Microbiology Laboratory

Microbiology is the study of microorganisms, which are unicellular and cluster forming entities not visible to the naked eye as individual organisms. The study of microorganisms has become important, especially in foods, due to their ability to cause food spoilage and bring about pathogenic reactions in humans and animals. Controlling the microbial activities contribute to high hygienic standards in food preventing the spread of food borne illnesses especially those spread through food and water. Vast developments in the area of food microbiology have resulted in standardization of test methods and practices in the testing laboratories. The current emphasis in ensuring microbiological safety requires use of accredited laboratories, ensuring reliability of test results for global acceptance. The related titles in this document describe the basic requirements in establishing a microbiology testing laboratory.

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1.Key functions – Introduction

A microbiology laboratory deals with the qualitative and quantitative estimations of microorganisms of interest in a given situation. The interest may arise due to the need to assure the quality of products, the safety in handling and consuming them, probable spoilage a product may undergo and to recognize effective functioning of microorganisms employed in processing (fermenting) food.

The products examined microbiologically may be water (potable and for other uses), foods & feeds, and non-food items. The tests performed in a food microbiology testing laboratory are mostly to examine the following parameters or microorganisms, but are not limited to what is given below.

Total Plate Count or Viable Plate Count
Coliforms and Fecal (thermotolent) coliforms as a group
Escherichia coli
Staphylococcus aureus and their toxins
Salmonella
Listeria monocytogenes
Bacillus cereus
Yeasts & Molds
Pseudomonas aeruginosa in water

The testing may extend to the following microorganisms on specific situations where food are known to be vulnerable or exposed to reservoirs of pathogenic microorganisms such as

Clostridium botulinum
Clostridium perfringens
Vibrio cholera
Vibrio parahaemolyticus
Shigella

In a given situation the need for examination of the microorganisms are judged based on the intrinsic and extrinsic characteristics of the foods, that permit preferential growth of certain microorganisms, processing the food were subjected to, and the historical evidence of exposure to reservoirs of microorganisms. In all microbiological tests culture techniques are used in combination with examination of biochemical color reactions to identify and confirm the presence of microorganisms.

2.Design, development and layout

Design

The general guidelines for design of the chemical laboratories apply to microbiology laboratories (Waitro/LabnetChemfiles/LabnetChemDesign/) too. However, the microbiology laboratory design need special attention due to the nature of the test material handled. The design of a microbiology laboratory needs to pay attention to the following general characteristics of microorganisms. The microorganisms are invisible, they could be present in the air and various surfaces or carried by humans working in the laboratory, and they could grow into large populations, being live and active entities. During testing of microorganisms, the laboratories provide highly conducive conditions for microbial growth. The laboratories therefore need to develop techniques to manage areas of high microbial populations, precautionary controls to prevent release of microorganisms to environment and mechanisms to prevent the entry of microorganisms to aseptically protected areas. The initial design of a microbiology laboratory therefore needs to address physical separation of spaces to carryout functions and meet environmental and other requirements identified in Table 1.

Table 1- Basic physical infrastructure requirements for a microbiological testing laboratory

Identity & space (m^2) [min-max]	Functions	Specific requirements
Testing laboratory [30-50]	Testing of samples	Temperature and humidity controlled; maintain specified microbiological quality of air; used by staff only during testing; floor and bench surfaces cleaned on a daily basis; house testing equipment only.
Media Preparation room [15-25]	Preparation of microbiological media & storage	Meet the conditions specified above for testing laboratory; house media preparation equipment only; may include storage facility for unused microbiological media.
Incubator room [15-25]	House incubators	Temperature maintained below 33 ^{o}C; house only incubators operating at temperatures between 10 ^{o}C to 45 ^{o}C; microbiological quality of air monitored less rigorously.
Reference culture room [10]	Maintain reference cultures	Temperature and humidity controlled; maintain specified microbiological quality of air; used only for reference culture work; floor and bench surfaces cleaned before and after work each time; house lamina hood and a refrigerator.
Decontamination room [15-25]	Washing and decontamination	House only washing and decontamination equipment; cleaned on daily basis; disposal of used

	of glassware	test materials after decontamination on a daily basis.
Reference culture maintenance room [10-15]	Maintain reference cultures	House only a lamina hood used solely for this purpose and a refrigerator to keep the cultures under storage.
Sample receipt room [15-25]	Temporary storage	Conveniently located for public access and away from testing laboratories, with a refrigerator and a freezer.
Tested sample store [15-20]	Temporary storage	Located away from testing facilities, with refrigerator and freezer.
Store room for purchased media [0-10]	Permanent storage	Temperature controlled around 15 ^{o}C; may use cupboards in the media preparation room in small laboratories
Office [15-25]	General administration	Handling all the documents with communication and printing facilities; need to carry safety lockers to store test reports and confidential documents
Microbiologist [15-25]	Office	Room for the Technical manager with visibility to testing laboratories through transparent partitioning
Staff room [20- 35]	Office	Room for staff to rest and carry on duties when not engaged in bench work
Cleaning equipment [5]	Storage	Space to keep cleaning equipment for the laboratories
Changing room [10-15]	Laboratory clothes	On the passage to the testing laboratories
Wash room facilities [15-20]	Personal hygiene	Separate female and male facilities at a ratio of 1 unit per 8 staff members

Development and layout

It is important in developing a laboratory and preparing the layout to recognize the required work capacity of the laboratory, the number of staff engaged in testing, the services (electricity, water, gas) required and the mechanisms to control inadvertent release of microorganisms to the environment as well as cross contaminations. Two layouts for small capacity laboratory and an optimal capacity laboratory are described below.

General guidelines for a small microbiological testing laboratory

where funds and human resource are limiting, it is advisable to establish microbiological laboratories of minimum capacity, with future possibility of expansion, as increased work demand and availability of increased funds. In such situations, the three essential separate works spaces need to be identified, together with an office space. In small laboratories the technical staff .

Let us take a microbiological testing laboratory with a minimal capacity of 70 sq. m. It will have a separate room in the vicinity which allows public access for receiving and storing samples. This room may be adjacent to the laboratory or some distance from it, depending on the general layout of the institution and its areas of public access. The sample receipt room may serve as a common facility for other testing laboratories (chemical etc). The space suggested for each activity in a small laboratory is as follows:

Food testing laboratory	25 sq. m.	
Changing/entry room	10 sq. m.	
Media preparation room	10 sq. m.	
Washing/decontamination room	10 sq. m.	
Office	10 sq. m.	
Interior passage	05 sq. m.	
TOTAL		70 sq. m. (approx)

The layout plan in (Fig. 1) shows an arrangement of the different areas used to carryout functions preventing contamination. There are locations for the main testing laboratory, a media preparation room, glassware washing and an office and other facilities. The design specifications are given below in Fig 1-4.

