ETHIOPIAN BIODIVERSITY INSTITUTE

Microbial Biodiversity Directorate

A Laboratory Manual for the Preparation of Chemical Reagents, Solutions and Special Indicators

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Laboratory Safety Precautions

Laboratory Clothing:

- 👉 Wear shoes that fully cover the feet. Sandals and clogs are not adequate.
- 👉 Shoes provide a great deal of initial protection in the case of dropped containers, spilled chemicals, and unseen hazards on the floor. Use old clothes, which are not too loose, especially at the sleeves.
- 👉 Laboratory coats or aprons must be worn over clothes. Snaps or fasteners are preferable to buttons for quicker removal in case of an emergency.
- 👉 Tie back long hair so that it will not fall into flames or chemicals.
- 👉 Avoid shorts and miniskirts in the lab.
- 👉 Exposed body skin gives added risk to irritation and burns by corrosive chemicals and gases.

Aprons:

- 👉 Plastic or Rubber - protects against corrosive and irritating chemicals.

Lab coats:

- 👉 Cotton - good against flying objects, sharp or rough edges (Usually treated with a fire retardant) Wool - protects against molten splashes, small acid spills and small flames.
- 👉 Synthetic fibers - protects against IR and UV radiation but burns easily and can be ruined by strong solvents.

Safety Glasses:

- 👉 Wear safety glasses at all times in the laboratory. Goggles are required to be worn at each lab period and should also be worn over prescription glasses.
- 👉 Contact lenses should not be used during the lab. Goggles designed for contact wearers should be made available.

Working Alone in the laboratory:

- 👉 All work must be performed under the supervision of a laboratory instructor/demonstrator.

- ☞ The instructor should be aware of the exact nature of all work being done in the laboratory.

Unauthorized Experiments:

- ☞ Do only the experiment which has been assigned by the laboratory instructor. Never do any unauthorized experiment in place of the one assigned by the instructor.
- ☞ Do not change the designated procedure without the advice of the instructor.

Read up experiment procedure:

- ☞ Know exactly what you are to do. Occasionally incomplete directions or a misunderstanding of instruction causes accidents.
- ☞ Whenever you are in doubt, ask your instructor.
- ☞ Think about what you are doing and why you are doing it at all times.
- ☞ Do not start any experiment involving the use of an experimental set-up (apparatus) until it has been checked and approved by your laboratory instructor unless otherwise instructed.

Food:

- ☞ Do not eat, drink or smoke in the laboratory. For safety purposes, assume all chemicals to be poisonous either by themselves or because of impurities.
- ☞ Also avoid direct contact with organic chemicals. Many are absorbed directly through the skin.

Cleanliness:

- ☞ Keep the lab bench clean at all times. If a solution, a solid or liquid chemical is spilled on the bench or on the laboratory floor, clean up the spill immediately.
- ☞ Any chemical spilled on your skin or your clothing, should be washed immediately and thoroughly.
- ☞ Notify the laboratory instructor of the spill.
- ☞ When leaving the laboratory, wipe the bench top thoroughly.
- ☞ Make sure that your work area is clean and free of spilled chemicals or scraps of paper.
- ☞ Wash your hands with detergent or soap and water.

Waste Disposal:

- 🕒 Dispose of waste and excess materials in the proper manner.
- 🕒 Used matches, paper, broken glass, or porcelain ware should be placed in the appropriate containers but not in the sinks or cup sinks.
- 🕒 If you have any questions concerning the waste disposal, ask the appropriate person for the proper procedure.

Fume Hood:

- 🕒 Use fume hood when necessary.
- 🕒 Use the fume hood when you are so directed by the laboratory instructor, or when it is indicated to do soon the experimental procedure. Fume hoods remove toxic vapors and irritating odors from the laboratory.
- 🕒 The removal of these materials is essential for protecting the health and safety of those people working in the laboratory.

Burners:

- 🕒 Light burners only when needed. Properly extinguish any flame not being used.
- 🕒 Any open flame may ignite reagents being used by you or others near you.
- 🕒 Many organic liquids are highly flammable and these liquids should be heated only on hot plates or heating mantles.

Reactions:

- 🕒 Never look directly into the mouth of an open flask or test tube if it contains a reaction mixture.

Hot Objects:

- 🕒 Avoid touching hot objects. When heating a chemical in a container, the clamp holding the container and the burner will also become hot.
- 🕒 Place the object on a piece of Asbestos board or on wire gauze, which is not directly touching the bench top.
- 🕒 Glass objects take a long time to cool, so allow plenty of time to cool before touching them.

Glass Rods:

- 👉 Use extreme caution when inserting glass into stoppers.
- 👉 Be very careful when inserting glass tubing, glass rods, thermometers, funnels, or thistle tubes into rubber stoppers or corks. Protect your hands by holding the glass and stopper with a cloth towel or multiple layers of paper towels.
- 👉 Always lubricate the glass surface with water.

Glassware:

- 👉 Use only equipment which is in good condition.
- 👉 Defective equipment is an important source of accidents. Some defects to watch for include:
  - ✓ Chipped tips on burets, pipets, and funnels.
  - ✓ Chipped or broken rims on beakers, flasks, funnels, graduated cylinders and test tubes.
  - ✓ Cracks in beakers, flasks, graduated cylinders, test tubes and crucibles.
  - ✓ Star-shaped breaks in the bottom of test tubes or near the bottom edges of beakers and flasks.
  - ✓ Severe scratches in the bottom of beakers, flasks, and test tubes.
  - ✓ Sharp edges on glass tubing and glass rods.

These defects may be repaired by a glass blower or have they replaced. Also look for

- Inflexibility in rubber stoppers - (replace)
- Separations in the mercury column of thermometers - (replace)
- Non-working parts of screw clamps, buret clamps or rings. - (clean off corrosion, lubricate or replace)
- Replace all old and worn electrical cords.

Chemical Reagent Preparation

Ammonium molybdate reagent

Method A:

1. Dissolve 45 gms of the commercial salt or 40 gms of pure molybdenum trioxide in a mixture of 70 ml of concentrated ammonia solution and 140 ml of water.
2. When solution is complete, add it very slowly and with vigorous stirring to a mixture of 250ml of concentrated nitric acid and 500ml of water, and dilute to 1 liter.
3. Allow to stand 1 to 2 days and decant and use the clear solution.

Method B:

1. Dissolve 45 gms of pure commercial ammonium molybdate in mixture of 40ml concentrated ammonia solution and 60 ml of water.
2. Add 120gms of ammonium nitrate and dilute to a liter with water.

Note:

- ☞ The alkaline solution of ammonium molybdate keeps better than the nitric acid solution; there is little tendency for the separation of solid.
- ☞ Before using the alkaline solution, it is important that the test solution contains a slight excess of nitric acid.

Barfords reagent

Dissolve 13.3gm of crystallised neutral copper acetate in 200ml of 1% acetic acid solution.

Note: This reagent does not stay well.

Biuret reagent(qualitative)

1. Take enough urea to cover the bottom of a test tube.
2. Heat very gently, until the liquid which forms resolidifies. This white solid is biuret.
3. Dissolve the biuret in about 2mL water, and use this solution for the biuret test.

Or

Soln. A: 0.1m sodium hydroxide.

Soln. B: 0.01M copper (II) sulphate solution.

Note: Add soln. A first to sample, then add B. Pink or purple color confirms protein.

Biuret reagent (quantitative)

1. Dissolve in order, 3g copper (II) sulphate, 5g potassium iodide, 9g potassium sodium tartrate, and 8g sodium hydroxide in about 600 ml water.
2. Make up the dissolved solids to 1 liter solution.

Bradys reagent (2, 4 DNPH)

1. Dissolve 40g of 2, 4 di-nitro-phenyl-hydrazine in 80ml conc. Sulphuric acid.
2. Cool and add 900ml methanol and 100ml water.

Carr-Price reagent

1. Weigh an unopened bottle of antimony trichloride.
2. Open the bottle and empty the contents into a wide mouth glass stoppered amber bottle containing about 100 ml of chloroform.
3. By difference, obtain the weight of antimony trichloride and then add sufficient chloroform to supply 100 ml for each 25 g.
4. Dissolve by warming or shaking for several hours and filter through sodium sulphate into a dry clean amber bottle with ground stopper.
5. Store at room temperature and stay in dark when not in use.
6. Rinse all glassware coming into contact with this reagent with chloroform or a mixture of ethanol and ether, since the antimony oxychloride which forms is insoluble in water.

Cadion 2B reagent

Components	Amounts
4-nitronaphthalene-diazamino-azobenzene	0.25g
Distilled water	100mL

Deniges reagent

1. Dissolve 5 g of yellow mercuric oxide (HgO) in a mixture of 40 ml of water, and while stirring slowly
2. Add 20 ml of sulphuric acid.
3. Then add another 40 ml of water, and stir until completely dissolved (<0.5N).

Dipridyl reagent

Dissolve 0.1g in 0.5ml ethanol, or in 0.1M HCl

Dithiol reagent

4-methyl-1:2 dimercapo-benzene. Dissolve 0.2g in 100 ml of 1% NaOH

Dragendorff reagent

Solution 1: dissolve 0.85g of basic bismuth nitrate in 10 ml acetic acid and 40 ml water.

Solution 2: dissolve 8 g of potassium iodide in 20 ml water.

Mix 5 ml of Solution 1; 5 ml of Solution 2; 20 ml of acetic acid; and 100 ml of water before use.

