbic acid in 100 ml 4% Oxalic acid.

4. Working standard – 10 ml Standard Ascorbic acid into 90 ml 4% oxalic acid solution

Standardisation of Dye:

Pipette 10 ml of standard Ascorbic acid solution in a small flask and titrate with indophenol solution until a faint pink colour persists for 15 seconds. Express the concentration as mg Ascorbic acid equivalent to 1 ml of dye solution i.e 10 ml ofAscorbic acid solution = 0.002 gm ascorbic acid If 0.002 gm ascorbic acid requires V ml dye solution to neutralize it then 1 ml dye solution = 0.002 / V gm ascorbic acid.

Procedure

1. Add 5 mL working standard to 10 mL 4% oxalic acid solution. Titrate against dye solution (V_1)mL.

2. Add 5 mL sample solution or 1000mg sample powder to 95 mL 4% oxalic acid solution. Take 5 mL aliquote from this solution and titrate against dye solution (V_2) mL. 1ml of juice was taken and made up to 15 ml by the addition of 4% Oxalic acid.

3. End point is determined by the appearance of pink color which should persist for about 15 seconds.

Calculation

Vitamin C/ Ascorbic acid content mg/100g =

$$\frac{0.5\text{ mg}}{V_1} \times \frac{V_2}{5\text{ mL}} \times \frac{100}{\text{Sample Wt}} \times 100$$

Note:- Acetone may be omitted if sulphur dioxide is known to be absent. Its function is to form the acetone bisulphate complex with sulphur dioxide which otherwise interferes with the titration. Sometime a small proportion of the ascorbic acid in foods becomes reversibly oxidized during aging and forms dehydroascorbic acid. If this is suspected, first estimate the ascorbic acid as above, then through another portion of the solution pass a stream of Hydrogen sulphide for 10 minutes. Stopper the flask and allow it to stand overnight in a refrigerator. Then remove hydrogen sulphide by bubbling nitrogen through the mixture and titrated as before. The difference between the two titrations gives a measure of the dehydroascorbic acid. One international unit of vitamin C = 50 µg ascorbic acid.

(Ref :- F.A.O Manuals of Food Quality Control 14 / 8, page 194 / Pearson's Composition and Analysis of Foods 9th edn,1991, page 264 and AOAC Official Method 967.21 Ascorbic acid in Vitamin preparation and juices)

TEST NO.8 SOLUBLE SOLIDS IN FRUITS

Principle:- Measurement of the refractive index of the test solution at 20 0C, using a refractometer, and use of tables correlating refractive index with soluble solids content (expressed as Sucrose), or direct reading of the soluble solids content on the refractometer. **Apparatus** a) Refractometer - indicating the refractive index by means of a scale graduated in 0.001, in order to allow readings to be estimated to 0.0002. Refractometer - indicates the percentage by mass of Sucrose by means of a scale graduated in 0.5 %, in order to allow readings to be estimated to 0.25 %. This refractometer shall be adjusted so that at 20 0 C it registers for distilled water a soluble solid (Sucrose) content of zero.

b) Water circulating apparatus - to maintain the temperature of the prisms of the refractometer constant to within 0.5 0 C in the neighbourhood of 20 0C which is the reference temperature. If the temperature of circulating water is different from 20 0C use temperature correction as per table on page no. c) Beaker- capacity 250 ml

Procedure:

Preparation of test solution (a) Clear liquid products Thoroughly mix the sample and use it directly for determination. (b) Semi thick products (purees etc) thoroughly mix the sample. Press a part of the sample through a gauge/muslin cloth folded in four, rejecting the first drops of the liquid and reserving the remainder of the liquid for the determination (c) Thick products (jams, Jellies etc) Weigh into the tared beaker to the nearest 0.01 gm, a suitable quantity (upto 40 gm) of the sample and add 100 – 150 ml of distilled water. Heat the contents of the beaker to boiling and allow to boil gently for 2- 3 minutes, stirring with a glass rod. Cool the contents and mix thoroughly. After 20 minutes weigh to the nearest 0.01gm, then filter through a fluted filter paper or a Buchner funnel into a dry vessel. Reserve the filtrate for determination

Determination Adjust the water circulation in order to operate at the required temperature and allow it to flow to bring the prisms of the refractometer to the same temperature, which shall remain constant to within 0.5°C during the determination. Put a small quantity of the test solution (2- 3 drops are sufficient) on the fixed prism of the refractometer and immediately adjust the movable prism. Suitably illuminate the field of view. Bring the line dividing the light and dark parts of the surface in the field of view to the crossing of the threads and read the value of refractive index.

Determine percent sugar from the table If the determination has been carried out at a temperature other than 20°C ± 0.50C the following corrections are required (a) For the scale indicating refractive index apply the formula

$$n_D^{20} = n_D^t + 0{,}0013(t - 20)$$

where

n_D^{20} is the refractive index at 20 °C;

n_D^t is the refractive index at the temperature of measurement;

t is the temperature of measurement, in degrees Celsius.

(b) For the scale indicating percentage by mass or Sucrose correct the result according to the table 1

Calculation

(a) Refractometer with refractive index scale

Read from table 2 the percentage mass of sucrose corresponding to the value of refractive index corrected for temperature if necessary. In the case of liquid or semi thick products, the soluble solid content is equal to the number found. If the determination has been carried out on a diluted sample the soluble solid content is equal to

$$\frac{P \times m1}{m0}$$

Where, P is the percentage by mass of soluble solids in the diluted solution m0 is the mass, in gm of the sample before dilution m 1 is the mass in gm of the sample after dilution Take the result as the arithmetic mean of two determinations. Express the result to one decimal place

(b) Refractometer with sugar scale In the case of liquid or semi thick products the soluble solid content, as a percentage by mass of the sucrose is equal to the value read, corrected for temperature if necessary. If the determination has been made on a diluted solution calculate the soluble solids as shown above Take the result as the arithmetic mean of two determinations.

IS 13815 : 1993
ISO 2173 : 1978

TABLE 1 — Correction of readings of the refractometer with scale indicating sucrose for a temperature different from 20 ± 0,5 °C

Temperature °C	Scale reading for soluble solids content, % (m/m)									
	5	10	15	20	25	30	40	50	60	70
	Corrections to be subtracted									
15	0,29	0,31	0,33	0,34	0,34	0,35	0,37	0,38	0,39	0,40
16	0,24	0,25	0,26	0,27	0,28	0,28	0,30	0,30	0,31	0,32
17	0,18	0,19	0,20	0,21	0,21	0,21	0,22	0,23	0,23	0,24
18	0,13	0,13	0,14	0,14	0,14	0,14	0,15	0,15	0,16	0,16
19	0,06	0,06	0,07	0,07	0,07	0,07	0,08	0,08	0,08	0,08
	Corrections to be added									
21	0,07	0,07	0,07	0,07	0,08	0,08	0,08	0,08	0,08	0,08
22	0,13	0,14	0,14	0,15	0,15	0,15	0,15	0,16	0,16	0,16
23	0,20	0,21	0,22	0,22	0,23	0,23	0,23	0,24	0,24	0,24
24	0,27	0,28	0,29	0,30	0,30	0,31	0,31	0,31	0,32	0,32
25	0,35	0,36	0,37	0,38	0,38	0,39	0,40	0,40	0,40	0,40