The planned testing laboratory is 5.5 x 4.5 meters. The media preparation room and the glassware washing room, the office room and changing room need to be separated as indicated in Fig. 1. The guidelines given below indicate the constructions and modifications required to meet the technical standards expected in a testing laboratory.

i. Partition the area as indicated in Fig 1 using aluminium and glass paneling. Alternately, use aluminium or cement brick up to 1.2 meters from the floor and then put glass up to the ceiling. At ceiling level all the panels should fit tightly and be sealed with appropriate material to prevent air leaks that may lead to contaminations.

ii. Construct a workbench projecting into the middle of the laboratory, as indicated in the layout diagram (Fig. 2). The proposed dimensions for the bench are 90 cm (height), 400 cm (length)) and 125 cm (width) with a sink, power outlets and gas outlets as indicated

in Figure 3.

iii. Construct workbenches along the walls of the testing laboratory. One bench is to be located along the north wall, 90 cm (height), 95 cm (width) and 510 cm (length) and the other bench of the same height and width, and length 200 cm to be located along the south wall in the testing laboratory. The locations of the sinks are indicated in Figure 2.

iv. The center writing table in testing laboratory should be 100 cm x 100 cm x 75 cm (height).

v. The workbench in the washing room to be 510 cm (length), 75 cm (width) and 90 cm (height), and the one in the media preparation room to be 510 cm (length), 90 cm (width) and 90 cm (height).

vi. The testing laboratory and the media preparation room need to be air-conditioned, to maintain the temperature at 25 oC and RH (55 + 5)%

vii. The floor of the testing laboratory and the media preparation room should be laid with vinyl material (epoxy resin). Floor tiles are suitable for other areas.

viii. Fit an exhaust fan in the washing room.

ix. All ceilings should be smooth. There should be no ceiling fans in laboratories.

x. All laboratory doors to open outward as indicated in Fig. 1.

xi. The locations for electricity outlets for 5 amps, 15 amps and 30 amps supply, the gas outlets and the location of sinks are indicated in the Fig.3. The sink in the corner of the central workbench in the testing lab to be fixed at 70 cm height for washing hands.

xii. Stainless steel sinks are preferred. In the washing room a sink with a draining board will be more useful. It is preferable to fix two-way or three-way laboratory type taps for the sinks.

xiii. The gas lines should carry two-way or four-way taps. The gas supply tank to be located outside the laboratory protected and fixed with external control valves.

xiv. All exhaust water down pipes from the sinks should be of 50 mm diameter leading to 100 mm diameter horizontal pipes with no bends and opening out of the lab so that they can be cleaned from outside.

xv. All surfaces where dust could accumulate (window panes) and sharp corners in the floor

need to be curved in the testing laboratory and media preparation room.

xvi. The pipelines if laid on the surface of the floor and conduits along the walls in the laboratories, they should be covered with cement to avoid accumulation of dust and dirt. It is preferable to embed them in the walls and floor.

xvii. Storage cupboards under workbenches and above 1.6 meters to be arranged in appropriate locations in the washing room and media preparation room for storage of glassware and chemicals.

viii. The glass windows in the walls to be tinted to prevent direct sunlight entering the laboratory.

xix. Lighting as done in all rooms with double fluorescent bulbs, fixed at the level of ceiling leaving no room for accumulation of dust on their upper surfaces and providing a light intensity of 750 lux.

xx. It is preferable to use enamel paints on the walls

xxi. The locations for equipment in the laboratory are given in Fig. 4.

FIG 1 - LAYOUT PLAN FOR A SMALL FOOD MICROBIOLOGICAL TESTING LABORATORY

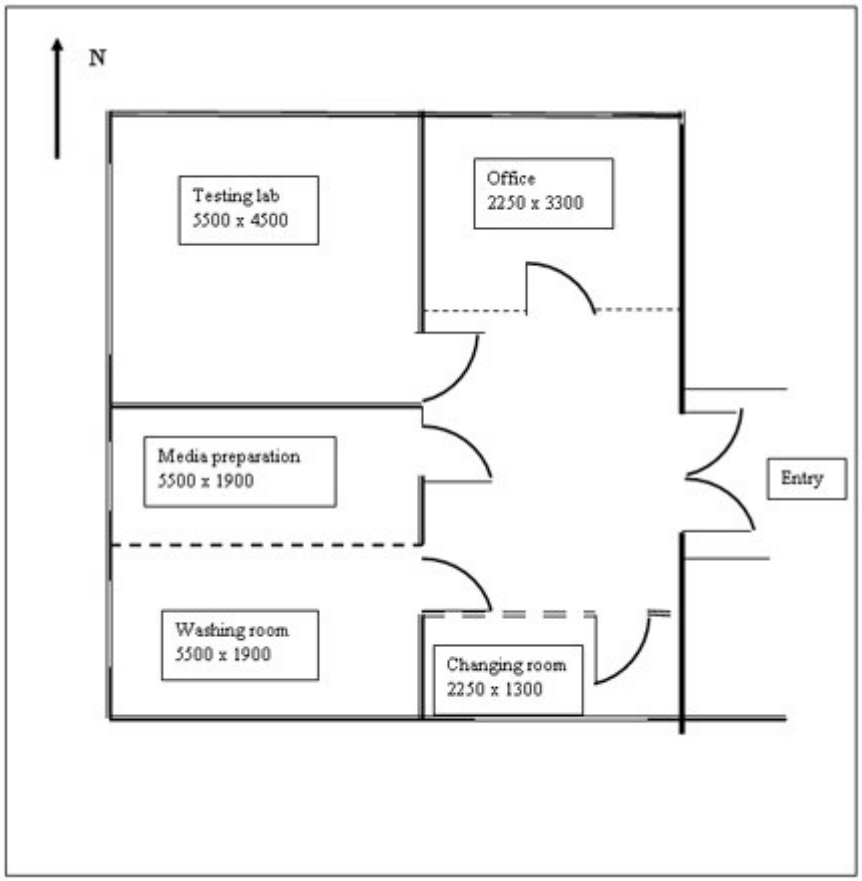

N
Testing lab
5500 x 4500
Office
2250 x 3300
Media preparation
5500 x 1900
Entry
Washing room
5500 x 1900
Changing room
2250 x 1300