Ethylenediamine reagent

Add copper sulphate to a solution of ethylenediamine until the color becomes darkblue violet.

Ferric thiocyanate reagent

Dissolve 1.5 g of ferric chloride and 2.0 g of potassium thiocyanate in 100 ml water.

Components	Amounts
Ferric chloride	1.5g
Potassium thiocyanate	2.0g
Distilled water	100mL

Ferron reagent (7-iodo-8-hydroxyquinoline-5-sulphonic acid)

Components	Amounts
7-iodo-8-hydroxyquinoline-5-sulphonic acid	0.2g
Distilled water	100mL

Furil-dioxime reagent

Dissolve 10 g in 100 ml ethanol.

Iodine reagent.

Dissolve 20g KI and 10g iodine crystals in 100ml water.

Jones reagent.

Mix 25g of chromium trioxide (chromic anhydride CrO_3) with conc. sulphuric acid to a paste, then dilute with water to 75ml.

Karl Fisher Reagent

Dissolve 762 g of iodine in 2,420 ml of pyridine in a 10 liter glass stoppered bottle, and add 6 liters of methanol.

To prepare the active stock:

- ☞ Add 3 liter of the foregoing stock to a 4 liter bottle
- ☞ Cool in ice bath.
- ☞ Add carefully 135 ml of liquid sulfur dioxide, collected in a calibrated cold trap, and stopper the bottle.
- ☞ Shake the mixture until homogeneous, and set aside for one or two days before use.

Kovacs' Reagent

Components	Amounts
<i>p</i> -di-methyl-amino-benzaldehyde	5g
Amyl alcohol (normal only)	75mL
HCl (conc)	25mL

Note:

- ☞ Dissolve *p*-di-methyl-amino-benzaldehyde in normal amyl alcohol
- ☞ Slowly add HCl
- ☞ Store at 4°C
- ☞ To test for indole, add 0.2-0.3mL reagent to 5mL of 24hr bacterial culture in tryptophan broth.
- ☞ Dark red color on surface layer is positive test for indole.

Lugol's Iodine solution

Components	Amounts
Potassium Iodide (KI)	10g
Iodine	5g
Distilled water	100mL

Note:

- ☞ Dissolve KI in about 20-30mL distilled water
- ☞ Add iodine and heat gently with constant mixing iodine is to be dissolved
- ☞ Dilute to 100mL with distilled water
- ☞ Filter the un-dissolved part of iodine
- ☞ Store in amber glass-stoppered bottle in the dark.

Magnesium chloride reagent

1. Dissolve 50 g of $MgC_2 \cdot 6H_2O$ and 100 g of NH_4Cl in 500 ml of water.
2. Add 10 ml of conc. NH_4OH
3. Allow to stand overnight and filter if a precipitate has formed.
4. Make acid to methyl red with if a dilute HCl.
5. Dilute to 1 liter of solution contains 0.25 M $MgCl_2$ and 2M NH_4Cl .
6. Solution may also be diluted with 133ml of conc. NH_4OH and water to make 1 liter.

Note: Such a solution will contain 2M NH_4OH .

Magnesium nitrate reagent

1. Dissolve 130gms of magnesium nitrate and 200gms of ammonium nitrate in water
2. Add 15-20ml concentrated ammonia solution
3. Dilute to 1 liter.

Mayers reagent (Mercuric-Potassium Iodide)

Dissolve 1.358 g of mercuric chloride in 60 ml water. Dissolve 5 g of potassium iodide in 10 ml water. Mix the two solutions and add water to make 100 ml.

Nessler's reagent (Molecular formula: K_2HgI_4)

Chemicals: Potassium iodide Solid, Mercuric chloride Solid, & 5N sodium hydroxide solution

Apparatus: Weighing balance, filter paper, china dish, funnel, volumetric flask, bottle, Beaker & stirrer

Method:

1. Dissolve 50 g of KI in the smallest possible quantity of water (50 ml).
2. Add a sat. Solution of mercuric chloride (about 22 g in 350 ml of water will be needed) in KI solution with continuous stirring until an excess is indicated by the formation of orange precipitation.
3. Then add 200 ml of 5N NaOH and dilute to 1L. Allow to stand until clear; then decant into an in brown bottle.

Another method that reacts promptly and consistently is to dissolve 143 g of sodium hydroxide in 700 ml water. Dissolve 50 g of red mercuric iodide and 40 g of potassium iodide in 200 ml water. Pour the iodide solution into the hydroxide solution, and dilute with water to 1 liter. Allow to settle, and use the supernatant liquid.

Storage and Handling:

- Stay bottle strongly closed
- Protect from light, together with direct sunrays
- Store in closed original bottle at temperatures between 15°C and 25°C

Safety advice:

1. In case of accident or if you're feeling unwell, look for medical recommendation instantly Do not discharge onto the bottom or into water course
2. Stay far from contact with skin and eyes
3. Wear suitable protective clothes, gloves and eye/face protection

Ninhydrin Reagent

- ☞ Dissolve 3.5g ninhydrine in 100mL of 1:1 mixtures of acetone and butanol.
- ☞ Store refrigerated

Nitrite detection reagent

A. Sulfanilic acid reagent

Components	Amounts
Sulfanilic acid	1g
5N acetic acid	125mL

B. N-(1-naphthyl)-ethylene-di-amine reagent

Components	Amounts
n-(1-naphthyl)-ethylene-di-amine	0.25g
5N acetic acid	200mL

C. α -Naphthol reagent

Components	Amounts
α -Naphthol	1g
5N acetic acid	200mL

Note:

- ☞ To prepare 5N acetic acid, add 28.75mL glacial acetic acid to 71.25 distilled water.

- ☞ Store reagents in glass-stoppered bottles.
- ☞ To perform test, add 0.1-0.5 mL each of reagent A and reagent B or reagent C (as specific in methods) to culture grown in liquid or semisolid medium.
- ☞ Development of red-violet color with reagent A and reagent B or orange color with reagent A and C indicates that nitrate has been reduced to nitrite.
- ☞ Since color produced with reagent A and B may fade or disappear within a few minutes, record reaction as soon as color appears.
- ☞ If no color develops, test for presence of nitrate by adding small amount of zinc dust. If color develops, nitrate has not been reduced.

Nitrate reduction test for entero-pathogenic E.coli

- ☞ To 3mL of 18-24hrs culture in indole-nitrite medium, add 2 drops of each reagents of A and B
- ☞ Red-violet color indicates that nitrate has been reduced to nitrite.
- ☞ Check negative test by adding small amounts of zinc dusts; if red-violet color does not appear, nitrate has reduced.

D. Alternative test reagent

- ☞ 5-amino-2-naphthylene sulfuric acid (cleve's acid) and N, N-di-methyl-1-naphthyl-amine is recommended as a substrate for preparation of reagent B.
- ☞ Absolute ethanol may be substituted for acetic acid in reagent C.
- ☞ However, comparative evaluations should be conducted before substitution of these alternative reagents.

CAUTION: *The α -naphthyl-amine reagent was recommended in previous times of the Bacteriological analytical method should not be used because of the possible hazard to laboratory personnel. Any supplies of α -naphthyl-amine on hand should be inventoried and secured by supervisory personnel because of the strict rules governing the use of carcinogenic substances in laboratories.*

Nitron reagent

Dissolve 5g nitron in 5 ml acetic acid and make up to 100 ml with water.

Oxidase Reagent

Components	Amounts
N,N,N',N'-Tetra-methyl-p-phenylen-di-amine.2HCl	1g
Distilled water	100mL

Note:

- ☞ This is the preferred reagent. Use freshly prepared. However, reagent can be used up to 7 days if stored in a dark glass bottle under refrigeration.
- ☞ Apply freshly prepared solution directly to young culture (24hrs) on either agar plate or slant.
- ☞ Oxidase positive colonies develop a pink color and progressively turn dark purple.
- ☞ If cultures are to be preserved, complete the transfer from plates to which reagent has been added within 3 minutes, since reagent is toxic to organisms.

Optional method:

1. Transfer small amounts of culture to reagent-impregnated filter paper.
2. Dark purple color within 10 seconds indicates that a positive test.
3. Use platinum wire containing iron (e.g. nichrome wire in ordinary inoculating needles and loops) gives false-positive reactions.
4. Alternatively, the test may be performed with a 1% solution of N, N-di-methyl-phenylene-di-amine hydrochloride. Apply solution directly to culture plate or slant.

PCR Reagents

1. **Lysozyme Lysis Buffer:** 100mL (this formulation is used for lysis of gram positive bacteria).

Components	Amounts
Lysozyme	2 g
Tris HCl Stock	2 mL
EDTA stock	2 mL
Triton	1.2mL

Note: Bring these components to 100mL with sterile distilled water (molecular grade).

2. **Tris HCl Stock Solutions:** 100 mL at 1M pH 8.0 (for use in lysozyme buffer).

Components	Amounts
Trizma base	5.7 g
Tris HCl	8.9 g
dH ₂ O	85.4 mL

3. **EDTA Stock Solution:** 100 mL at 0.1 M (for use in lysozyme buffer).

Components	Amounts
EDTA	3.72 g

Note: Bring to 100mL with sterile distilled water (molecular grade).

Quimociac reagent

Solution A:

Components	Amounts
sodium Molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)	70g
Water	150mL

Solution B:

- ☞ Dissolve 60 g of acetic acid in a mixture of 85ml nitric acid and 150 ml of water and cool.