TABLE 2 — Refractive index and corresponding percentage by mass of soluble solids (sucrose)

Refractive index	Soluble solids (sucrose) content	Refractive index	Soluble solids (sucrose) content	Refractive index	Soluble solids (sucrose) content	Refractive index	Soluble solids (sucrose) content
n_D^{20}	% (m/m)	n_D^{20}	% (m/m)	n_D^{20}	% (m/m)	n_D^{20}	% (m/m)
1,333 0	0	1,367 2	22	1,407 6	44	1,455 8	66
1,334 4	1	1,368 9	23	1,409 6	45	1,458 2	67
1,335 9	2	1,370 6	24			1,460 6	68
1,337 3	3	1,372 3	25	1,411 7	46	1,463 0	69
1,338 8	4			1,413 7	47	1,465 4	70
1,340 3	5	1,374 0	26	1,415 8	48		
		1,375 8	27	1,417 9	49	1,467 9	71
1,341 8	6	1,377 5	28	1,420 1	50	1,470 3	72
1,343 3	7	1,379 3	29			1,472 8	73
1,344 8	8	1,381 1	30	1,422 2	51	1,475 3	74
1,346 3	9			1,424 3	52	1,477 8	75
1,347 8	10	1,382 9	31	1,426 5	53		
		1,384 7	32	1,428 6	54	1,480 3	76
1,349 4	11	1,386 5	33	1,430 8	55	1,482 9	77
1,350 9	12	1,388 3	34			1,485 4	78
1,352 5	13	1,390 2	35	1,433 0	56	1,488 0	79
1,354 1	14			1,435 2	57	1,490 6	80
1,355 7	15	1,392 0	36	1,437 4	58		
		1,393 9	37	1,439 7	59	1,493 3	81
1,357 3	16	1,395 8	38	1,441 9	60	1,495 9	82
1,358 9	17	1,397 8	39			1,498 5	83
1,360 5	18	1,399 7	40	1,444 2	61	1,501 2	84
1,362 2	19			1,446 5	62	1,503 9	85
1,363 8	20	1,401 6	41	1,448 8	63		
		1,403 6	42	1,451 1	64		
1,365 5	21	1,405 6	43	1,453 5	65		

(Ref: - I.S 13815: 1993 / I.S.O 2173: 1978 Fruit and Vegetable Products Determination of Soluble solid Content - Refractometer method)

TEST NO. 9.0 TITRATABLE ACIDITY IN FRUITS

9.0.0	**Title**	Titratable Acidity in Fruits
9.0.1	**Objective**	To Estimate Titratable Acidity in Fruits
9.0.2	**Scope**	Estimation of Titratable Acidity in Fruits by Indicator Method
9.0.3	**Related or Relevant Procedure**	AOAC, 19th Edn, 2012; Ch-37; 37.1.37; 942.15; Estimation of titratable acidity in fruits by Indicator Method, pp:10.

9.0.4 Requirement for Procedure

9.0.4.1 Apparatus

Filter paper

Burette

Burette Stand

500ml Beakers

Erlenmeyer Flasks 250ml

9.0.4.2 Reagents

Phenolphthalein Indicator

9.0.5 Procedure

(a) Colourless or slightly coloured solutions: Dilute approximately 250ml with neutralized or recently boiled H_2O, 10g prepared juice (Mix thoroughly by shaking to ensure uniform test sample, and filter through absorbent cotton or rapid paper. Prepare fresh juices by pressing well-pulped fruit and filtering. Express juice of citrus fruit by commercial device and filter) or 25ml prepared solution

(I) For jellies, syrups: Mix thoroughly to ensure uniform test sample. Prepare solution by weighing 300g thoroughly mixed test sample into 2L flask and dissolve in H_2O, heating on steam bath if necessary. Apply as little heat as possible to minimize inversion of sucrose. Cool, dilute to volume, mix thoroughly by shaking and use aliquots for the various determinations. If insoluble material is present, mix thoroughly and filter first).

(II) For fresh fruits, dried fruits, preserves, jams and marmalades: Pulp by passing through food chopper, or by use of soil dispersion mixer, Hobart mixer, or other suitable mechanical mixing apparatus, or by grinding in large mortar, and mixing thoroughly, completing operation as quickly as possible to avoid loss of moisture. With dried fruits, pass test sample through food

chopper 3 times, mixing thoroughly after each grinding. Set burns or blades of food chopper as close as possible without crushing seeds. Grind entire contents of No.10 or smaller container. Mix contents of larger containers thoroughly by stirring and remove portion of grinding. With stone fruits, remove pits and determine their proportion in weighed test sample.

Prepare solution by weighing into 1.5-2 L beaker 300g fresh fruit, or equivalent of dried fruit, preserve, jams and marmalades, well-pulped and mixed in blender or other suitable type of mechanical grinder; add approximately 800ml H_2O; and boil 1h replacing at intervals H_2O lost by evaporation. Transfer to 2L volumetric flask, cool dilute to volume, and filter. With unsweetened fruit, ashing is facilitate by addition of sugar before boiling; therefore weigh 150g fruit, add 150g sugar and 800ml H_2O and proceed as above.

(III) Canned fruit: Carefully invert by hand all fruits having cups or cavities if they fall on sieve with cups or cavities up. Cups or cavities in soft products may be drained by tilting sieve, but no other handling of these products while draining is permissible. Separate liquor by draining and treat as in juices.

Titrate with 0.1M alkali, using 0.3ml phenolphthalein for each 100ml solution being titrated, to pink persisting 30s. Report as ml 0.1M alkali/100g or 100ml original material.

(b) Highly colored solution: Dilute test portion of known weight with neutralized H_2O and titrate to just before end point with 0.1M alkali, using 0.3ml phenolphthalein for each 100ml solution being titrated. Transfer measured volume (2 or 3 ml) of solution into approximately 20ml neutral H_2O in small beaker. (In this extra dilution, color of fruit juice becomes so pale that phenolphthalein color is easily seen.) If test shows that end point is not reached, pour extra diluted portion back into original solution, add more alkali, and continue titration to end point.

9.0.6 Determination

By comparing dilutions in small beakers, differences produced by few drops 0.1M alkali can be easily observed.

9.0.7 Expression of Result

Titratable acidity can be expressed conventionally in g acid per 100g or per 100ml product, as appropriate, by using the factor appropriate to the acid; for malic acid use 0.067 as factor; oxalic acid, 0.045; citric acid monohydrate, 0.070; tartaric acid,0.075; sulfuric acid, 0.049; acetic acid, 0.060; lactic acid, 0.090.