FIG 2 - LAYOUT PLAN FOR WORK BENCHES IN A SMALL FOOD MICROBIOLOGICAL TESTING LABORATORY

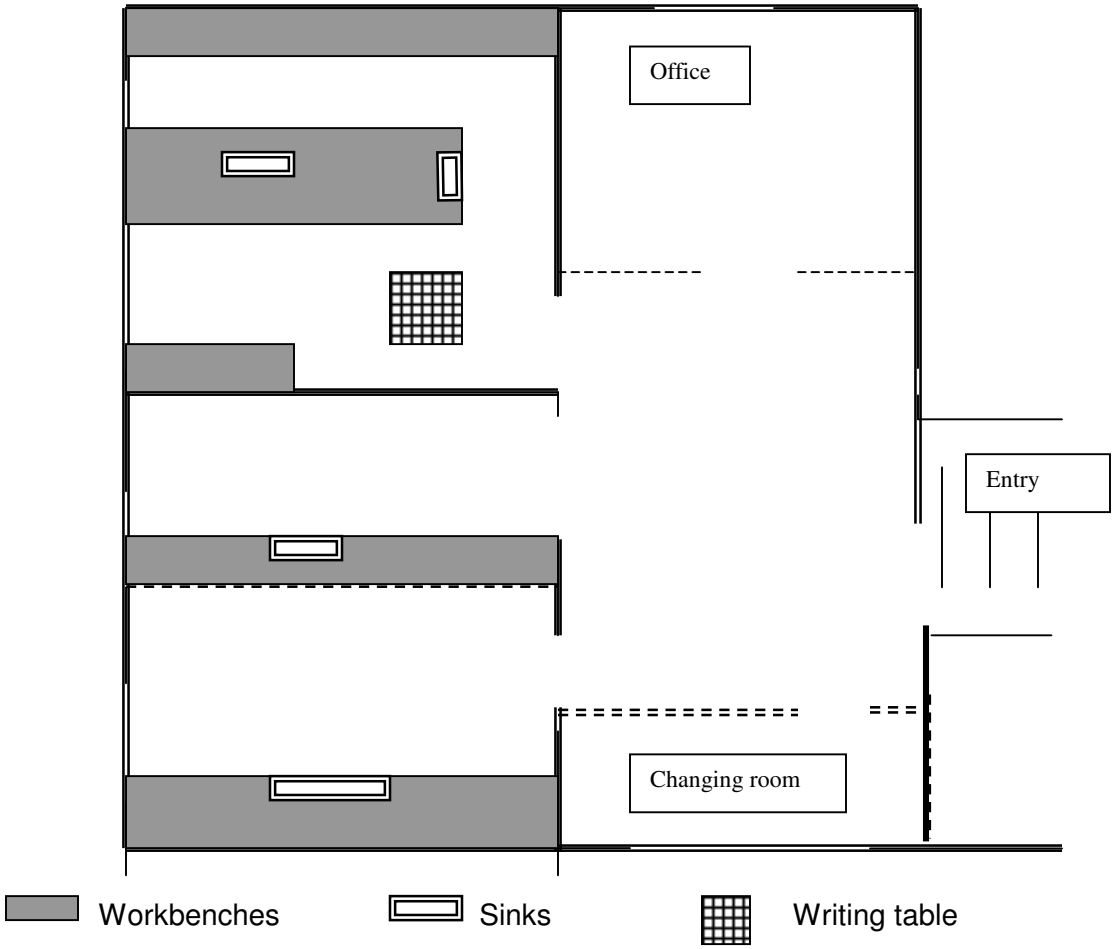


FIG 3 - LAYOUT PLAN FOR ELECTRICITY AND GAS SUPPLIES IN A SMALL FOOD MICROBIOLOGICAL TESTING LABORATORIES

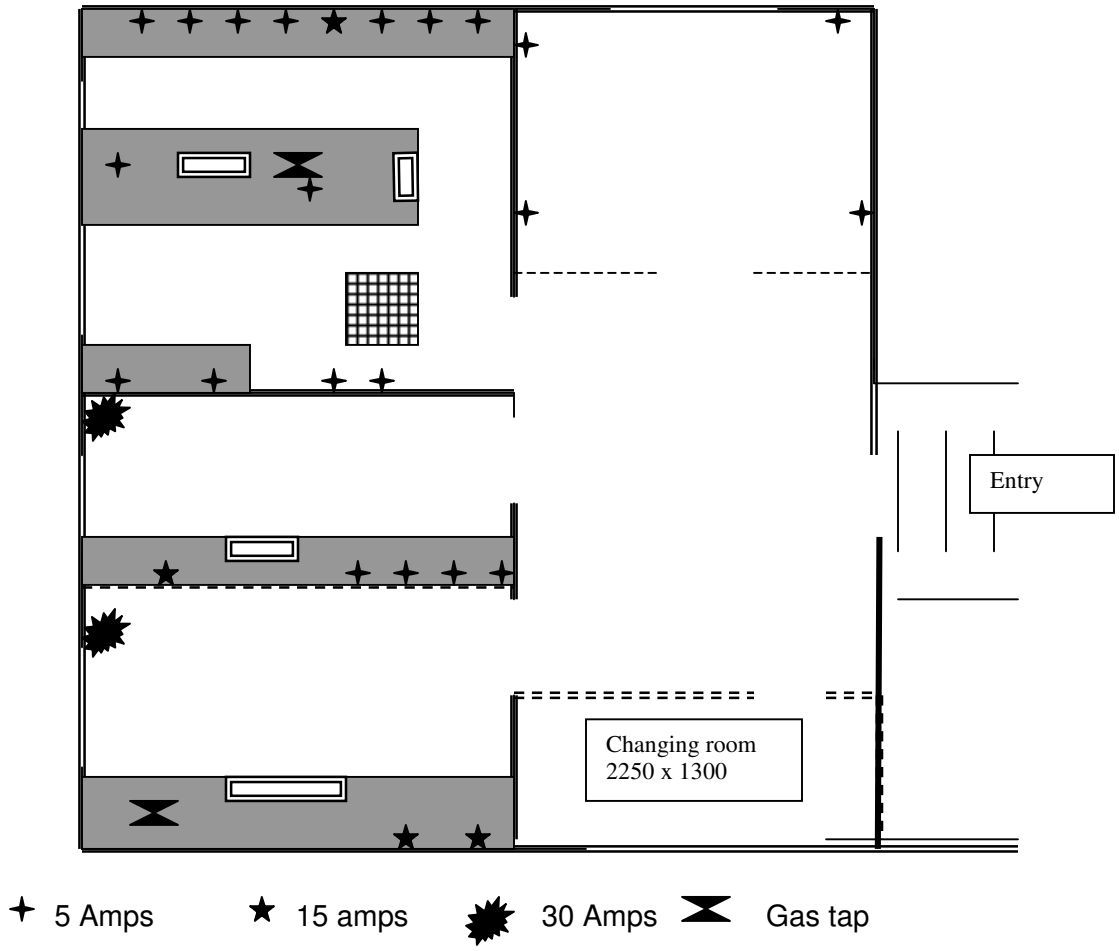
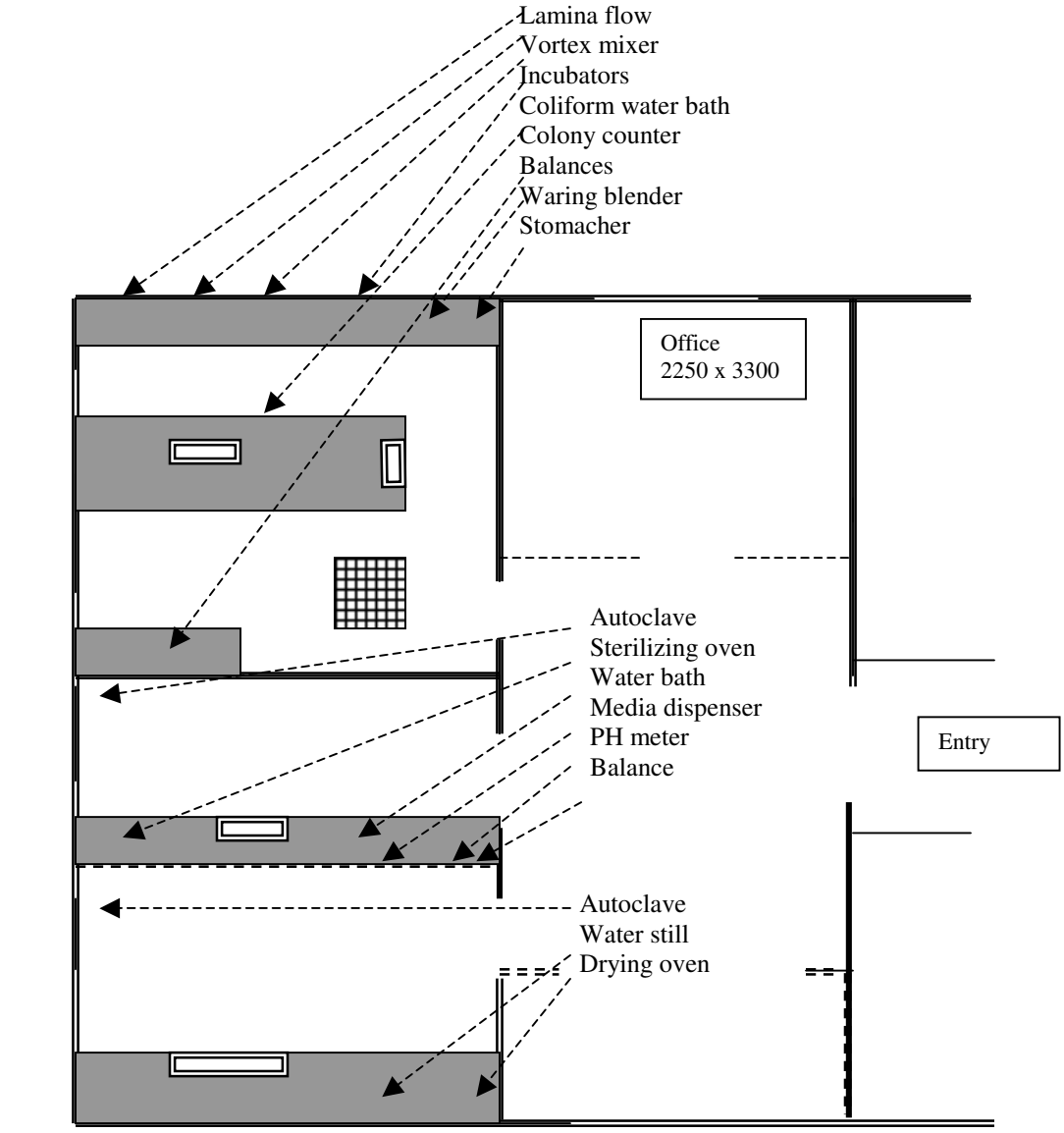