Note: Gradually add Solution A to Solution B, with stirring, to produce Solution C.

Solution D:

Dissolve 5 g of synthetic quinoline in a mixture of 35 ml nitric acid and 100 ml water.

Note:

1. Gradually add Solution D to Solution C, mix well, and allow standing overnight.
2. Filter the mixture, add 280 ml of acetone to the filtrate, dilute to 1 liter with water, and mix. Store in polythene bottle. (**Caution:** Flammable).

Quinalizarin reagent

Dissolve 0.02g in 100 ml ethanol, or dissolve 0.05g in 100 ml of 0.01M sodium hydroxide.

Components	Amounts
Quinalizarin	0.02g
Ethanol	100mL

Or

Components	Amounts
Quinalizarin	0.05g
0.01M Sodium hydroxide	100mL

Schiffs reagent

Method 1:

- ☞ Dissolve 0.2gm of pure p-rosaniline hydrochloride in 20ml of a cold, freshly prepared, saturated aqueous solution of sulphur dioxide.
- ☞ Allow the solution to stand for a few hours until it becomes colourless or pale yellow.
- ☞ Dilute the solution to 200ml and stay it in a tightly stoppered bottle.
- ☞ The solution stays well, and should not be exposed to light or air.
- ☞ Store in the dark.

Method 2:

- ☞ Add 2gm. of sodium bisulphite to a solution of 0.2gm.of p-rosaniline hydrochloride and 2ml of concentrated hydrochloric acid in 200ml. of water.

Sodium rhodizonate reagent :

Components	Amounts
Sodium rhodizonate	0.5g
Water	100mL

Tollens reagent.

Add sodium hydroxide solution to silver nitrate soln. to form a precipitate then add dilute ammonia solution until precipitation dissolves.

Voges-Proskauer (VP) Test Reagent

Solution 1

Components	Amounts
α -Naphthol	5g
Alcohol (Absolute)	100mL

Solution 2

Components	Amounts
Potassium hydroxide	40g
Distilled water	To make 100mL

Voges-Proskauer (VP) test

1. Transfer 1mL of 48 hrs culture to test tube.
2. Add 0.6mL solution 1 and 0.2mL solution 2.
3. Shake after adding each solution.

☞ To intensify and speed reaction, add a few creatine crystals to mixture.

4. Let stand at room temperature.
5. Read result 4 hrs after adding reagents.
6. Development of eosin pink color is positive test.

Zirconyl nitrate reagent (for fluoride test)

Components	Amounts
zirconyl nitrate	0.1g
Concentrated HCl	20mL
Water	100mL

Note: Dissolve 0.1g of zirconyl nitrate in 20ml concentrated HCl and dilute with water to 100ml.

Zirconyl nitrate reagent (for phosphate separations)

1. Heat 10gms of commercially pure zirconium nitrate and 100ml of 1M nitric acid to the boiling point with constant stirring.
2. Leave to stand for about 24hrs and decant the clear solution.

Zwikk's reagent

- ☞ Mix 1 ml of pyridine with 4 ml of a 10% aqueous solution of copper sulphate and 5 ml of water.

Buffer and Chemical Solution Preparation

0.1M Bicarbonate buffer (pH 9.6)

Components	Amounts
Na ₂ CO ₃	1.59 g
NaHCO ₃	2.93 g
Distilled water	1L

Note: Store at room temperature for not more than 2 weeks.

1-Amino-1-Naphthol-4-sulfonic acid

1. Dissolve 0.2 g in 195 ml of sodium bisulphite solution (3 in 20) and 5 ml of anhydrous sodium sulphite solution (1 in 5) and filter if necessary.
2. Store in a cool dark place. Use within 10 days.

Bovine serum Albumin (BSA) (1mg/mL)

Components	Amounts
Nuclease-free bovine serum albumin	10mg
Distilled water	10mL

Note: Place 0.5mL portions into 1.5mL plastic conical centrifuge tubes. Store frozen.

1% Bovine serum Albumin in cholera toxin ELISA buffer

Components	Amounts
Bovine serum albumin (BSA)	1g
ELISA buffer (for cholera toxin), pH 7.4	100mL

Note: Dissolve BSA in ELISA buffer. Aliquot and store at -20°C.

1% Bovine serum Albumin in PBS

Components	Amounts
Bovine serum albumin (BSA)	1g
Phosphate buffer solution, pH 7.4	100mL

Note: Dissolve BSA in PBS buffer. Aliquot and store at -20°C

Chlorine solution (200 PPM) (containing 0.1% sodium dodecyl sulfate)

Components	Amounts
Commercial bleach (5.25% sodium hypochlorite)	8mL
dH ₂ O containing 1g sodium dodecyl sulfate	992mL

Note: Dissolve 1g sodium dodecyl sulfate in 992mL distilled water then add 8mL commercial bleach and mix well. Make immediately before use.

Coating solution for *V.vulnificus*

Components	Amounts
Phosphate buffered solution	100mL
Triton X-100 (α -poly-oxy-ethylene ether)	20 μ L

Note: Mix Triton X-100 with PBS, pH 7.4

Dithizone solution

1. Dissolve 30 mg of dithizone in 1 liter chloroform.
2. Add 5 ml alcohol.
3. Store in refrigerator.

50X denhardt's solution

Components	Amounts
Ficoll	2g
Poly-vinyl pyrrolidone	2g
Bovine serum albumin	2g

Note: add distilled water to make 200mL. Store at -20⁰C in 10mL aliquots.

Disinfectants (for preparation of canned foods for microbiological analysis)

1. Alcoholic solution of iodine

Components	Amounts
Potassium Iodide	10g
Iodine	10g
Ethanol (70%)	500mL

2. Sodium hypochlorite solution*

Components	Amounts
Sodium hypochlorite	5.0-5.25g
Distilled water	100mL

*Laundry bleach, which is 5.25% sodium hypochlorite (NaOCl), may be used.

Dulbecco's phosphate-buffered saline (DPBS)

Components	Amounts
NaCl	8g
KCl	200mg

Na ₂ HPO ₄	1.15g
KH ₂ PO ₄	200mg
CaCl ₂	100mg
MgCl ₂ .6H ₂ O	100g
Distilled water	1L

Note: dissolve ingredients in water. Sterilize by filtration. Final pH, 7.2

ELISA Buffer (for cholera toxin test)

Components	Amounts
Bovine serum albumin	1.0g
NaCl	8.0g
KH ₂ PO ₄	0.2g
Na ₂ HPO ₄	2.9g
KCl	0.2g
Distilled water	1L
Tween 20	0.5mL

Note: adjust pH to 7.4 and add tween 20. Store frozen. Thaw before use.

Fehlings solution

Solution A

Components	Amounts
Pure copper sulphate	34.6gm
Distilled water	Dilute to 500ml

Blur

Solution B:

Components	Amounts
Sodium potassium tartrate	173gm
Pure sodium hydroxide	30gm
Distilled water	Dilute to 500ml

Alternately,

Components	Amounts
Pure sodium hydroxide	121gm
Pure tartaric acid	93.1gm
Distilled water	Dilute to 500ml

☞ Colorless

Note: Mix equal volumes of solutions A and B immediately before use, and then use as the reagent.

Fuchsin solution

Dissolve 0.15gm of fuchsin in 100ml. water.

Formalinized physiological saline solution

Components	Amounts
Formaldehyde solution (36-38%)	6mL
NaCl	8.5g
Distilled water	1L

Note:

- ☞ Dissolve 8.5g NaCl in 1L distilled water
- ☞ Autoclave 15 minutes at 121°C
- ☞ Cool at room temperature
- ☞ Add 6mL formaldehyde solution

Caution: do not autoclave after addition of formaldehyde.

Gel-phosphate buffer

Components	Amounts
Gelatin	2g
Na ₂ HPO ₄	4g
Distilled water	1L

Note:

- ☞ Use gentle heat to dissolve ingredients.
- ☞ Sterilize 20 minutes at 121⁰C
- ☞ Final pH, 6.2

Glycerin salt solution (buffered)

Components	Amounts
Glycerin (reagent grade)	100mL
K ₂ HPO ₄ (anhydros)	12.4g
KH ₂ PO ₄ (anhydros)	4
NaCl	4.2g
Distilled water	900mL

Note:

- ☞ Dissolve in water NaCl and bring the volume to 900mL with water.
- ☞ Add glycerin and phosphates.
- ☞ Adjust pH to 7.2
- ☞ Autoclave 15 minutes at 121⁰C
- ☞ For double strength (20%) glycerin solution, use 200mL glycerin and 800mL distilled water.