TEST NO. 10.0 WATER-INSOLUBLE SOLIDS IN FRUITS

10.0.0	**Title**	Water-insoluble solids in fruits
10.0.1	**Objective**	To estimate water-insoluble solids in fruits
10.0.2	**Scope**	Estimation of Water-Insoluble Solids in fruits.
10.0.3	**Related or Relevant Procedure**	AOAC, 19th Edn, 2012; Ch-37; 37.1.13; 922.10; Estimation of water-insoluble solids in fruits, pp: 6.
10.0.4	**Requirement for Procedure**	
10.0.4.1	**Apparatus**	Whatman No.4 Filter Paper Moisture Box or Aluminium Dish with lid Desiccator Hot Air Oven
10.0.5	**Procedure**	For use with büchner, prepare filtering medium consisting of either circular disk of absorbent cotton approximately 80mm diameter, weighing approximately 1.5g, or coarse, qualitative filter paper (7-15cm diameter, Whatman No.4 or 41-H, or equivalent). For use with 60°C funnel, prepare absorbent cotton approximately 12.5 cm diameter weighing approximately 2g, 0r 12.5 cm filter paper. Wash filtering medium with hot water and dry overnight at 100-110°C in open, flat bottom Al dish of suitable size provided with tight-fit cover. Cool closed dish and contents 1h in desiccator and weigh to nearest mg. Weigh 25 or 50g well-mixed test portion (as per - II For fresh fruits, dried fruits, preserves, jams and marmalades in titratable acidity in fruits), to nearest 10mg, transfer tp 400ml beaker.
10.0.6	**Determination**	
10.0.7	**Expression of Results**	Express Result as Percent water-insoluble solids.

TEST NO. 11 MICROBIOLOGICAL ANALYSIS OF FOOD AND WATER

The bacteriological quality of food and water can be tested by;

1. Total plate count at 20°C and at 37°C.
2. Presence of coliform bacteria as indicators of sewage contamination
3. Identification of specific pathogenic bacteria
4. Employing miscellaneous indicators and serological methods

Identification normally uses techniques known as 'isolation' and 'cultivation'. Isolation is the separation of a particular organism from a mixed population while cultivation is the growth of that organism in an artificial environment.

Coliform Bacteria

Coliform bacteria can serve as indicators of fecal contamination. They are not themselves pathogenic but are denizens of the digestive systems of animals, and thus abundant in feces. Coliform are defined as "Gram-negative aerobic or facultative anaerobes, nonspore-forming, rod shaped bacteria that ferment lactose with acid and gas production." ***E. coli***, the most abundant bacterium of the human colon, is the most important indicator of human fecal contamination. However, some coliform bacteria, such as *Enterobacter aerogenes*, are of non-fecal origin and may be present in uncontaminated samples. Thus testing for coliform bacteria involves several tests to minimize the possibility of false positive results. Bear in mind that the presence of indicator bacteria does not mean that human pathogens are definitely present, but their presence suggests that the fecal contamination has occurred, and that pathogens may be present.

Methods of counting bacteria

There are two standard methods of counting bacteria: the standard plate count (SPC) and the most probable number (MPN) methods. Either technique can be used with selective or non-selective technique – the method of choice depends largely upon the number of bacteria to be counted in the sample. The SPC is routinely used for samples that have a relatively large number of bacteria, which can be diluted down and grown as a countable number of colonies in a petri plate. The MPN method is used when the number of bacteria to be counted is so low that the cells could not be detected if a sample were applied to a plate, which is often the case when counting coliform bacteria.

I. Standard Plate Count of Bacteria in Food Products

The **Standard Plate Count** is the most common method used to quantify bacteria in foods. To perform a standard plate count, the food to be tested is suspended in liquid and a sample is then spread over the surface of a solid medium in a petri plate. Bacterial cells present will form colonies that can be counted to determine the number of cells in the original sample. When the objective is to estimate the total number of bacteria, a complex medium called **Plate Count Agar** is commonly used since it will support growth of many different types of bacteria. We call the results the number

of **Colony Forming Units (CFU)**, not total bacteria. This is because no single culture medium will support all different types of bacteria, we can only count those that do grow to form a visible colony.

Serial Dilution of samples

When performing a bacteria count, **between 30 and 300 bacterial colonies** need to be on the plate. A minimum of 30 assures that the data is statistically reliable; however, if there are more than 300 colonies are present, competition for nutrients can suppress growth of colonies. For example, if a sample were to contain 10^6 cells\ml, a 1 ml sample would contain 10^6 bacteria – far more than the 300 cell limit of a standard plate count – and the sample must be serially diluted:

The standard way to dilute a sample in microbiology is through Serial Dilution. The sample is diluted step-wise in a series of tubes containing sterile diluent. Each step yields a particular dilution factor. Since the original concentration of bacteria in sample is unknown, we will plate several dilutions, and hope that at least one will yield between 30- 300 colonies on the plate.

Materials required

- Sterile 1 ml pipets
- 90 ml and 9 ml of sterile distilled water
- Sterile plates containing Plate Count Agar.

Note:

- Be sure to observe aseptic technique, and use a new sterile pipet for each dilution step.
- Thoroughly mix each tube before proceeding with the next dilution step. However, do not mix so violently that the contents splash near the cap.