FIG 4 - LAYOUT PLAN FOR EQUIPMENT FOR A SMALL FOOD MICROBIOLOGICAL TESTING LABORATORIES



3. Equipment and Consumables

Purchase of equipment

The quality and performance characteristics of equipment for microbiological testing vary widely. When purchasing equipment it is important to examine the specifications in detail to ensure that they deliver the anticipated quality of test results. A general list of equipment needed to operate a microbiology laboratory successfully is given below. The quantities and capacities of the equipment required depends on the daily work load. The prices quoted are based on bids in December 2007. In costing, appropriate percent increases may be worked out for a given year. When purchasing equipment for the measurement of mass, volume and temperatures, it is important to obtain calibration certificates, and to purchase the reference material needed to carry out regular intermediate checks on the equipment. The equipment list includes certified masses and certified calibration thermometers for intermediate checks.

Suggested list of equipment for a microbiological testing laboratory

No	Specifications	Quantity	Estimated cost US$
1.	Electronic balance, capacity 400g, top loading readability 0.1g, 230V, 50-60Hz. Calibration certificate, 2-year warranty, user manual, installation instruction manual	1	250
2.	Electronic balance, semi-micro analytical 100g, readability 0.001 g, 230V, 50-60Hz. Calibration certificate, 2 year warranty, user manual, installation instruction manual	1	600
3.	Sliding triple beam balance, single pan, capacity 2 kg, readability 1g, user manual, installation instruction manual	1	150
4.	Certified calibration weights 5g and 50g with certificate	1 each	70
5.	Autoclave capacity 60-80 litres, high precision, front loading model, with digital display, microprocessor controlled, together with baskets /shelves, 230V, 50-60Hz. Sterilization indicator tapes 3 rolls Calibration certificate, 2 year warranty, user manual, installation instruction manual	2	22,000 2020
6.	Lamina hood, width 1 metre, with HEPA filter system 230V, 50-60Hz 2 year warranty, user manual, installation instruction manual	1	11,000
	Waring blender two speed complete unit Max 12000 rpm, Calibration certificate, 2 year warranty	1	1700

	User manual, installation instruction manual 1 liter autoclavable jars with lids for above -100 ml autoclavable jars with lids for above	2	300
8.	Stomacher blender, sample capacity 400 - 500 ml, variable speed and time, 230V, 50-60Hz. Calibration certificate, 2 year warranty, user manual, installation instruction manual - Bags for the stomacher (5 packs)	1	5000 50
9.	Coliform water bath, high precision, capacity 10-12 liter, microprocessor controlled, with lid 230V, 50-60Hz. Calibration certificate, 2 year warranty, user manual, installation instruction manual - rubber coated test tube racks for above (10 x 4 holes)	1	1400
10.	Water bath capacity 8-10 liter, with lid, 230V, 50-60Hz. 2 year warranty, user manual, installation instruction manual	1	700
11.	Incubators ambient to 70 oC, microprocessor controlled, capacity 200 liter, 230V, 50-60Hz. Calibration certificate, 2 year warranty, user manual, installation instruction manual	3	9000
12.	Cooling incubators 5 to 30 oC, microprocessor controlled, capacity 200 liter, 230V, 50-60Hz. Calibration certificate, 2 year warranty, user manual, installation instruction manual	2	10000
13.	Sterilizing oven with fan circulated, temp. up to 250 oC, capacity 300 liter, 230V, 50-60Hz. 2 year warranty, user manual, installation instruction manual	1	3000
14.	Glassware drying cabinet Max 120 oC, capacity 300 liter, 230V, 50-60Hz. 2 year warranty, user manual, installation instruction manual	1	3000
15.	Manesty type water distillation unit, 230V, 50-60Hz. 2 year warranty, user manual, installation instruction manual	1	1500
16.	Vortex mixer, 230V, 50-60Hz. 2 year warranty, user manual, installation instruction manual	1	200
17.	Refrigerators capacity 250 liter, 230V, 50-60Hz. 2 year warranty, user manual, installation instruction manual	3	2000
18.	Freezer capacity 300 liter. minimum temperature - 18 oC, 230V, 50-60Hz. 2 year warranty	2	2000
19.	Colony counter with bulb and magnifier lens, 230V, 50-60Hz. user manual, installation instruction manual	1	900
20.	Binocular microscope, [1 unit] Eye piece lenses x10 or x15, Objectives x4, x10,x40, x100, Operated with transmitted light from a bulb, including condenser Include two extra bulbs, graticule to be fixed on eye piece, Stage micrometer 230 V 50 - 60 Hz., user manual, installation instructions, two year warranty	1	1200
21.	Plastic pipette bath external siphon Inner baskets for above	1	200
22.	Pipette jars to be used for chromic acid	1	40