Hippurate Solution (1%)

- ☞ Dissolve 0.1g sodium hippurate in 10mL distilled water.
- ☞ Filter-sterilize and store refrigerated (or in 0.4mL aliquot) at -20⁰C

Horseradish peroxidase (color development solution)

Solution A

Components	Amounts
HRP color development reagent	60mg
Ice cold methanol	20mL

Note:

- ☞ Mix to dissolve
- ☞ Protect from light and prepare fresh

Solution B

Components	Amounts
Ice cold H ₂ O ₂ (30%)	60mL
Tris buffered solution	100mL

Note:

- ☞ Prepare fresh before use.
- ☞ Mix ice cold solution A with room temperature solution B.
- ☞ Use immediately

10X kinase buffer

Components	Amounts
2.0M tris, pH 7.6	2.5mL
1.0M MgCl ₂	1mL

0.5M di-thio-threitol	1.0mL
10 mM spermidine	1.0mL
0.5M EDTA, pH 8.0	20mL
Distilled water	4.5mL

Note: store at 4⁰ C

Lysis solution (0.5M NaOH, 1.5M NaCl)

Components	Amounts
Sodium hydroxide	20g
NaCl	87g
Distilled water	1L

Monoclonal Antibody solution

- ☞ Dispense 10 µL ascetic fluids into 1mL 1% gelatin-Tris-buffered saline (TBS).
- ☞ Add 3 µl horseradish peroxidase-proteins A conjugates, and stir mixture at 4⁰C for 1hr.
- ☞ Then dilute to 10mL with 1% gelatin TBS.
- ☞ Sufficient for 1 hydrophobic grid membrane filter.

Neutralizing solution (Maas II)

Components	Amounts
Tris	121.14g
NaCl	117g
HCl (concentrated)	As needed for pH7
Distilled water	1L

Note:

- ☞ Dissolve in several hundred mL distilled water.
- ☞ Adjust pH 7.0 with concentrated HCl
- ☞ Bring volume to 1L by addition of distilled water.

N,N-dimethyl-p-phenylenediamine

Method a

1. Measure 89 mL of distilled water and pour into a 250 mL beaker.
2. Stir on a magnetic stirrer.
3. Carefully add 15 mL of concentrated sulfuric acid.
4. Add and dissolve 1.0 g n,n-dimethyl-p-phenylenediamine sulfate.
5. Add 5 g of Florisil and stir the mixture until all is absorbed.
6. Allow the adsorbent to settle and decant the supernatant solution.

Method b.

1. Add 200 ml sulphuric acid to 700 ml water, cool.
2. Add 1g of N, N-dimethyl-p-phenylenediamine (p-aminodimethylaniline) and dilute in 1 liter.

Novobiocin Solution (100mg/mL)

Components	Amounts
Novobiocin (sodium salt)	100mg
Deionized water	1.0mL

Note:

- ☞ Dissolve novobiocin
- ☞ Filter-sterilize, using 0.2 µm filter and syringe
- ☞ May be stored for several months in dark bottle at 4°C.

ONPG test

Monosodium phosphate solution (1.0M, pH 7)

Components	Amounts
NaH ₂ PO ₄ .H ₂ O	6.9g
Distilled water	145mL
NaOH solution (30%, w/v)	30mL

Note:

- ☞ Dissolve NaH₂PO₄.H₂O in distilled water
- ☞ Add 30% NaOH solution and adjust the pH to 7.
- ☞ Bring volume to 50mL with distilled water and store in refrigerator (about 4⁰C).

O-Nitro-phenyl-D-galactoside (ONPG) (0.0133M)

Components	Amounts
ONPG	80mg
Distilled water (37 ⁰ C)	15mL
Mono-sodium phosphate solution (1.0M, pH 7)	5mL

Note:

- ☞ Dissolve ONPG in distilled water at 37⁰C
- ☞ Add 1.0M NaH₂PO₄ solution.
- ☞ Solution should be colorless
- ☞ Store in refrigerator (about 4⁰C)
- ☞ Before use, warm appropriate portion (sufficient for number of tests) of ONPG solution to 37⁰C

Procedure

1. Inoculate cultures to be tested onto triple sugar iron agar slants and incubate for 18hrs at 37⁰C (or other appropriate temperature, if required).
2. Nutrient (or other) agar slants containing 1.0% lactose may also be used.
3. In sterile 13X 100mm tube containing 0.25mL of physiological saline, emulsify large loopful of culture growth to form heavy suspension.
4. Add 1 drop of toluene to each tube and shake well to liberate enzyme
5. Let tubes stand for 5 minutes at 37⁰C.
6. Add 0.25mL buffered 0.0133M ONPG solution to each suspension to be tested.
7. Incubate tubes at 35-37⁰C and examine tubes at intervals up to 24hrs.
8. Yellow color is positive test.

ONPG disks are available commercially. Emulsify growth from 18hrs lactose-containing medium in 0.2mL sterile physiological saline in sterile 13x100mm tubes. Add disks and agitate gently. Incubate and read tubes as above.

Peptone-Tween salt dilution (PTS)

Components	Amounts
Peptone	1.0g
Tween 80	10.0g
NaCl	30.0g
Distilled water	1L

Note:

- ☞ Dissolve ingredients in distilled water, adjust pH to 7.2 and dispense into bottles of desired volume.
- ☞ Autoclave for 15 minutes at 121⁰C.

Peroxidase substrate for membrane ELISA

Solution A

Components	Amounts
4-chloro-1-naphthol	60mg
Methanol (cold)	20mL

Note: Dissolve 4-chloro-1-naphthol in cold methanol and store at -20°C

Solution B

Components	Amounts
H ₂ O ₂ (30%)	60mL
Tris-buffer saline (TBS)	100mL

Note:

- ☞ Add 30% H₂O₂ to TBS at room temperature.
- ☞ Immediately before use, mix ice cold solution A with room temperature solution B.

Peoxidase substrate solution (ABTS) 2,2'-azino-bis(3-ethyl-benzo-thiazoline-6-sulfonic acid) (ABTS) substrate

Components	Amounts
ABTS	10mg
Citric acid (0.05M)	10mL
H ₂ O ₂ (30%)	30mL

Note: prepare 5 minutes before use. One recipe is sufficient for one 96-well micro-well plate.

Phosphate Saline Buffer (0.02M, pH 7.3-7.4)

Prepare stock solution of 0.2M mono-and di-sodium phosphate in 8.5% salt solutions and dilute 1:10 for preparation of 0.02M phosphate saline water.

Stock solution 1

Components	Amounts
Na ₂ HPO ₄ (anhydrous, reagent grade)	28.4g
NaCl (reagent grade)	85g
Distilled water	To make 1L

Key: Na₂HPO₄, Sodium phosphate dibasic anhydrous

Stock solution 2

Components	Amounts
NaH ₂ PO ₄ .H ₂ O (monohydrate, reagent grade)	27.6g
NaCl (reagent grade)	85g
Distilled water	To make 1L

Note:

- ☞ To obtain 0.02M phosphate buffer saline (0.85%), make 1:10 dilution of each stock solution. *For example:*

Components	amount	Components	Amounts
Stock solution 1	50mL	Stock solution 2	10mL
Distilled water	450mL	Distilled water	90mL
Approximate pH 8.2		Approximate pH 5.6	

- ☞ Using pH meter, titer diluted solution 1 to pH 7.3-7.4 by adding about 65mL of diluted solution 2.
- ☞ Use resulting 0.02M phosphate saline buffer solution in the lysostaphin susceptibility test on *S.aureus*.

NOTE: do not titer 0.2M phosphate buffer to pH 7.3-7.4 and then dilute to 0.02M strength. This results in a drop of pH of approximately 0.25. Additions of 0.85% salt before pH adjustment also results in a drop of approximately 0.2.

Phosphate saline solution (for Y-1 LT assay)

Components	Amounts
Na ₂ HPO ₄	1.07g
NaH ₂ PO ₄	0.24g
NaCl	8.9g
Distilled water	1L

Note: dissolve ingredients in water. Adjust pH to 7.5

Phosphate-Buffered Saline (0.01M, pH 7.5)

Stock Solution (0.1M)

Components	Amounts
Na ₂ HPO ₄ (anhydrous)	12.0g
NaH ₂ PO ₄ .H ₂ O	2.2g
NaCl	85.0g
Distilled water	1L

Note:

- ☞ Dissolve the ingredients in distilled water and bring volume to 1L.
- ☞ Dilute stock solution 1+9 in double distilled water.
- ☞ Mix well, adjust pH to 7.5 with 0.1N HCl or 0.1N NaOH if necessary.

Phosphate-Buffered Saline (PBS, pH 7.4)

Components	Amounts
Na ₂ HPO ₄ (anhydrous)	0.724g
KH ₂ PO ₄	0.210g
NaCl	7.650g
Distilled water	1L

Note:

- ☞ Dissolve ingredients in distilled water.
- ☞ Adjust pH to 7.4 (with 1N NaOH).
- ☞ Autoclave for 15 minutes at 121⁰C.
- ☞ This buffer may be used for steps in the *Vibrio spp.* methods when PBS is needed.

Physiological saline solution (0.85%, strile)

Components	Amounts
NaCl	8.5g
Distilled water	1L

Note:

- ☞ Dissolve 8.5g NaCl in water.
- ☞ Autoclave for 15 minutes at 121⁰C.
- ☞ Cool at room temperature at the time of using the solution

Saline Solution (0.5%)

Components	Amounts
NaCl	5g
Distilled water	1L

Note: Autoclave at 121⁰C for 15 minute.

Salts-Phosphate buffered Saline Solution (Salts-PBS)

Components	Amounts
NaCl	121g
KCl	15.5g
MgCl ₂	12.7g
CaCl ₂ .2H ₂ O	10.2g
NaH ₂ PO ₄ .H ₂ O	2.0g

Na ₂ HPO ₄ ·7H ₂ O	3.9g
Distilled water	1L

Note: Adjust pH to 7.4

Sarkosyl Solution (10%)

Components	Amounts
N-lauroyl-sarcosine, sodium salt	10g
Distilled water	100mL

Scintillation Fluid

Components	Amounts
2,5-Di-phenyl-oxazole	5.0g
Toluene	1L

FAT Mounting Fluid (pH 9.0)

Components	Amounts
Glycerol	90.0 mL
DABCO*	2.5 g
PBS	10.0 mL

Note:

- ☞ Suspend the DABCO in glycerol over low heat.
- ☞ Then add 1X PBS (see below).
- ☞ Adjust pH to 8.6 to 9.0 with 1N hydrochloric acid or 0.1N sodium hydroxide. The pH of the mounting media is important, as an acid pH will quench fluorescence. Check the pH frequently. Store at room temperature.