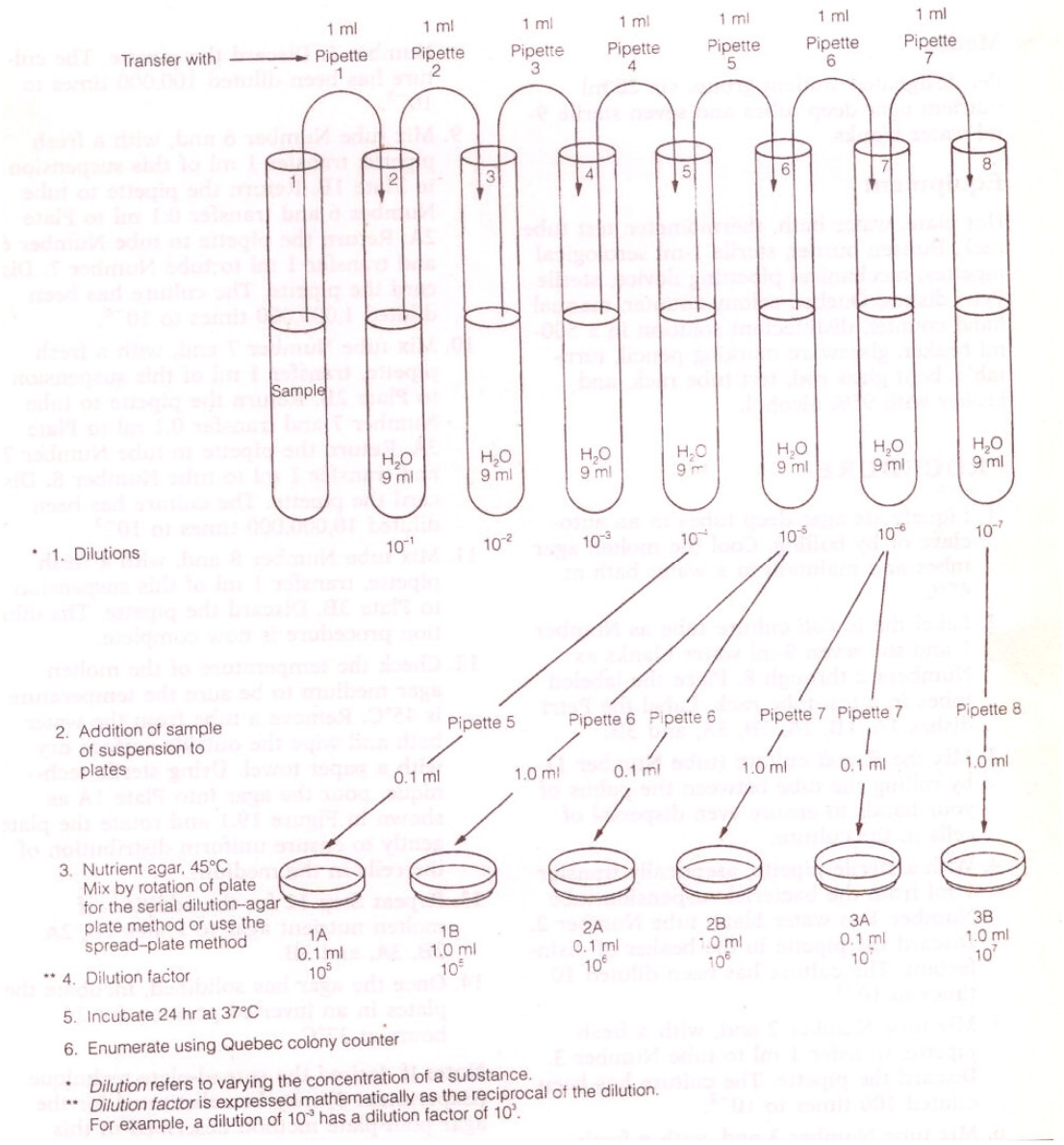
Plating the diluted samples

Two methods of these examinations are differentiated:

1) In the **pour–plate method,** a sample from an accurate dilution of microbes/sample ispipetted onto a Petri - dish, then agar medium is poured over the liquid and mixed.

2) In the **spread–plate method,** generally 0.1ml of the diluted sample is pipetted onto thesurface of a solidified agar medium and spread with a sterilized, bent, glass rod.

Preparing the serial dilutions



To report a final count, the average number of colonies per plate is multiplied by the reciprocal of the dilution factor for those plates to obtain the count per gram or per ml of the original sample. The following formulas can be used in making calculations:

Dilution factor (DF) = Initial dilution x Subsequent dilutions x Amount plated

Count/g = (1/ DF) x Colonies counted

Quantifying Bacteria.

The end point of our analysis is the number of colony forming units per mL (CFU/mL) sincewe are counting the number of colonies rather than the actual number of bacteria. CFU/mL isactually a more useful determination than counting all the bacteria under a microscope, because in many bacterial populations, a significant number will be dead cells and thus of no interest.

Diluting the bacteria.

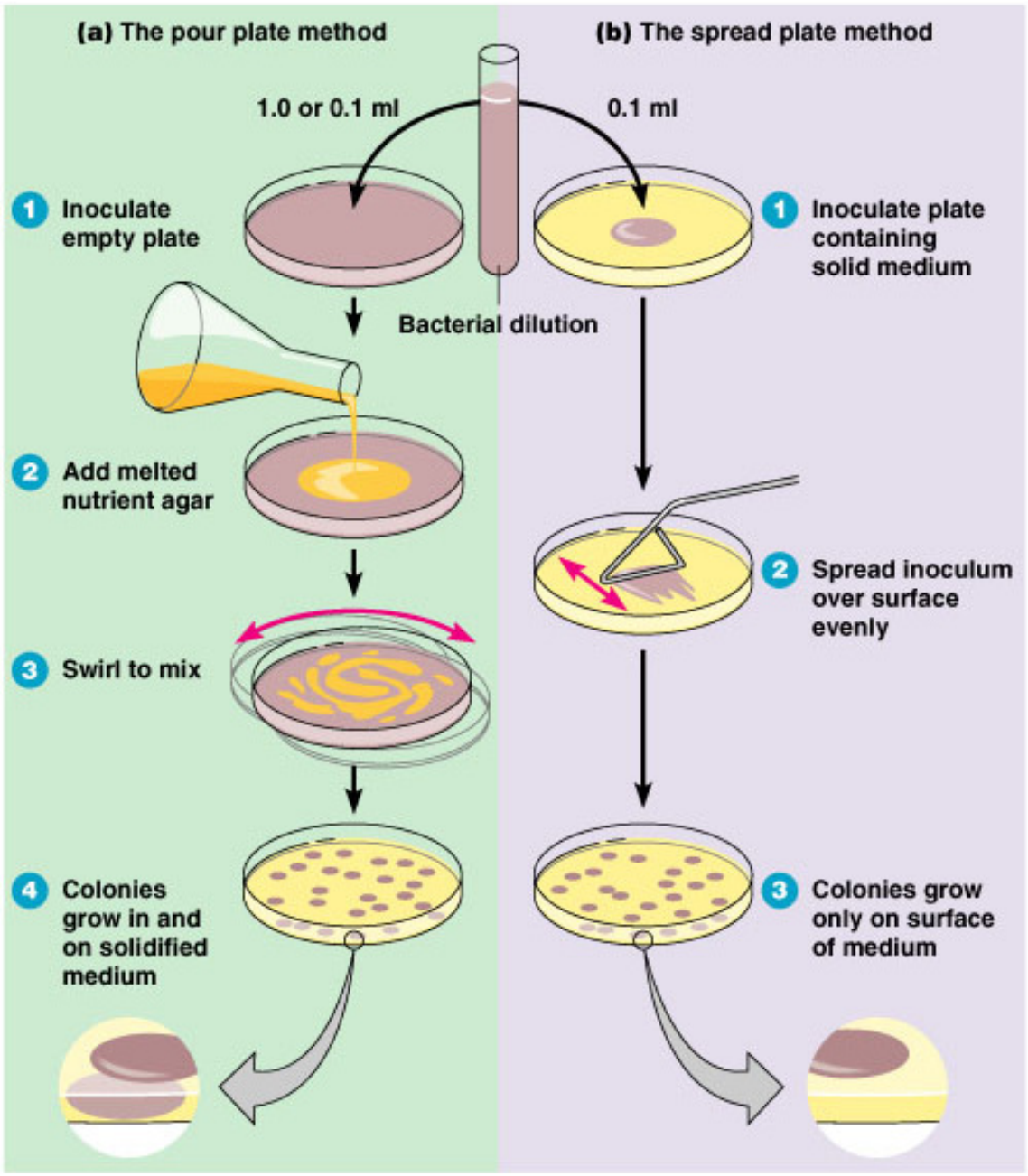
Bacteria commonly grow up to densities around 10^9 CFU/mL, although the maximum densities vary tremendously depending on the species of bacteria and the media they are growing in. Therefore, to get readily countable numbers of bacteria, we have to make a wide range of dilutions and assay all of them with the goal of having one or two dilutions with countable numbers. We do this by making serial 10-fold dilutions of the bacteria that cover the entire probable range of concentrations. Then we transfer 0.1 mL of each dilution to an agar plate, which in effect makes another 10-fold dilution, since the final unit is CFU/mL and we only streak 0.1 mL.