23.	Glass fully automatic water still, Output 4 liter / hr, Electrical heating with overheat cut out. Power supply 230 V 50 - 60 Hz. [1 unit] Also supply: One additional heating element, user manual, installation instructions, Two year warranty Additional heating element and gasket Additional heating vessel	1 1 1	2000 100 50
24.	pH meter working range 0-14 with temperature control and electrodes, 230V, 50-60Hz., user manual, installation instruction manual 2 year warranty - Extra pH electrode for above - Buffer sachets pH 4, 7 and 9 for above	1 2 4sets	800
25.	Manifold filter holders to hold membranes of size 47 mm, three holder unit for filtration under suction with control values to use each port independently. Sulphonated Nalgene or similar material, autoclavable at 15 psi for 30 minutes. Also supply: - Polysulphone filter funnels (autoclavable) with clamps including cap, silicone gasket, No.8 rubber stopper and aluminium clamps to hold 47 mm membrane with upper reservoir capacity of 250 ml [Four packs; each pack containing 4 units] - 47 mm membrane filters Membrane filters for the filter flasks 45 nm, user manual, installation instruction manual 2 year warranty	1 4 5boxes	3000 1000 1000
26.	NAMAS calibration thermometers 0-50 oC, with calibration certificate		150
27.	NAMAS calibration thermometers 100 - 150 oC, with calibration certificate		130
28.	Eppendorf type pipettor adjustable maximum capacity 1000 µl, user manual, 2 year warranty	1	300
29.	Autoclavable pipette tips for 1000 µl pipettor	5 boxes	100
30.	Eppendorf type pipettor adjustable maximum capacity 10 ml, user manual, 2 year warranty	1	300
31.	Autoclavable pipette tips for the 10 ml Eppendorf type pipettor	2 boxes	20
32.	Media dispenser / syringe to deliver 1ml to 10 ml volumes, with autoclavable delivery tubes, manual	1	300
33.	Wet and dry bulb hygrometers	2	60
34.	Maxima-minima thermometer	1	10
35.	Desiccators 250 mm internal diameter, plastic	3	300
36.	Wire baskets, autoclavable, 25 cm X 25 cm [10 No]	10	250
37.	Plastic pipette pumps (pi-pumps), 2 ml	4	50
38.	Plastic pipette pumps (pi-pumps), 10 ml	2	30
39.	Tripod stands	3	20

40.	Wire gauze with asbestos sheet for tripod stands	10	20
41.	Burners to be used with liquid petroleum gas	3	50
42.	Gloves (heat resistant), for use with autoclave	1	20
43.	Thermometers 0 to 100 oC, divisions 1oC	6	90
444.	Thermometers 0 to 100 oC, divisions 0.1oC	3	180
45.	Thermometers 100 - 300 oC,	3	30
46.	Stainless steel spatula, flat ends 200 mm	10	40
47.	Stainless steel scissors, 300 mm	10	150
48.	Stainless steel forceps, 200 mm	10	30
49.	Stainless steel scalpels, 200 mm	10	30
50.	Test tube racks stainless steel for 16 mm x 1/50 tubes (10 x 4 holes)	5	160
51.	Test tube racks stainless steel for 16 mm x 1/50 tubes (12 x 10 holes)	5	150
52.	Tongs with PTFE coated tips 200-250 mm	3	70
53.	Petridish sterilizing cans, aluminium (102 outer diameter)	10	700
54.	Pipette sterilizing cans, aluminium	10	300
55.	Hand lens 100 mm diameter (magnifying glass)	1	10
56.	Handles for inoculating loops for microbiological use, metal	3	20
57.	Nickel chromium wire to make inoculating loops 1 metre length	1	20
58.	Plastic corrosion-resistant filter pump to create vacuum using water tap connection (water jet pump), connection suitable 7 mm internal diameter tubing and water inlet connection 10 mm bore plastic corrosion-resistant filter pump to create vacuum using water tap connection (water jet pump). Connection suitable 7 mm internal diameter tubing and water inlet connection 10 mm bore.	2	90
59.	Safety goggles, clear plastic	3	20
60.	Enamel trays 200 mm x 300 mm	5	100
61.	Haemocytometer	1	40
62.	Anaerobic jars	3	800
63.	Table lamp (angle poise)	1	70
64	Dissecting set	1	30
65.	Timer with alarm, mechanical	1	10

After use, the glassware in microbiology laboratories requires intense decontamination, cleaning and sterilization prior to reuse. The cleaning and preparation of the glassware for reuse may take more than a day. Some of the glassware, such as culture tubes and petri-dishes, may become available for use again only after a week. Adequate stocks of glassware are necessary in a microbiology laboratory for uninterrupted operations. The glassware required for a microbiology laboratory is listed below.

Suggested glassware for a microbiology laboratory

No	Items with specifications	Quantity	Estimated cost US $
1.	Test tubes without rim borosilicate, 20 X 150 mm	500	200
2.	Autoclavable caps for above test tubes	500	400
3.	Test tubes without rim borosilicate, 16 X 150 mm	500	300
4.	Autoclavable caps for above test tubes	500	400
5.	Test tubes without rim borosilicate, 12 X 150 mm	500	150
6.	Autoclavable caps for above test tubes	500	400
7.	Burette borosilicate 50 ml	2	100
8.	Burette stands	2	60
9.	Measuring cylinders graduated, 10 ml	10	50
10.	Measuring cylinders graduated, 100ml	10	50
11.	Measuring cylinders graduated, 500 ml	5	70
12.	Measuring cylinders graduated, 1000 ml	4	100
13.	Petridishes, 15 X 90 mm borosilicate	1000	1800
14.	Universal bottles, 28 ml	200	200
15.	McCartney bottles, 28 ml	200	300
16.	Funnels glass, diameter 100 mm, stem 50 mm	4	20
17.	Buchner flasks, 1 liter	3	100
18.	Beakers, borosilicate / Pyrex, 1000 ml	2	30
19.	Beakers, borosilicate / Pyrex, 500 ml	10	60
20.	Beakers, borosilicate / Pyrex, 250 ml	10	60
21.	Beakers, borosilicate / Pyrex, 2000 ml	3	50
22.	Conical flasks (Erlenmeyer), Pyrex 100 ml	10	60
23.	Conical flasks (Erlenmeyer), Pyrex 500 ml	10	60
24.	Conical flasks (Erlenmeyer), Pyrex 1000 ml	10	80
25.	Conical flasks (Erlenmeyer), Pyrex 2000 ml	5	100
26.	Volumetric flasks, Grade A, 500 ml	3	50
27.	Volumetric flasks, Grade A, 250 ml	3	50
28.	Volumetric flasks, Grade A, 10 ml	3	40