* Optional ingredient - DABCO is 1, 4-diazabicyclo-(2,2,2)-octane. Its addition to mounting fluid can reduce quenching of fluorescence.

Slide Preserving Solution

Prepare 1% acetic acid solution (10mL glacial acetic acid, reagent grade + 990mL distilled water). Add 1mL glycerin to each 100mL of solution.

Sodium Bicarbonate solution (10%)

Components	Amounts
Sodium bicarbonate	100 g
Distilled water	1L

Sodium Chloride Dilution water, 2% and 3%

2% solution

Components	Amounts
NaCl	20g
Distilled water	1L

3% solution

Components	Amounts
NaCl	30g
Distilled water	1L

Note:

- ☞ Dissolve NaCl in water
- ☞ Autoclave for 15 minutes at 121⁰C
- ☞ Final pH 7.0

0.2 M Sodium Chloride Solution

Components	Amounts
NaCl	11.7g
Distilled water	1L

Note: Dispense in suitable containers. Autoclave for 15 minutes at 121°C

Sodium Desoxycholate 0.5% in sterile dH₂O (String test)

Components	Amounts
Sodium deoxyscholate	0.5g
Distilled water	100mL

Note: pH of solution does not have to be adjusted and can be stored in screw cap bottle at room temperature.

Sodium Dodecyl sulphate (10%) in strile dH₂O

Components	Amounts
Sodium Dodecyl Sulphate	10g
Distilled water	100mL

Note: Sterility of the solution is not important

1N Sodium Hydroxide Solution

Components	Amounts
NaOH	40g
Distilled water	1L

Note: Used for the adjusting pH of culture media.

10N Sodium Hydroxide Solution

Components	Amounts
NaOH	400g
Distilled water	1L

Spicer-Edwards EN complex Antibody Solution

1. Add 0.1mL Spicer-Edwards EN complex (Difco) to 0.07 mL horseradish peroxidase protein A.
2. Conjugate in 1mL 1% gelatin-Tris-Buffered saline (TBS)
3. Stire at 4⁰C for 1hr.
4. Dilute to 40mL 1% gelatin-TBS.
5. Use within a few hours.
6. Sufficient for 4 hydrophobic grid membrane filters.

Standard Saline Citrate Solution (SSC) (20%)

Components	Amounts
NaCl (Reagent grade)	175.3g
Sodium Citrate	88.2g

Note:

- ☞ Dissolve in 800mL deionized water and adjust to pH with 10N NaOH
- ☞ Bring volume to 1L

6X SSC

Components	Amounts
NaCl (Reagent grade)	52.6g
Sodium Citrate	26.5g

Note:

- ☞ Dissolve in 800mL deionized water and adjust to pH with 10N NaOH
- ☞ Bring volume to 1L

3X SSC

Components	Amounts
6X SSC	500mL
deionized water	500mL

2X SSC

Components	Amounts
6X SSC	333mL
deionized water	667mL

Tris-Buffered Saline (TABS), 1% or 3% Gelatin or Tween 20

Components	Amounts
Tris	2.42g
NaCl	29.24g
deionized water	1L

Note:

- ☞ Dissolve ingredients in distilled water by heating and stirring.
- ☞ Adjust pH to 7.5 with HCl
- ☞ Autoclave for 15 minutes at 121⁰C

For 1% and 3% Gelatin-TABS: add 10g and 30g gelatin, respectively, to ingredients before autoclave. Adjust final pH to 7.5 with HCl.

For tween-TABS: add 0.5mL tween 20 to ingredients and adjust pH to 7.5 before autoclaving.

Tris-Buffered Saline (TBS) pH 7.5

Components	Amounts
Tris	2.42g
NaCl	29.24
distilled water	Make it 1L

Note: Dissolve ingredients, adjust pH to 7.5 with HCl and bring the volume to 1L.

Tris-Buffered Saline (TBS), with Gelatin

1% solution

Components	Amounts
Gelatine	1g
TBS, pH 7.5	100mL

3% Solution

Components	Amounts
Gelatine	3g
TBS, pH 7.5	100mL

Note: Add gelatin to TBS at 40⁰C, stir to dissolve, cool to at room temperature before use.

Tris-Buffered Saline (TBS)-Tween

Components	Amounts
Tween 20	50mL
TBS, pH 7.5	100mL

Note: Dissolve Tween 20 in TBS

Tris-EDTA-Triton X-100 (TET) buffer

Components	Amounts
Triton X-100	1.0mL
1.0M Tris (mentioned above)	5.0mL
0.5M Na ₂ EDTA	12.5mL

Note: Distilled water to make 100mL.

Trypsin-EDTA Solution 1X

Components	Amounts
Trypsin (1:250)*	0.05g/L
EDTA, disodium salt	0.02g
NaCl, 0.9%	100mL

Note:

☞ Dissolve trypsin and EDTA in NaCl diluents.

☞ Filter-sterilize through 0.22 µm membrane.

* 1g Trypsin (1:250) will digest 250g casein substrate under standard condition.

Staining Solution Preparation

Clark's flagellar stain

Solution A

Components	Amounts
Basic fuchsin	1.2g
95% ethanol	100mL

Note: Mix and let stand overnight at room temperature.

Solution B

Components	Amounts
Tannic acid	3 g
NaCl	1.5g
Distilled water	200mL

Note:

- ☞ Mix solution A and B. Adjust pH to 5.0 with 1N NaOH or 1N HCL, if necessary.
- ☞ Refrigerate 2-3 days before use.
- ☞ Stain is stable 1 month at 4⁰C or may be stored frozen indefinitely (50mL portions).
- ☞ To use, thaw stain, and store at 4⁰C. Optimum staining time for each batch varies 5-15 min. to determine staining time (after 2-3 days refrigeration at 4⁰C), stain a known flagellated organism on 3 or more cleaned slides for various time (e.g. 5, 10, 10 min).

Basic Fuchsin staining Solution

1. Dissolve 0.5g basic fuchsin dye in 20mL 95% ethanol.
2. Dilute to 100mL with distilled water
3. Filter if necessary with whatman No.31 filter paper to remove any undissolved dye.

Gram Stain

Hucker's Crystal Violet

Solution A

Components	Amounts
Crystal violet (90% dye conten)	2g
Ethanol (95%)	20mL

Solution B

Components	Amounts
Ammonium oxalate	0.8g
Distilled water	80mL

Note: Mix solution A and B. store 24hrs and filter through coarse filter paper.

I. Gram's iodine

Components	Amounts
Iodine	1g
Potassium iodide (KI)	2g
Distilled water	300mL

Note:

- ☞ Place KI in mortar, add iodine and grind with pestle for 5-10 minutes.
- ☞ Add 1mL water and grind.
- ☞ Add 5mL of water and grind, then 10mL and grind.
- ☞ Pour this solution into reagent bottle.
- ☞ Add amount of water needed to bring total volume to 300mL
- ☞ Rinse mortar and pestle with water

II. Hucker's counter stain (stock solution)

Components	Amounts
Safranin O	2.5
Ethanol (95%)	100mL

Note:

- ☞ Working solution: add 10mL stock solution to 90mL distilled water
- ☞ Staining procedure (gram stain)
- ☞ Fix air-dried films of food sample in moderate heat.
- ☞ Stain films 1 minute with crystal violet-ammonium oxalate solution.
- ☞ Wash briefly in tap water and drain.
- ☞ Apply gram's iodine for 1 minute.
- ☞ Wash in tap water and drain
- ☞ Decolorize with 95% ethanol until blue color is no longer released (30 s).

- ☞ Alternatively, flood slides with ethanol, pour off immediately, and re-flood with ethanol for 10s.
- ☞ Wash briefly with water, drain and apply Hucker's counter-stain (safranin solution) for 10-30s.
- ☞ Wash briefly with water, drain blot or air dry and examine.

Giemsa Stain

Components	Amounts
Giemsa stain	1g
Glycerol	66mL
Methanol (absolute)	66mL

Note:

- ☞ Dissolve Giemsa stain in glycerol by heating 1.5-2.0 hours at 55-60⁰C.
- ☞ Add methanol
- ☞ Store stain in tightly stored bottle at 22⁰C for at least 2 weeks.
- ☞ Dilute stock solution with distilled water (1+9) before use.

May-Grunwald Stain

Components	Amounts
May-Grunwald stain	2.5g
Methanol (absolute)	1L

Note:

- ☞ Weight May-Grunwald stain and mix in to 50mL methanol and dissolve by grinding.
- ☞ Dilute to 1L with methanol

- ☞ Stir 16hrs at 37⁰C
- ☞ Hold stain for 1 month at 22⁰C (room temperature).
- ☞ Filter before use.