Inoculating the plate.

Streaking in this technique is done using a bent glass rod. 0.1 mL ofbacterial suspension is placed in the center of the plate using a sterile pipette. The glass rod issterilized by first dipping it into a 70% alcohol solution and then passing it quickly throughthe Bunsen burner flame. The burning alcohol sterilizes the rod at a cooler temperature thanholding the rod in the burner flame, thus reducing the chance of you burning your fingers.When all the alcohol has burned off and the rod has air-cooled, streak the rod back and forthacross the plate working up and down several times. Unlike streaking for isolation, you wantto backtrack many times in order to distribute the bacteria as evenly as possible. Turn theplate 90 degrees and repeat the side to side, up and down streaking. Turn the plate 45 degreesand streak a third time. Do not sterilize the glass rod between plate turnings. Cover the plateand wait several minutes before turning it upside down for incubation. This will allow thebroth to soak into the plate so the bacteria won't drip onto the plate lid.

Counting bacteria.

Colonies are most readily counted using a plate counter. The platecounter has a light source and a magnifying glass making colonies easier to see. If at allpossible, you don't want to count plates with more than 300 or less than 30 colonies. In theformer case, the colonies, run together, and, in the latter, there are too few to allowstatistically accurate counts. Once you count the colonies, multiply by the appropriate dilutionfactor to determine the number of CFU/mL in the original sample.The concentration of bacteria in the original samples is calculated by multiplying the colony counts by the total dilution factor. Only plates that contain between 30 and 300 colonies should be used to calculate the original concentration. If multiple plates fall within this range, then average the concentrations calculated for each plate.



II. Using the Most Probable Number Technique to Count Coliform in Water

In water, only a few coliform per liter can represent a potential health hazard. In this situation, the concentration of cells is too low to count with a standard plate count (no cells may be present within any particular 1 ml sample). The **most probable number (MPN)** technique is one solution to this problem.

Theory of Most Probable Number counts

The MPN technique is a statistical method of estimating the concentration of bacteria. Imagine a hypothetical situation where a sample of water contains 10 bacteria evenly dispersed in 100 ml of water. If different sample volumes were analyzed, each 10 ml sample withdrawn would contain 1 cell, but only 1 out of 10 1 ml samples would contain a cell. If the samples were added to culture media, the samples containing a cell would show growth. *Conversely, knowing the volume of the samples and the number of tubes showing growth, we could know the concentration of bacteria in an unknown original sample.*

For example, suppose a water sample were analyzed with the following results:

Volume cultured	Number of samples cultured	Number of cultures showing bacterial growth
10	10	10
1.0	20	2
0.1	20	0

Since the smallest sample size that yielded growth was 1.0 ml, and growth occurred in 2 out of the 20 samples tested, the number of bacteria in the original could be calculated as 2 cells per 20 ml (i.e., 5 cells per /100ml).

In the real world bacteria are randomly but not evenly distributed in a water sample. Thus, in our example above, if twenty 1 ml samples were cultured, growth might occur in fewer or more than 20 tubes. In addition, some tubes in which growth occurs might actually start with 2 or more cells. If growth occurs, all we know is that at least one viable bacterial cell was present. What a bummer!

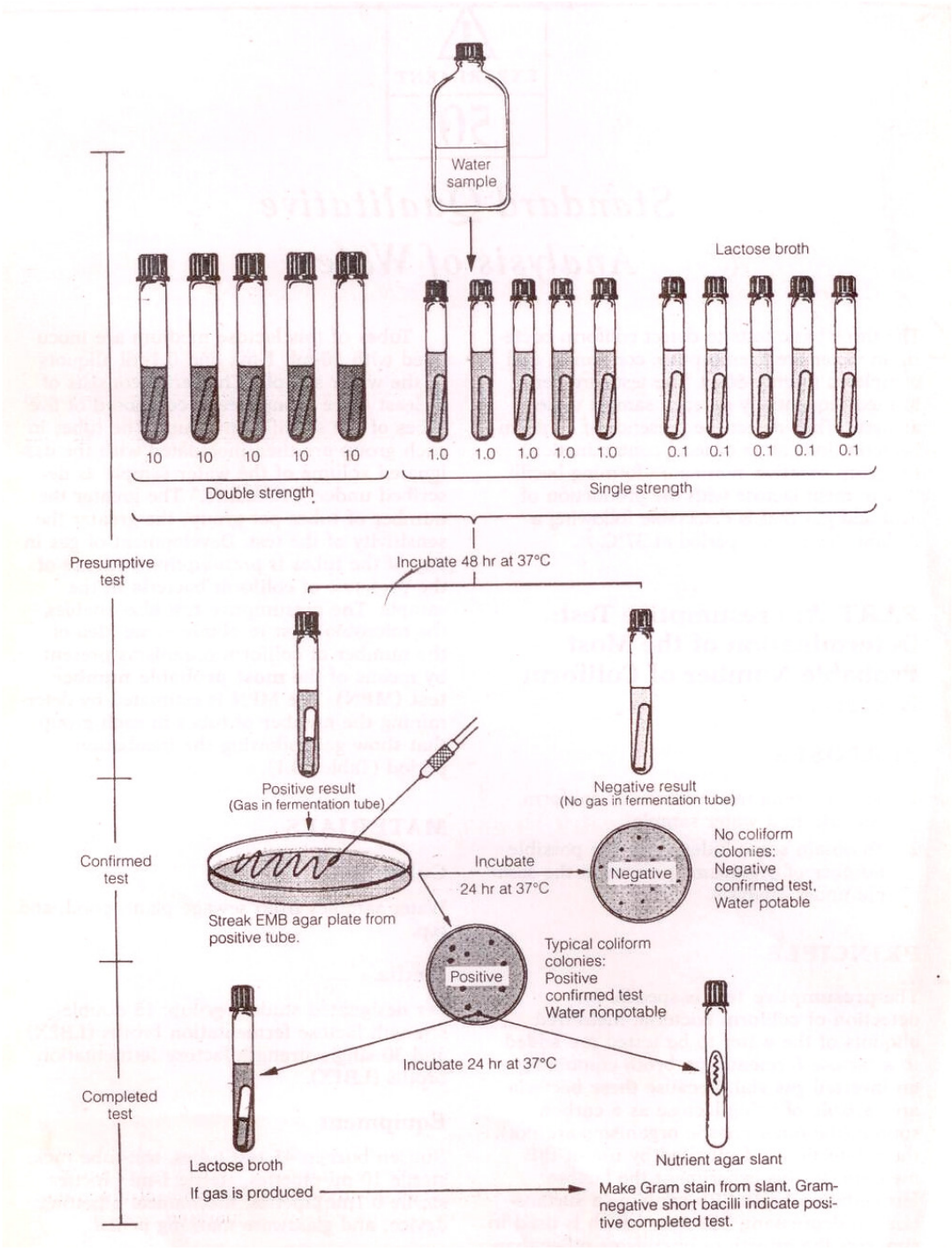
However, because the cells are *randomly* distributed, statistical techniques can be used to estimate the number of bacteria in the original sample, i.e., the "most probable number" A statistical estimate of the number of bacteria can be obtained from an appropriate statistical table, such as the one shown below.