29.	Milk dilution bottles with screw caps, 160 ml	10	60
30.	Pipettes, 1ml with 0.1 ml graduations - Grade A	50	150
31.	Pipettes, 2 ml with 0.1 ml graduations - Grade A	40	100
32.	Pipettes, 5 ml with 0.5 graduations - Grade A	20	100
33.	Pipettes, 10 ml with 1 ml graduations - Grade A	10	40
34.	Glass bottles with polypropylene (autoclavable) screw caps, 500 ml	20	80
35.	Glass bottles with polypropylene (autoclavable) screw caps, 1000 ml	30	150
36.	Glass bottles with polypropylene (autoclavable) screw caps, 2000 ml	10	200
37.	Durham tubes 50 X 7.5 mm	500	150
38.	Cotton wool - non absorbent	2 Kg	30
39.	Weighing boats, plastic 1 ¾"	5 boxes	70
40.	Weighing boats, plastic 3 5/16"	5 boxes	70
41.	Brushes for bottle washing 40 cm	10	20

Microbiological media and reagents

The microbiological media and the reagents required are based on the tests that the laboratory intends to perform. The media required to carry out Total Plate Counts, tests for Coliforms, Salmonella, Staphylococcus aureus and Yeasts & Molds are listed below. Ensure that the purchased media meet the quality requirements to generate reliable results. This may be done by checking the media using standard cultures exhibiting positive and negative growth and anticipated colony appearances and colour reactions. The work load of checking the media could be reduced if quality assurance certificates are obtained from the producers indicating that the media have been tested with relevant cultures. It is also important to address the specified storage conditions and expiry dates of the media.

Suggested reagents and growth media for a microbiological testing laboratory

No	Chemical/microbiological media	Quantity	Estimated cost US$	Tests
1.	Potassium dihydrogen phosphate	2 Kg	20	G
2.	Peptone	500 g	60	G
3.	Rappaport-Validis medium	200 g	60	G
4.	Sodium chloride	1 Kg	10	G/SA
5.	Potassium dichromate (GPR)	1 Kg	30	G
6.	Conc. Sulphuric acid (GPR)	5 Litre	60	G
7.	Cottonwool nonabsorbent	2 Kg	60	G
8.	Sterile sampling bags 200 ml (boxes of 250)	2	180	G
9.	aluminium foil	6 boxes	50	G
10.	Agar	500 g	200	G
11.	Plate count agar	500 g x 2	300	T
12.	Yeast and mold agar	500 g	180	F
13.	Potato dextrose agar	500 g	150	F
14.	Chlortetracycline hydrochloride	5 g	70	F
15.	Tartaric acid	100 g	10	F
16.	Lauryl tryptose broth	500 g	200	F
17.	Brilliant green bile broth 2%	500 g	170	c
18.	EC medium	500 g	180	C
19.	Levines Eosin methylene blue agar	500 g	150	C
20.	Tryptone	200g	80	C
21.	MRVP test reagent	500g	50	C
22.	Koser Citrate medium	500g	140	C
23.	p-dimethyl aminobenzaldehyde (for Kovacs reagent)	100g	50	C
24.	n-Amyl alcohol (for Kovacs reagent)	1 L	50	C
25.	Conc. Hydrochloric acid (for Kovacs reagent)	1 L	60	C
26.	Baird-Parker agar	500 g	180	SA
27.	Trypticase soy broth	500g	50	SA
28.	Sodium pyruvate	100 g	100	SA
29.	Brain heart infusion broth	500g	90	SA
30.	Coagulase plasma	100g	900	SA
31.	Toluidine blue-DNA agar	100g	600	SA
32.	Lysostaphin	10 mg	900	SA

33.	Ethylene diamine tetracetate	500g	20	SA
34.	Mannitol	100g	20	SA
35.	Paraffin oil	100 ml	10	SA
36.	Catalase test reagents	100 g	1500	SA
37.	Lactose broth	500 g	100	S
38.	Brilliant green water	500g	100	S
39.	Trypticase soy broth	500G	140	S
40.	Selenite cystine broth	500g	200	S
41.	Tetrathionate broth	500g	200	S
42.	Bismuth sulphite agar	500g	220	S
43.	Hektoen enteric agar	500g	190	S
44.	Xylose lysine desoxycholate agar	500g	210	S
45.	Triple sugar iron agar	500g	180	S
46.	Lysine iron agar	500g	180	S
47.	Simmons citrate agar	500g	170	S
48.	Urea broth (base)	500g	90	S
49.	Malonate broth (base)	500g	80	S
50.	Lysine desoxycholate broth (base)	100g	220	S
51.	Potassium cyanide broth (base)	100g	200	S
52.	Phenol red	1 g	40	S
53.	Dulcitol	100g	30	S
54.	Glucose	200g	10	S
55.	Lactose	100 g	20	S
56.	MacConkey agar	500 g	140	S
57.	Brain heart infusion broth	200g	120	S
58.	Salmonella polyvalent somatic (o) antigen	1 ampoule	400	S
59.	Salmonella polyvalent somatic (H) antigen	1 ampoule	400	S
Note on use of above microbiological media for tests				
G = General reagents	T = Total Plate Count	F = Fungi (Moulds)		
C = Coliforms/ Fecal coliforms/ E.coli	SA = Staphylococcus aureus	S = Salmonella		

In assessing a microbiology testing laboratory for accreditation against the ISO 17025 International Standard, the records of calibration, maintenance and intermediate checks on the equipment, and the records of preparation of the media and reagents provide valuable evidence of the performance of the laboratory. This information should be maintained well. It is discussed in the section on record keeping.

4. Staff, skills and training

The accreditation of a laboratory is primarily dependent on the competence of the staff to maintain the required work standards. Competence arises through a combination of background knowledge, skills, planned training and continuous practice. The laboratories are expected to maintain staff files providing this information and future staff training plans.

Qualifications and job descriptions of the staff

The qualifications required of the staff members of a microbiology testing laboratory, and their job descriptions, are given in the table below.

Job titles	Qualifications	Job descriptions
Technical manager	(a) Bachelor's degree in microbiology or biological sciences with postgraduate degree in microbiology, together with 5 years of work experience (b) Bachelors degree in Microbiology with 7 years of work experience (c) Biology related degree with 10 years of experience in microbiological testing	1) Administration of laboratory 2) Decision making on all technical matters 3) Interpretation of test results 4) Ability to provide guidance to the industry when required 5) Serve in national committees addressing food safety issues 6) Staff improvement activities
Quality manager	Bachelor's degree in science with training in laboratory quality management	1) Maintaining quality system 2) Arranging internal audits 3) Arranging management review meetings
Analysts	Bachelors degree in Microbiology, Biochemistry, Chemistry or Biology	1) Testing 2) Preparation of media & reagents 3) Managing equipment 4) Record keeping 5) Supervision of

		5) Supervision of house keeping 6) Assisting technical manger in procurement
Attendants	Education up to year 10 with aptitude and liking for laboratory work	1) Moving heavy equipment and test samples 2) Cleaning of laboratory and equipment 3) Assisting analysts
Help staff	Reading and writing knowledge preferably in English (or working language of the laboratory) with work commitment	1) Moving documents inside and outside laboratory 2) Assisting in photocopying and filing 3) Helping in general maintenance work 4) Handling transfer of samples between laboratory and receipt room / stores

External training

Staff need to undergo regular training and have access to new literature if they are to keep up with developments in testing techniques, the modernization of test methods and changing concepts of laboratory quality management. Planning for future training and keeping evidence of training are a part of the improvement process that helps maintain high standards. Each laboratory is expected to document training plans to remove the weaknesses observed in staff performance. The training needs are discussed and finalized at management review meetings of laboratories accredited against the ISO 17025 international standard.