Methylene blue stain (Loeffler's)

Solution A

Components	Amounts
Methylene blue (90% dye content)	0.3g
Ethanol (95%)	30mL

Solution B

Components	Amounts
Diluted potassium hydroxide (0.01%)	100mL

Note: Mix solution A and B

North Aniline (Oil)-methylene blue stain

Components	Amounts
Aniline oil	3mL
Ethanol (95%)	10mL
HCl (Concentrated)	1.5mL
Methylene blue (saturated solution)	30mL
Distilled water	4.5mL

Note:

- ☞ Mix aniline oil with ethanol
- ☞ Slowly add HCl with constant agitation

- ☞ Add saturated methylene blue solution.
- ☞ Dilute to 100mL with water and filter

FITC Conjugated Rabbit Anti-X /Rhodamine Counter Stain

Components	Amounts
Rhodamine stock	10.0 μ L
FITC conjugated antibodies	10.0 μ L
Phosphate buffered saline	480.0 μ L

Store at 4°C.

Note: Evans Blue or Eriochrome Black T can also be used as counterstain in FAT in a separate step during the staining process, but neither can be added directly to the conjugate solution.

Thiazine Red R Stain (for *S.aureus* enterotoxin gel diffusion technique)

Dissolve Thiazine Red R in 0.1 % in 1.0 acetic acid.

1.0M Tris (pH 8.0)

Components	Amounts
Tris	121.14g
Con. HCl (for pH 8.0)	
deionized water	Make it 1L

Note:

- ☞ Dissolve in several hundred mL of distilled water.
- ☞ Adjust pH to 8.0 with concentrated HCl.
- ☞ Bring volume to 1L.

Indicators and Dye Solution Preparation

1. Bromopyrogallol Red:

Preparation: Dissolve 0.05g of the reagent in 100ml of 50% ethanol.

Colour change: The indicator is coloured orange-yellow in strongly acid solutions, claret red in nearly neutral solutions; and violet to blue in basic solutions.

2. Brilliant green dye solution (1%)

Components	Amounts
Brilliant green dye	1g
Distilled water (serile)	10mL

Note: Dissolve 1g dye in sterile water. Dilute to 100mL. Before use, test all batches of dyes for toxicity with known positive and negative test microorganisms.

3. Bromocresol purple dye solution (0.2%)

Components	Amounts
Bromocresol purple dye	0.2g
Distilled water (sterile)	100mL

Note: Dissolve 0.2g dye in sterile water and dilute to 100mL.

4. Bromthymol blue indicator (0.04%)

Components	Amounts
Bromthymol blue	0.2g
0.01N NaHO	32mL

Note: Dissolve bromthymol blue in NaHO and dilute to 500mL with distilled water.

5. Diphenylamine:

Preparation: Dissolve 1 g solid in 100 ml conc. H_2SO_4

Colour change: blue-violet to colorless

6. Eriochrome Black T (EBT) or Solochrome Black T

Preparation: Dissolve 0.2 g of the solid in 15ml. of ethanolamine and add 5ml of absolute ethanol to reduce the viscosity; the reagent is stable for several months. A 0.4% solution of the pure dye in methanol may last for about a month.

Colour change: From blue to red.

7. Eriochrome Red B:

Preparation: Dissolve 0.1g of solid in 50ml, ethanol. It is stable indefinitely.

Colour change: from pink to pale yellow.

8. Evan's Blue dye solutions

Components	Amounts
Evan's blue dye	2g
NaCl	0.5g
Distilled water	100mL (to make 100mL final volume)

Caution: Evans blue dye is a suspected carcinogen.

9. Fast Sulphon Black F:

Preparation: The indicator solution is 0.5% solution in water.

Colour change: For copper is from magenta to pale blue to bright green.

10. Murexide:

Preparation: Suspend 0.5 g of the powdered solid in water, shake thoroughly and allow to settle. The saturated supernatant is used as the indicator.

Colour change: Towards a blue endpoint.

11. Methylene blue:

Preparation: Dissolve 0.1 to 0.5g in 100 ml water

Colour change: blue to colorless

12. Methyl red indicator

Components	Amounts
Methyl red	0.10g
Ethanol (95%)	300mL
Distilled water	To make 500mL

Note:

Dissolve methyl red in 300mL methanol
Bring volume to 500mL with distilled water

13. Methyl orange:

Preparation: Dissolve 0.05 g of the Methyl orange in 100 ml of water, add 8 ml of 0.1M hydrochloric acid, and filter if necessary.

Color change: Pink/red towards yellow-orange

14. Xylenol Orange:

Preparation: Dissolve 0.5 g of xylenol orange indicator in 100ml of water. The solution is stable indefinitely.

Colour change: Acid solutions are colored lemon yellow and those of the metal complexes intensely red.

Preparation of special reagents

1. 1-Amino-1-Naphthol-4-sulfonic acid

Dissolve 0.2g in 195 ml of sodium bisulphite solution (3 in20) and 5 ml of anhydrous sodium sulphite solution (1 in 5) and filter if necessary. Stopper and store in a cool dark place. Use within 10 days.

2. Ammonium citrate, lead free

Dissolve 40g of citric acid in 100 ml of water and make alkaline to phenol red with ammonium hydroxide. Remove lead by shaking with small portions of dithizone extraction solution in chloroform until the dithizone solution retains its original green color. Discard the extraction solution.

3. Ammonium mercurithiocyanate

Dissolve 9g of ammonium thiocyanate and 8g of mercuric chloride in 100ml of water.

4. Ammonium metavanadate

Dissolve 2.5g of NH_4VO_3 in 500 ml of boiling water, cool, and add 20 ml of nitric acid. Dilute to 1 liter and store in polythene bottle.

5. *Ammonium molybdate reagent*

Method A: Dissolve 45 gms of the commercial substance or 40 gms of pure molybdenum trioxide in a mixture of 70 ml of concentrated ammonia solution and 140 ml of water; when solution is complete, add it very slowly and with vigorous stirring to a mixture of 250ml of concentrated nitric acid and 500ml of water, and dilute to 1 liter. Allow to stand 1 to 2 days and decant and use the clear solution.

Method B: Dissolve 45 gms of pure commercial ammonium molybdate in mixture of 40ml concentrated ammonia solution and 60 ml of water, add 120gms of ammonium nitrate and dilute to a liter with water.

Note: The alkaline solution of ammonium molybdate stays better than the nitric acid solution; there is little tendency for the separation of solid. Before using the alkaline solution, it is important that the test solution contains a slight excess of nitric acid.

6. *Ammonium sulfanilate*

To 2.5 g of sulfanilic acid add 15 ml water and 3 ml ammonia and mix. If necessary add more ammonia until the acid dissolves. Adjust the pH to 4.5 with dilute HCl and dilute to 25 ml.

7. *Amylase*

To 0.2 g of amylase crystal, add 100ml water, shake well and filter. Prepare fresh.

8. *Anthrone*

Dissolve about 0.1 g in 100 ml sulphuric acid. Prepare fresh.

9. *Aqua Regia*

Mix one part by volume of conc. nitric acid with three (3) parts by volume of conc. hydrochloric acid in a pyrex beaker and allow to stand until a bright red color develops.

10. *Benedict's solution (qualitative)*

Dissolve 86.5gm of sodium citrate and 50gm of anhydrous sodium carbonate in about 350ml of water. Filter if necessary. Add a solution of 8.6gm of copper sulphate in 50ml of water

with constant stirring. Dilute to 500ml. The resulting solution should be perfectly clear; if not, filter through a fluted filter paper.

11. *Benedicts solution (quatitative)*

Dissolve 200 g sodium citrate, 75g sodium carbonate and 125g potassium thiocyanate in about 600 ml water. Dissolve separately, 18g copper (II) sulphate in 100 ml water. When the solutions have cooled, mix them together with stirring. Now add 5 ml of a 5% potassium ferrocyanide to the solution, and make up to 1 liter.

12. *Benzidine*

Dissolve 50 g in 10 ml glacial acetic acid, dilute to 100 ml with water and mix. **caution:** Toxic!.

13. *Benzodine*

Dissolve 1g in 10ml acetic acid and dilute to 100 ml with water.

14. *2, 2-bipyridine*

Dissolve 0.100 g in 50ml purified absolute ethanol.

15. *Cacotheline (α -nitroso- β -naphthol reagent)*

Dissolve 0.25 g in 100 ml of water.

16. *Chromic acid*

Weigh out 10 g of sodium dichromate crystals, make it into a slurry with a few ml of water, then dissolve in 250ml conc. sulphuric acid with stirring and cooling (icebath), to give a thick syrupy dark brown mixture. (**very corrosive!**)

17. *N,N-dimethyl-p-phenylenediamine*

- a. Measure and pour into a 250 ml beaker 89 ml of dH₂O. Stir on a magnetic stirrer. Carefully add 15 ml of concentrated sulfuric acid. Add and dissolve 1.0 g n,ndimethyl- p-phenylenediamine sulfate. Add 5 g of Florisil and stir the mixture until all is absorbed. Allow the adsorbant to settle and decant the supernatant solution.
- b. Add 200 ml sulphuric acid to 700 ml water, cool, and add 1g of N,N-dimethyl-pphenylenediamine (p-aminodimethylaniline) and dilute ti 1 liter.

18. Fehlings solution

Solution A; Dissolve 34.6gms of pure copper sulphate in distilled water and dilute to 500ml (blue)

Solution B: Dissolve 173gms of sodium potassium tartrate and 30gms of pure sodium hydroxide in water and dilute to 500ml. Alternately, dissolve 121gms. Of pure sodium hydroxide and 93.1gms. of pure tartaric acid in water, then dilute the solution to 500ml. (colorless).