MPN Index when five 10 ml samples, one 1 ml sample, and one 0.1 ml sample are tested.

Number of tubes giving Positive Reaction (acid + gas)*			95% confidence limits		
10 ml	1 ml	0.1 ml	MPN cellsper 100 ml	Lower	Upper
0	0	0	<2	0	5.9
0	1	0	2	0.050	13
1	0	0	2.2	0.050	13
1	1	0	4.4	0.52	14
2	0	0	5.0	0.54	19
2	1	0	7.6	1.5	19
3	0	0	8.8	1.6	29
3	1	0	12	3.1	30
4	0	0	15	3.3	46
4	0	1	20	5.9	48
4	1	0	21	6.0	53
5	0	0	38	6.4	330
5	0	1	96	12	370
5	1	0	240	12	3700
5	1	1		88	

*This table only includes those combinations of positive tubes that occur with a significant frequency. If the other nine possible combinations occur with a greater frequency than 1%, than faulty technique should be suspected.

MPN Technique



Counting coliform bacteria involves three steps

The MPN technique is used to estimate coliform bacteria (not total bacteria), and therefore selective and differential media must be used. There are 3 stages to this method:

1.The Presumptive test.

The samples are first inoculated into **lactose broth** containing a Durham tube. In a positive culture, growth will occur with the production of BOTH acid and gas. Because few other types of bacteria can ferment lactose and yield acid and gas, a positive presumptive test is a very good indication of the presence of coliform.

2.The Confirmed test.

Some non-coliform bacteria can yield a false positive in the presumptive test. Thus, all positive lactose broth cultures are subjected to the confirmed test: a loopful of each culture is streaked onto **Eosine Methylene Blue agar (EMB)** and inoculated into **Brilliant Green Lactose Bile Broth (BGLBB)**. EMB is a differential medium; eosine and methylene blue combine to yield a distinctive coloration pattern for coliform bacteria. The presence of bile and brilliant green in BGLBB make this medium selective for Gram-negative bacteria. A confirmed test is positive when colonies with a green metallic sheen form on EMB and gas occurs in BGLBB.

3.The Completed test.

Cells from an isolated colony on EMB are transferred to an agar slant andagain transferred to lactose broth. If acid and gas are again observed, and Gram-negative rods are found in the slant culture, then the identification of coliform is considered positive.

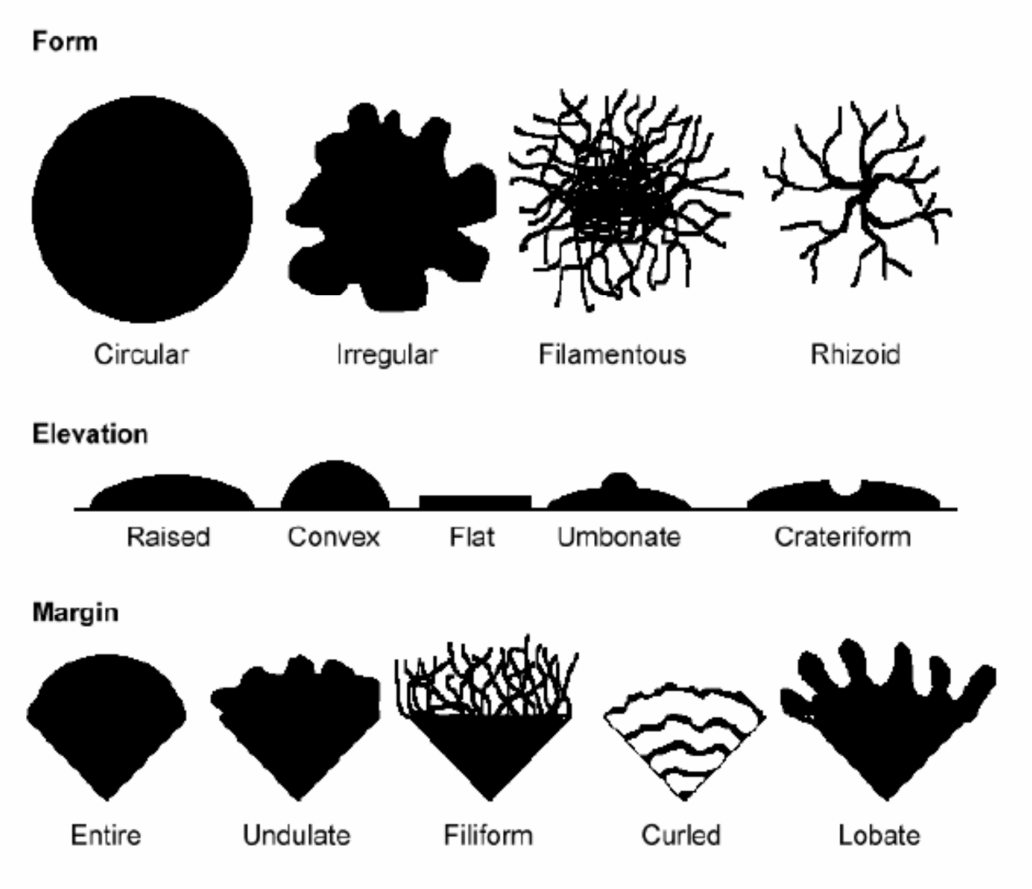
IDENTIFICATION OF UNKNOWN BACTERIA

To accurately describe a colony the following traits should be taken into account:

- Size: A mm ruler should be held under a plate and used to measure the diameter of several representative colonies, these measurements should then be averaged and recorded.
- General Shape: The shape of a colony will have one of three general shapes when viewed from above; round, irregular and spreading, and concentric.
- Margin: The margin should be observed from above also and will represent the outside edge of the general shape. The margin will be one of the following; entire, undulate lobate or filamentous
- Elevation: The elevation of a colony should be observed from the side of the plate and will be characterized in the following ways; effuse (like water on a flat surface), flat (discrete colony with a flat top), convex (water on wax paper), umbonate (with a nipple in the center), or umbilicate (sunken center)
- Surface: The surface of the colony should be observed from the top and will have one of the following characteristics; smooth, filamentous, powdery, wrinkled or ringed.
- Density: This quality describes if the colony is opaque or translucent
- Pigment: There are three ways a colony may be pigmented; (1) Non pigmented colonies may be described as colorless, white, or off-white, (2) Nondiffusible pigment means that

just the cell is pigmented, while (3) Diffusible/water soluble pigments will color the agar around the colony

Colony Morphology



Microorganism Measuring Procedure

Procedure:

1. Place a slide micrometer on the stage of the microscope with the ruled area directly over the center of the condenser lens.
2. Look through the eyepiece with the low power objective in place and identify the ocular micrometer, check by turning the eyepiece. Note the number of gradations between numbers.
3. Focus the stage micrometer scale and note the gradations that are 0.01 mm apart and those which are 0.1 mm apart (if present). Adjust the two scales so that they are parallel and partially superimposed.
4. Align an ocular micrometer line with a stage micrometer line at the left side of the field. Look for another ocular micrometer line toward the right side of the field which is aligned similarly with a stage micrometer line.