In-house training

Performance in a laboratory is a process of continuous improvement. The engagement of analytical staff on regular intra-laboratory and inter-laboratory testing experiences, the examination of observations from internal audits and complaints, and the generation of preventive actions based on work experiences form an important part of in-house training. These activities also provide the opportunity for the technical and quality manager to identify the external training needs of their staff. Systematic implementation of the quality management system in the laboratory, and vigilance on the part of the quality manager do much to develop in-house training.

5. Proficiency Test

The principles and process of entering into proficiency testing programs is discussed under Chemical Testing (www.WaitroLabnetChemfilesLabnetChemProfTest). The reliability of a laboratory test result depends mainly on a combination of the accuracy of the performance of the equipment, the quality of the reagents and microbiological media and the performance of the analysts. The first of these parameters is observed by calibrating, using and maintaining the equipment, conducting intermediate checks on it, and keeping records of its performance. The quality of reagents and microbiological media are assured through quality control checks on the media, quality assurance certificates and adherence to stipulated storage, handling and discard conditions documented at purchase, and the bench practices. Analyst performance is normally monitored through intra-laboratory comparisons on testing and the performance of individual operations (weighing, delivering and diluting solutions, observing colors and colony counting).

Proficiency testing allows one to see how successful the combined application of the three types of activities has been, especially in the overall competency of the analysts—in general inaccuracies in the performance of equipment are easily detectable within the laboratory since they result in very high deviations from the expected test results. The variations in the performance of individuals (especially in microbiology, which requires high bench skills and measurements based on eye estimations and judgments) play an important role in deciding how close a test result is to the expected “true” value. Proficiency testing examines the latter aspect in recognizing the standard deviation observed for a given test in a laboratory.

In entering into a proficiency testing scheme it is important to select the tests most commonly performed and the food matrices more frequently tested in the laboratory.

Common problems in proficiency testing in microbiology

Microbiological testing deals with living organisms which are continuously growing and highly susceptible to storage temperatures. The temperature and the duration of transport of samples therefore contribute the activity test cultures. This tends to be beyond the control of the laboratories and even of the proficiency testing service providers operating internationally. Participating in a proficiency testing scheme essentially involves the airlift of cultures, customs clearance and performing the tests on the expected day. Delays in the transport and customs clearance of test matrices containing live cultures, difficulties arising from non-adherence to storage temperatures in transport and storage leave can raise doubts about the validity of the test results, especially in the case of quantitative estimations. It is important that the proficiency test service provider and the

laboratory participating in the testing scheme take note of these difficulties, communicate them to each other and design a means to minimize errors that may arise as a result.

Proficiency test results provide the best evidence for an accrediting body on the performance of the laboratories prior to any kind of assessment. However, it must be noted that good results in proficiency testing, which brings in a good rating for the laboratory, does not necessarily mean that the laboratory is meeting the high international performance standards. Judgment on the standard can only be made after close examination of performance at the site of the testing laboratory.

Proficiency testing service providers for microbiology laboratories

United Kingdom- FAPAS/FEPAS -http://www.fapas.com/fepas/cfm/
United States of America - AOAC http://www.aoac.org/proficiencytesting/
Australia - NATA - http://www.nata.asn.au/

6. Reference Materials (CRM)

General concepts and principles guiding the use of reference materials is discussed in the section on chemical testing

The analysts rely on the performance of equipment, the reagents and his/her own performance on a long-term basis in generating reliable test results. However, all these parameters could show variations in their performance over short intervals and continuous discrete variation with time. It is important to eliminate effects arising from such variations on the test results. Use of reference materials gives an opportunity to compare the test results on a daily basis, or even at more frequent intervals, and decide on the acceptability of results. A laboratory may prepare their own reference materials to achieve the anticipated level of homogeneity of the test results. The results from this activity, however, carry limitations arising from the competence of the laboratory in preparing their own reference materials. It is even more difficult in case of microbiology where the main reference materials tend to be microbial cultures, which first needs to be checked against internationally accepted standard microbial cultures performing a wide variety of tests based on biochemical, serological and molecular biological methods.

Reference cultures used more commonly in microbiology testing laboratories

No	Name of microorganism and reference number	Approx cost US$
1	Candida albicans ATCC 10231	500
2	Enterobacter aerogenes ATCC 13048	100
3.	Enterococcus fecalis ATCC 33186.	100
4	Escherichia coli ATCC 8739.	500
5	Pseudomonas aeruginosa ATCC 27853.	100
6	Saccharomyces cerevisiae ATCC 9763	100
7	Salmonella typhimurium ATCC 14028	500
8	Staphylococcus aureus ATCC 25923	100
9	Bacillus cereus ATCC 11778	100
10	Enterobacter cloacae ATCC 13047	100
11	Proteus mirabilis ATCC 29906	100

Source of bacterial reference cultures:

American Type Culture Collectionhttp://www.atcc.org/

Address:

American Type Culture Collection

12301 Parklawn drive

Rockville, Maryland 20852

USA

These cultures are used for two purposes. First, they are used to assess the quality of microbiological media prior to use. For this purpose, certain cultures that grow well in the media (positive) and those do not (negative) are used. This provides a mechanism to assess the selectivity of a given microbiological medium in isolation and identification of the microorganism separated from the matrices under tests. The catalogues of microbiological media suppliers indicate the ATCC cultures they use to establish the characteristics of the media. Secondly, the cultures are used to generate the color reactions which are considered typical. These are then visually compared with the color reactions shown by the test microorganisms. If they correspond this will confirm the test results generated using the microbiological media.

7. Test Methods

Some of the basic points that need to be addressed and the principles involved in selecting test methods are discussed under the section on chemical laboratories.

In selecting methods for microbiological testing, consider the requirements of the clients, the requirements of the regulatory organizations and the suitability of the method for the purpose, assuming there are no other limiting factors such as cost, competency, duration to perform the tests etc.

There are several sources from which to select test methods. In selecting methods, the analysts need to be mindful of the conditions under which the microorganisms are present in the matrix, specially the stresses arising from storage, processing and use of chemicals. These stresses results in debilitation of some enzymes in the microorganisms, resulting in reduced responses to the biochemical tests. This requires regeneration of the microorganisms using enrichment and selective enrichment media. Different test methods described in literature vary in their techniques of enrichment, and this could cause variations in test results. The test methods used generally in medical diagnostic tests for microorganisms may not work effectively with food microorganisms that have undergone stresses.

It is advisable to select well-validated test methods, written by organizations engaged in writing test methods. Among the different sources of test methods the following are usually accepted as more reliable.