Mix equal volumes of solutions A and B immediately before use, and then use as the reagent.

19. Fluorescein

Make a saturated solution in 50% ethanol.

20. Formaldehyde

Dilute the commercial 40% solution (1 part) with water (7 parts)

21. Indigo solution

Gently warm 1gm. of indigo with 12ml. of concentrated sulphuric acid, allow standing for 48hrs and pouring into 240ml. of water. Filter if necessary.

22. Magnesia mixture

Dissolve 100gms of magnesium chloride and 100gms. of ammonium chloride in water, add 50ml. of concentrated ammonia solution and dilute to 1 litre with dH₂O.

23. Malachite green

A 1% solution of malachite green oxalate in glacial acetic acid.

24. Manganese sulphate

Dissolve 90 g manganese sulphate in 200 ml water, 175 ml phosphoric acid and 350 ml of diluted sulphuric acid (1 in 2). Add water to make up 1 liter.

25. Ninhydrin

A 0.2% solution of ninhydrin (triketohydrindene hydrate, C₉H₄O₃.H₂O) in water. Prepare fresh.

26. *α -Nitroso- β -naphthol*

Dissolve 10 g in 100 ml of either 1:1 acetic acid, ethyl alcohol or acetone.

27. *Orthophenanthroline*

Dissolve 0.15 g orthophenanthroline ($C_{12}H_8N_2 \cdot H_2O$) in 10 ml solution of ferrous sulphate, prepared by dissolving 1.48 g of ferrous sulphate in 100 ml water. The ferrous sulphate solution must be prepared immediately before dissolving the orthophenanthroline.

28. *Salicylaldehyde*: A 20% solution in ethanol.

29. *Starch solution*

Triturate 0.5gm. of soluble starch with a little cold water into a thin paste and add 25ml. of boiling water. Boil until a clear solution is obtained (5-mins.). This solution should be freshly prepared as required. A more stable starch solution is obtained by adding 0.5gm. of potassium iodide and 2-3 drops of chloroform. A more satisfactory starch solution for use as an indicator is prepared as follows: Mix 5.0 gm. of powdered sodium starch glycollate with 1-2 ml. ethyl alcohol, add 100ml. of cold water and boil for a few minutes with stirring. This 5% stock solution is stable for many months; it is diluted to 0.1% strength when required for use.

30. *Zinc amalgam*

Add about 10 g of granulated zinc to 20 ml mercury, to produce a liquid amalgam on cooling, and heat to 150 degrees with stirring until the zinc is dissolved.

31. *Zinc amalgated (Jones Reductor)*

The zinc is amalgated by immersing it in a solution of mercuric chloride in hydrochloric acid. A quantity of 250 g of 20 mesh zinc is covered with water in a 1 liter flask, and a solution of 11 g of mercuric chloride in 100 ml of hydrochloric acid is poured into the flask.

The system is slowly mixed and shaken for about 2 minutes. The solution is poured off, and the amalgam is cleaned thoroughly with hot tap water, then D.W.

Known and Suspected List of Carcinogens

A

A-alpha-C (2-Amino-9H-pyrido[2,3-b]indole)
 Acetaldehyde
 Acetamide
 Acetochlor
 2-Acetylaminofluorene
 Acifluorfen
 Acrolein
 Acrylamide
 Acrylonitrile
 Actinomycin D
 Adriamycin (Doxorubicin hydrochloride)
 AF-2;[2-(2-furyl)-3-(5-nitro-2-furyl)]acrylamide
 Aflatoxins
 Agaritine
 Alachlor
 Aldrin
 Allyl chloride
 Aallyl isothiocyanate
 Allyl isovalerate
 Aluminium products
 2-Aminoanthraquinone
 p-Aminoazobenzene

o-Aminoazotoluene [solvent yellow 3]
 4-Aminobiphenyl (4-aminodiphenyl)
 3-Amino-9-ethylcarbazole hydrochloride
 1-Amino-2-methylantraquinone
 2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole amino-5-nitrothiazole, 2-Amitrole
 Analgesic mixtures containing phenacetin
 Androgenic (anabolic) steroids
 Aniline
 ortho-Anisidine ortho-Anisidine hydrochloride
 para-anisidine anthanthrene
 Antimony oxide (antimony trioxide)
 Aramite
 Arsenic (inorganic arsenic compounds)
 Asbestos
 Auramine
 Azaserine
 Azathioprine
 Azacitidine
 Azobenzene
 Azathioprine

B

Benz[a]anthracene
 Benzene
 Benzdine [and its salts]
 Benzdine-based dyes
 Benzo[b]fluoranthene
 Benzo[j]fluoranthene
 Benzo[k]fluoranthene
 Benzofuran
 Benzo[a]pyrene

Benzotrichloride
 Benzyl chloride
 Benzyl violet 4B
 Beryllium and beryllium compounds
 Bis(2-chloroethyl)ether
 N,N-Bis(2-chloroethyl)-2-naphthylamine (Chlornapazine)
 Bischloroethyl nitrosourea (BCNU) (Carmustine)

Bis(chloromethyl)ether and technical-grade
chloromethyl methyl ether
Bitumens, extracts of steam-refined and air
refined
Bleomycins
Bracken fern
Bromodichloromethane

C

Cadmium and cadmium compounds
Caffeic acid
Captafol
Captan
Carbazole
Carbon tetrachloride
Carbon-black extracts
Carrageenan, degraded
Ceramic fibers (airborne particles of
respirable size)
Chlorambucil
Chloramphenicol
chlorbenzilat
Chlordane
Chlordecone (Kepone)
Chlordimeform
Chlorendic acid
Chlorinated Paraffins
alpha-Chlorinated toluenes
p-Chloroaniline
Cchlormadinone aceate Chlornaphazine[n,n-
bis(2-chloroethyl)-2-naphthylamine]
Chlorodibromomethane
Chloroethane (ethyl chloride)
1-(2-Chloroethyl)-3-cyclohexyl-1-
nitrosourea (CCNU) (Lomustine)
1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-
1-nitrosourea (Methyl-CCNU)
Chloroform
Chloromethyl methyl ether
3-Chloro-2-methylpropene

Bromoform
1,3-Butadiene
1,4-Butanediol dimethanesulfonate
(Busulfan, myleran)
Butylated hydroxyanisole (BHA)
t-butyl methyl ether
beta-Butyrolactone

4-Chloro-ortho-phenylenediamine
p-Chloro-o-toluidine
Chlorophenols
Chlorophenoxy herbicides
Cloroprene
Chlorothalonil
Chlorozotocin
Chromim
Chromium (hexavalent compounds)
Chrysene
C. I. Acid Red 114
C. I. Basic Red 9 monohydrochloride
Ciclosporin (Cyclosporin A; Cyclosporine)
Cinnamyl anthranilate
Cisplatin
Citrus Red No. 2
Clofibrate
Coal gasification products
Coal-tars and pitches
Cobalt metal powder
Cobalt [II] oxide
Conjugated estrogens
Creosotes
Crystal violet
para-Cresidine
cupferron
cycasin
cyclamates
1,4-cyclohexadiene
cyclophosphamide (anhydrous)
cyclophosphamide (hydrated)

D

D&C Orange No. 17	3,3'-Dichloro-4,4'-diaminodiphenyl ether
D&C Red No. 8	1,1-Dichloroethane
D&C Red No. 9	1,2-Dichloroethane
D&C Red No. 19	Dichloromethane (Methylene chloride)
Dacarbazine	1,2-Dichloropropane
Daminozide	1,3-Dichloropropene
Dantron (Chrysazin; 1,8-	Dieldrin
Dihydroxyanthraquinone)	Dienestrol
dapsone	Diepoxybutane
Daunomycin	Di(2-ethylhexyl)phthalate
DCM	1,2-Diethylhydrazine
DDD (Dichlorodiphenyldichloroethane)	Diethyl sulfate
DDE (Dichlorodiphenyldichloroethylene)	DES, Diethylstilbestrol
DDT (Dichlorodiphenyltrichloroethane)	Diglycidyl resorcinol ether (DGRE)
DDVP (Dichlorvos)	Dihydrosafrole
decabromodiphenyl oxide	Diisopropyl sulfate
N,N'-Diacetylbenzidine	3,3'-Dimethoxybenzidine (ortho-
2,4-Diaminoanisole	Dianisidine)
2,4-Diaminoanisole sulfate	3,3'-Dimethoxybenzidine dihydrochloride
4,4'-Diaminodiphenyl ether (4,4'-	(ortho-dianisidine
Oxydianiline)	dihydrochloride)
2,4-Diaminotoluene	para-Dimethylaminoazobenzene
Diaminotoluene (mixed)	4-Dimethylaminoazobenzene
Dibenz[a,h]acridine	trans-2-[(Dimethylamino)methylimino]-5-
Dibenz[a,j]acridine	[2-(5-nitro-2-furyl)vinyl]-1,3,4 - oxadiazole
Dibenz[a,h]anthracene	7,12-Dimethylbenz(a)anthracene
7H-Dibenzo[c,g]carbazole	3,3'-Dimethylbenzidine (ortho-Tolidine)
Dibenzo[a,e]pyrene	3,3'-Dimethylbenzidine dihydrochloride
Dibenzo[a,h]pyrene	Dimethylcarbonyl chloride
Dibenzo[a,i]pyrene	1,1-Dimethylhydrazine (UDMH)
Dibenzo[a,l]pyrene	1,2-Dimethylhydrazine
1,2-Dibromo-3-chloropropane (DBCP)	Dimethyl sulfate
1,2-Dibromoethane	Dimethylvinyl Chloride
2,3-Dibromo-1-propanol	1,6-Dinitropyrene
p-Dichlorobenzene	1,8-Dinitropyrene
3,3'-Dichlorobenzidine	2,4-Dinitrotoluene
3,3'-Dichlorobenzidine dihydrochloride	2,6-Dinitrotoluene
1,4-Dichloro-2-butene	1,4-Dioxane