5. Count the number of graduations between the two aligned marks. Count the number of ocular micrometer divisions which span the observed number of mm.

6. Divide the distance by the number of divisions to determine the distance between each two ocular micrometer divisions. Record the results on the chart provided.

7. Repeat the procedure using the high dry objective and the oil immersion objective. Note that with the higher magnifications the stage micrometer lines look thick. It is necessary at these magnifications to align an ocular micrometer line along one edge of a stage micrometer line and choose a second ocular micrometer line also lying along the corresponding edge of a stage micrometer line.

8. Record on the board the calibration of your assign microscope using the oil immersion objective.

9. Measure the diameter of the microscope field on low power, high dry, and oil immersion using the stage micrometer.

10. Return the stage micrometer to its case after carefully wiping off the oil.

11. Measure a rod-shaped bacterium, a coccus, a yeast cell, a protozoan, and a human red blood cell. Record the results.

STAINING

Simple staining

1. Clean and dry microscope slides thoroughly.

2. Flame the surface in which the smear is to be spread.

3. Flame the inoculating loop.

4. Transfer a loop full of tap water to the flamed slide surface.

5. Reflame the loop making sure the entire length of the wire that will enter the tube has been heated to redness

6. Remove the tube cap with the fingers of the hand holding the loop.

7. Flame the tube mouth.

8. Touch the inoculating loop to the inside of the tube to make sure it is not so hot that it will distort the bacterial cells; then pick up a pinhead size sample of the bacterial growth without digging into the agar.

9. Reflame the tube mouth, replace the can, and put the tube back in the holder.

10. Disperse the bacteria on the loop in the drop of water on the slide and spread the drop over an area the size of a dime. It should be a thin, even smear.

11. Reflame the inoculating loop to redness including the entire length that entered the tube.

12. Allow the smear to dry thoroughly.

13. Heat-fix the smear cautiously by passing the underside of the slide through the burner flame two or threetimes. Test the temperature of the slide after each pass against the back of the

hand. It has been heatedsufficiently when it feels hot but can still be held against the skin for several seconds. Overheating willdistort the cells.

14. Stain the smear by flooding it with one of the staining solutions and allowing it to remain covered with thestain for the time designated below.

    Methylene blue - 1 minute

    Crystal violet - 30 seconds

    Carbolfuchsin - 20 seconds

    During the staining, the slide may be placed on the rack or held in the fingers.

15. At the end of the designated time rinse off the excess stain with gently running tap water. Rinse thoroughly.

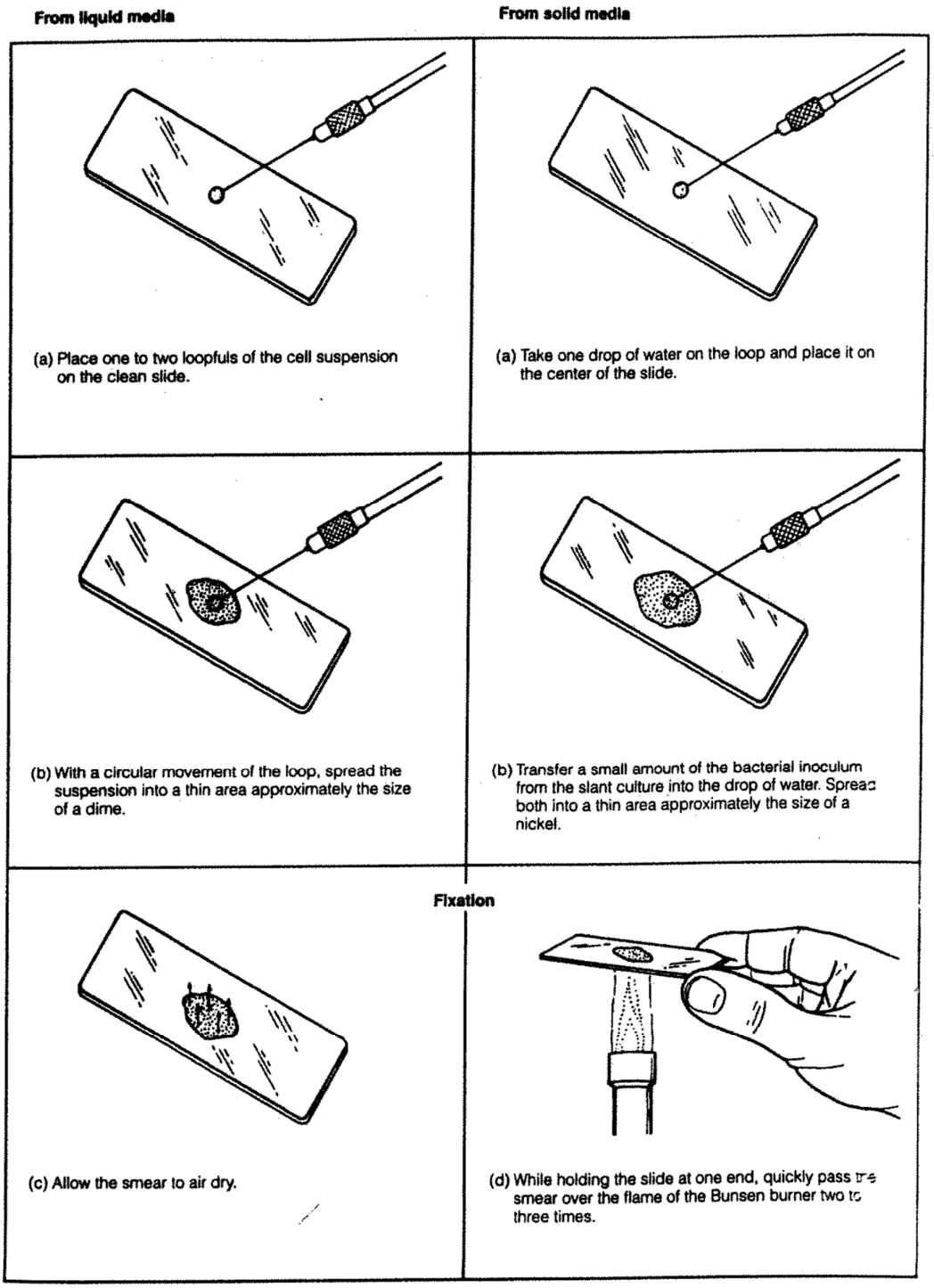
16. Wipe the back of the slide and blot the stained surface with bibulous paper or with a paper towel.

17. Place the stained smear on the microscope stage smear side up and focus the smear using the 10X objective.

18. Choose an area of the smear in which the cells are well spread in a monolayer. Center the area to be studied, apply oil directly to the smear, and focus the smear under oil with the 100X objective.

19. Draw the cells observed.

Bacterial Smear Preparation



Gram Stain Procedure

Procedure:

A. Slant Cultures

1. Prepare and heat-fix smears.

2. Prepare the smears on a second slide. Heat-fix.

3. Stain the slides as follows:

   a. Flood the crystal violet for one minute.
   b. Pour off excess dye, wash gently in tap water, and drain the slide against a paper towel.
   c. Expose the smears to Gram's iodine for one minute by washing with iodine, then adding more iodine and leaving it on the smear until the minute is over.
   d. Wash with tap water and drain carefully. (Do not blot.)
   e. Wash with 95% alcohol for 30 seconds.
   f. Wash with tap water at the end of the 30 seconds to stop the decolorization. Drain.
   g. Counterstain with 0.25% safranin for 30 seconds.
   h. Wash, drain, blot, and examine under oil.
   i. Draw the cells showing morphology, grouping, and relative sizes. Color a few of the cells of each bacterial species to show the Gram reaction.
   j. Save these slides and the ones from parts B & C of this exercise to use at the next lab period.

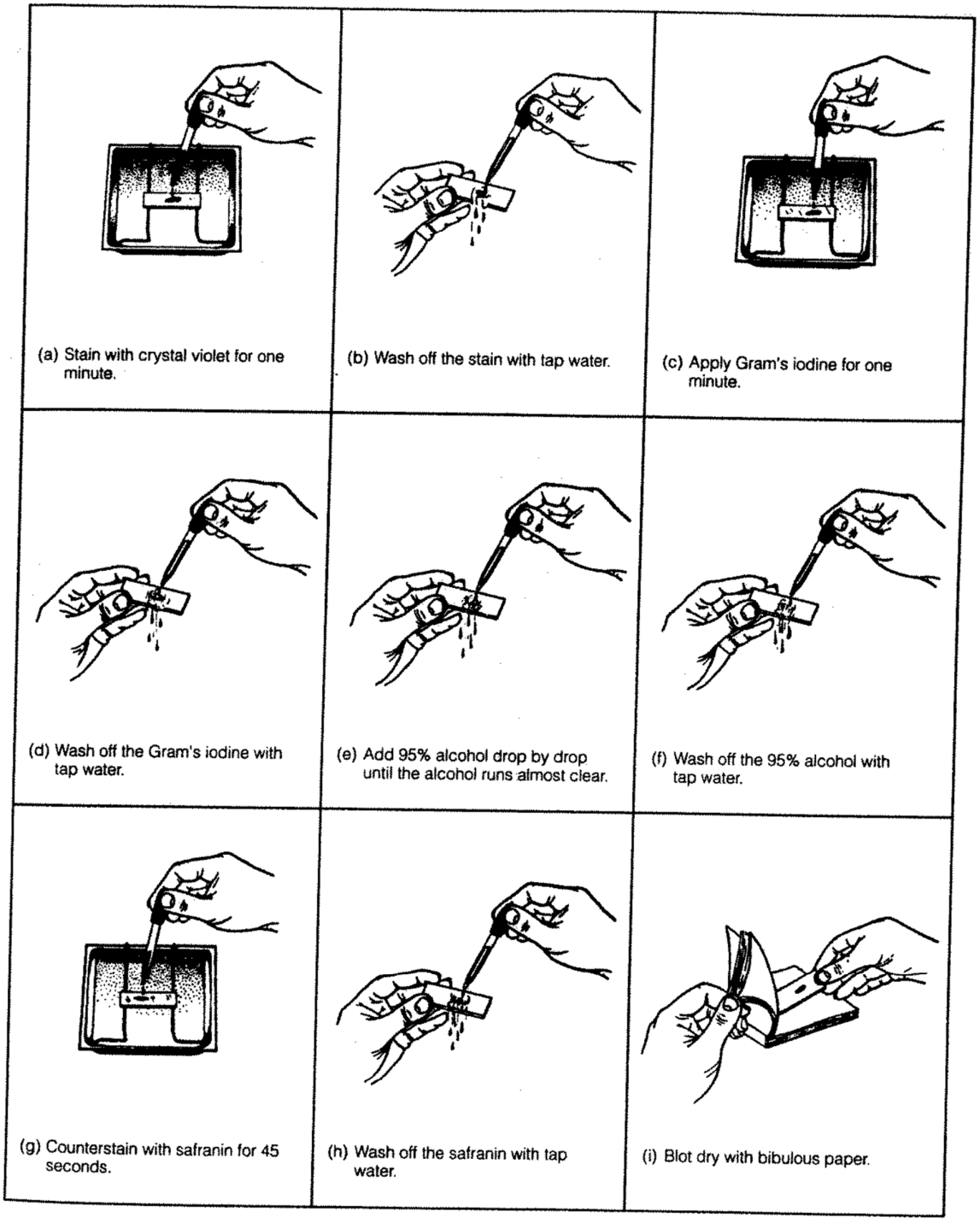
B. Broth Cultures

1. Because the smear made from the broth will be a thin smear and nearly invisible to the naked eye even after staining, it may be advisable to draw a ring with a felt open on the under side of the slide to mark the area in which the broth smear will be made. Also, when making a smear from broth do not add a drop of water to the slide.

2. Hear-fix the smears, Gram stain them with the above procedure, and examine them. When focusing the broth smear use the technique suggested for thin smears.

3. Compare the appearance of the cells in the two smears.

Gram Staining



GOOD LABORATORY PRACTICE

The use of good laboratory practice is an important factor in safeguarding the health and safety of laboratory personnel. It should be remembered that many of the bacteria which are cultured in aquatic microbiological laboratories are capable of producing disease in humans. This, coupled with the fact that, potentially more virulent, pure strains of such bacteria are often being produced, means that there is considerable risk to the health of microbiology laboratory workers if adequate precautions are not taken. The basis of good practice in a microbiological laboratory can be summed up by thefollowing:

- ensure all necessary equipment and media is sterilised prior to use
- ensure that all sterilised equipment and media is not re-contaminated after sterilization by allowing it to touch, or rest on, any unsterilised surface
- frequently disinfect hands and working surfaces
- as far as possible, eliminate flies and other insects which can contaminate surfaces, equipment, media and also pass organisms to laboratory personnel
- never pipette by mouth samples which are suspected to have high bacterial concentrations
- wear appropriate protective clothing
- do not eat, drink or smoke in the laboratory
- sterilize contaminated waste materials prior to disposal
- take care to avoid operations which result in bacterial aerosols being formed

*****

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www.marietta.edu/~spilatrs/biol202/labexercises/9-**Food_and_water**.pdf

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