Diphenylhydantoin (Phenytoin)
Diphenylhydantoin (Phenytoin), sodium salt
Direct Black 38

Direct Blue 6
Direct Brown 95
Disperse Blue 1

E

Epichlorohydrin
Erionite
Estradiol 17B
Estrogens (not conjugated)

- Estradiol-17
- Estrone
- Ethinylestradiol
- Mestranol

Estrone

Ethinylestradiol
Ethyl acrylate Ethyl methanesulfonate
Ethyl-4,4'-dichlorobenzilate
Ethylene dibromide
Ethylene dichloride (1,2-Dichloroethane)
N-Ethyl-N-nitrosourea
Ethylene oxide
Ethylene thiourea
Ethyleneimine

F

Folpet
Formaldehyde (gas or aqueous solution)
2-(2-Formylhydrazino)-4-(5-nitro-2-furyl) thiazole
Furan
Furazolidone
Furmecyclox
Fusarin C

G

Glasswool fibers (airborne particles of respirable size)
Glu-P-1 (2-Amino-6-methyldipyrdo[1,2- a:3', 2'-d]imidazole)
Glu-P-2 (2-Aminodipyrdo[1,2-a:3',2'-d]imidazole)
Glycidaldehyde
Glycidol
Griseofulvin
Gyromitrin (Acetaldehyde methylformylhydrazone)

H

HC Blue 1
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Hexachlorocyclohexanes
Hexachlorodibenzodioxin

Hexachloroethane
Hexamethylphosphoramide
Hydrazine
Hydrazine sulfate
Hydrazobenzene (1,2-Diphenylhydrazine)
Hydroxybutyric acid lactone

I

Indeno [1,2,3-cd]pyrene
Indium trichloride
IQ (2-Amino-3-methylimidazo[4,5-f]quinoline)
Iron dextran complex
Isosafrole

K

Kepone (Chlordecone)

L

Lactofen
Lasiocarpine
Lead
Lead acetate
Lead phosphate
Lindane and other hexachlorocyclohexane isomers

M

Mancozeb	(MOCA)
Maneb	4,4'-Methylene bis(N,N-dimethyl)benzenamine
Me-A-alpha-C (2-Amino-3-methyl-9H-pyrido[2, 3-b]indole)	4,4'-Methylene bis(2-methylaniline)
Medroxyprogesterone acetate	4,4'-Methylenedianiline
MeIQ(2-Amino-3,4-dimethylimidazo[4,5-f]quinoline)	4,4'-Methylenedianiline dihydrochloride
MeIQx(2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline)	Methylhydrazine and its salts
Melphalan	Methyl chloromethyl ether
Merphalan	Methyl-CCNU
Mestranol	Methyl iodide
2-Methylaziridine (Propyleneimine)	Methyl methanesulfonate
Methylazoxymethanol	2-Methyl-1-nitroanthraquinone
Methylazoxymethanol acetate	N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)
3-Methylcholanthrene	N-Methyl-N-nitrosourea
5-Methylchrysene	N-Methylolacrylamide
4,4'-Methylene bis(2-chloroaniline)	Methylthiouracil
	Metiram

Metronidazole

Michler's ketone Mineral Oils, untreated and mildly treated

Mirex

Mitomycin C

N

Nafenopin

1-Naphthylamine

2-Naphthylamine

3-Naphthylamine

Nickel and certain nickel compounds

Nickel carbonyl

Nickel subsulfide

Niridazole

Nitrilotriacetic acid

Nitrilotriacetic acid, trisodium salt monohydrate

5-Nitroacenaphthene

5-Nitroacenaphthene

5-Nitro-o-anisidine

o-Nitroanisole

4-Nitrobiphenyl

6-Nitrochrysene

Nitrofen

2-Nitrofluorene

Nitrofurazone

1-[(5-Nitrofurfurylidene)amino]-2-imidazolidinone

1-[(5-Nitrofurfurylidene)-N-[4-(5-Nitro-2-furyl)-2 thiazolyl]acetamide

Nitrogen mustard (Mechlorethamine)

Nitrogen mustard hydrochloride

O

Ochratoxin A

Oestrogen, nonstreoidal

Oestrogen, steroidal

Oil Orange SS

MOPP

Monocrotaline

5-(Morpholinomethyl)-3-[(5-nitrofufurylidene)-amino]-2-oxazolidinone

Mustard gas

(Mechlorethamine hydrochloride)

Nitrogen mustard N-oxide

Nitrogen mustard N-oxide hydrochloride

2-Nitropropane

4-Nitropyrene

N-Nitrosodi-n-butylamine

N-Nitrosodiethanolamine

N-Nitrosodiethylamine

N-Nitrosodimethylamine

p-Nitrosodiphenylamine

N-Nitrosodiphenylamine

N-Nitrosodi-n-propylamine

N-Nitroso-N-ethylurea

3-(N-Nitrosomethylamino)propionitrile

4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)

N-Nitrosomethylethylamine

N-Nitroso-N-methylurea

N-Nitroso-N-methylurethane

N-Nitrosomethylvinylamine

N-Nitrosomorpholine

N-Nitrosornicotine

N-Nitrosopiperidine

N-Nitrosopyrrolidine

N-Nitrososarcosine

Norethisterone (Norethindrone)

4,4'-Oxydianiline

Oxadiazon

Oxymetholone

Oxazepam

P

Panfuran S	Polychlorinated dibenzo-p-dioxins
Pentachlorophenol	Polychlorinated dibenzofurans
Phenacetin	Polycyclic aromatic hydrocarbons
Phenazopyridine hydrochloride	Polygeenan
Phenesterin	Ponceau MX
Phenobarbital	Ponceau 3R
Phenoxybenzamine	Potassium bromate
Phenoxybenzamine hydrochloride	Procarbazine
Phenyl glycidyl ether	Procarbazine hydrochloride
Phenylhydrazine and its salts	Procymidone
o-Phenylphenate, sodium	Progesterone
Phenytoin	Progestins
PhiP(2-Amino-1-methyl-6-phenylimidazol[4,5-b]pyridine)	1,3-Propane sultone
Polybrominated biphenyls	Progargite
Polychlorinated biphenyls	beta-Propiolactone
Polychlorinated biphenyls (containing 60 or more percent chlorine by molecular weight)	Propylene oxide
	Propylthiouracil
	Pyridinium chlorochromate

R

Radionuclides	Residual (heavy) fuel oils
Radon	Rhodamine 101

Reserpine

S

Saccharin	Sodium ortho-phenylphenate
Saccharin, sodium	Sterigmatocystin
Safrole	Streptozotocin
Selenium sulfide	Styrene
Shale-oils	Styrene oxide
Silica, crystalline (airborne particles of respirable size)	Sulfallate
Sodium dichromate	Sulphur trioxide
Sodium hexafluoroarsenate(V)	Sulphur trioxide N,N-dimethylformamide complex

T

Talc containing asbestiform fibers
 Terrazole
 Testosterone and its esters
 2,3,7,8-Tetrachlorodibenzo-para-dioxin (TCDD)
 1,1,2,2-Tetrachloroethane
 Tetrachloroethylene (Perchloroethylene)
 p-a,a,a-Tetrachlorotoluene
 3,3',5,5'-tetramethylbenzidine
 Tetranitromethane
 Thioacetamide
 4,4'-Thiodianiline
 Thiourea
 Thorium dioxide
 Tobacco, oral use of smokeless products
 Tobacco smoke
 Toluene diisocyanate
 p-toluenesulphonic acid
 ortho-Toluidine
 ortho-Toluidine hydrochloride
 para-Toluidine
 Toxaphene (Polychlorinated camphenes)
 Treosulfan (Tresoluphan)
 Trichlormethine (Trimustine hydrochloride)
 2,4,6-Trichlorophenol
 1,2,3-Trichloropropane
 Triphenyltin hydroxide
 Trichloroethylene
 Tris(aziridiny)-para-benzoquinone (Triaziquone)
 Tris(1-aziridiny)phosphine sulfide (Thiotepa)
 Tris(2-chloroethyl) phosphate
 Tris(2,3-dibromopropyl)phosphate
 TRIZMA base
 Trp-P-1 (Tryptophan-P-1) (3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole)
 Trp-P-2 (Tryptophan-P-2) (3-Amino-1-methyl-5H-pyrido[4,3-b]indole)
 Trypan blue

U

Uracil mustard

Urethane (Ethyl carbamate)

V

Vinyl bromide

Vinyl chloride

4-Vinyl-1-cyclohexene diepoxide (Vinyl cyclohexene dioxide)

n-vinyl pyrrolidone

Vinyl trichloride (1,1,2-Trichloroethane)

X

2,6-Xylidine (2,6-Dimethylaniline)

Z

Zineb

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