Official Methods, Association of Official Analytical Chemists (AOAC)
Microbiology Manual of the Food and Agriculture Organization (FAO)
Bacteriological Analytical Manual (FDA)
American Public Health Association (APHA)
International Standards Organization (ISO)
Even with the methods developed and published by the above sources, the biochemical reactions shown by the microorganisms could vary due to matrix effects. The "Compendium of methods for microbiological examination of foods" provides helpful information for the more meaningful interpretation of test data. Some documents that could provide useful information on test methods are listed below, but there are also other sources among various organizations that provide acceptable test methods.

Suggested books for a microbiology laboratory

(a) Compendium of methods for microbiological examination of foods (seek the most

recent edition),

American Public Health Association
Publication sale, P. O. Box 933019
Atlanta, GA 31193-3019
apha@pbd.com

(b) Lenore Clasceri, Arnold E Greenberg, Andrew W Eaton. Standard Methods for Examination of water and wastewater. 20th edition or more recent edition.

American Public Health Association
Publication sale, P. O. Box 933019
Atlanta, GA 31193-3019
apha@pbd.com
http://www.apha/

(c) FAO manual on Microbiological Analysis, (1995), Nutrition paper 14/4.

(d) Official Methods of Analysis, Association of Official Analytical Chemists [Available in the form of Text Book or Compact Disc]

Address: AOAC International
481, North Federick Avenue
Suite 500
Gaithersburg MD 20877-2417
USA
Email: http://aoac@aoac.com/

(e) H. H. Huss et al. (2003) Assessment and management of Seafood safety and Quality.

FAO Fisheries Technical paper 444 ISBN - 92-5- 104954-8

Some of the important ISO standards for food and water for microbiology laboratories

No	Standard
1.	ISO 6887-1, 1999 Sample preparation
2.	ISO 6222 Total Plate Count for drinking water
3.	ISO 9308-1 Total coliforms, thermotolerant coliforms & E. coli in water
4.	ISO 26461 -2 1993 Sulphite reducing bacteria in water
5.	ISO 12780, 2005 Pseudomonas aeruginosa in water
6.	ISO 7899 Enterococci in water
7.	ISO 1925 Salmonella in water
8.	ISO 4833 Total Plate Count for foods
9.	ISO 4832 Coliforms and thermotolerant bacteria in foods
10.	ISO 6579 Salmonella identification in food
11.	ISO 7973 Clostridium perfringens in food
12.	ISO 6888-2 Staphylococcus aureus identification in foods
13.	ISO 11290-1 Listeria monocytogenes detection in foods
14.	ISO 11290-2 Listeria monocytogenes enumeration in foods
15.	ISO 16649-1 Escherichia coli in foods
16.	ISO 8523 Enterobacteria in foods
17.	ISO 7932 Bacillus cereus in foods

Other test methods

There are other sources that describe non-standard methods, in-house methods and methods associated with equipment. While these are important and are accepted by ISO 17025 International Standard, the laboratories using such methods are required to generate validation information that will satisfy the assessors when they are seeking accreditation. It is much safer for a microbiological testing laboratory to use test methods that are already validated and more commonly practiced.

Competence

Validation guarantees the ruggedness of the methods and provides evidence of their suitability. However, an analyst using a validated method still needs to provide evidence

of his/her competence gained through regular and long practice, including performance checks in intra- and inter-laboratory comparison studies, as well as engaging in proficiency testing.

8. Method Validation

Method validation involves a series of technical activities carried out with careful planning to identify the suitability of a test method for examining an identified matrix. The principles governing method validation are discussed in the section on chemical laboratories (Waitro\LabnetChemfiles\LabnetChemMtdsValida_files). Even a validated method has to be validated if changes were made to the procedure (diluents, test media, reagents, temperatures, or a combination of elements from different methods) or to the matrix. Non-validated methods and in-house methods need to be validated by a laboratory seeking accreditation for such methods. Before applying a testing method, the analyst needs to be confident that it works for the analyte and the matrix. This may take the form using spiked samples and comparing them against controls, blanks and closely related analytes. In microbiological testing, judgment of the identity of a microorganism must be based on all the documented features. For example, if a laboratory is using the morphological and biochemical characteristics of salmonella on Triple Sugar Iron Agar slants, it is important to have agreement on all the four parameters given in the table below to avoid misdiagnosis, since the microorganisms are closely related.

Microorganism	**Slant**	**Butt**	**Gas production**	**Hydrogen sulphide**
Escherichia species	+- <SUB< td>	+	+	-
Shigella species	-	+	-	-
Salmonella typhi	-	+	-	+
Salmonella enteriditis	-	+	+	+

Even the slightest deviation in observations from this validated test could lead to a wrong decision. It is essential to use only validated methods or to follow a method validation that rigorously addresses the criteria discussed in the section on chemical laboratories.

In validating a method it is important to address the following:

Selectivity & specificity
Range of products to which the method is applicable
Linearity
Sensitivity and range
Accuracy & precision
Limit of detection
Limit of quantification

Ruggedness and robustness
Bias
Method validation needs the commitment of a group of laboratories and a group of experts to establish the ruggedness of a test method.

9. Uncertainty of Measurement

The corresponding section under chemical laboratories discusses what analysts need to know about uncertainty measurements and about the principles and basis of estimating uncertainty. This document describes the application of uncertainty measurement in microbiological testing.

When repeated measurements are made on an analyte using an identified test method, there can be a dispersion of measured values attributed to the quantity that is being measured. In making estimations it is possible to think of a "true" value for the analyte in the matrix. Individual estimations however, will not show the "true" value. The difference between the "true" value and the observed value is described as the error in estimation. The uncertainty indicates how big the error might be.

Some of the key features that need to be considered in estimating uncertainty during microbiological analysis are identified in the document European Co-operation for Accreditation EA 4/10 as:

Microbiological tests generally belong to the category of tests that preclude rigorous, metrologically and statistically valid calculation of uncertainty.
It is generally appropriate to base estimation of uncertainty on repeatabilityand reproducibility data alone, but ideally including bias (from PT results).
Some individual components like pipetting, weighing and diluting may be evaluated to demonstrate their negligible contribution to overall uncertainty.
Sample stability and sample preparation cannot be measured directly and evaluated statistically. However, their importance should also be considered.

On the basis of the above, the best approach a microbiological testing laboratory could take is to first identify and document the causes of uncertainty of measurements associated with testing. There are causes among those listed below that cannot be handled in the laboratories with their present level of practice. They need to be understood. The laboratory then needs to take steps to estimate the measurement uncertainty associated with activities that could be controlled and devise a means to minimize these uncertainties.

The more important activities leading to uncertainty of measurement in microbiological testing are,

Size of the original sample from which sub-samples are taken for testing
Sampling and sub-sampling in selection of the required quantity of matrix for testing
Time taken to homogenize the sample with diluents in a blender

Volume of homogenate used for blending
The volume of diluents used in serial dilution of the blended sample
The number of dilutions carried out before subjecting the sample to tests
Variations in incubation temperature and time
Counting of the colonies
Techniques of preparation of microbiological media and reagents
The sterilization temperatures, the time and the total duration of exposure of media to heat

It is possible for an analyst to identify the uncertainties arising from the above and take steps to minimize the degree of uncertainty. The following factors are not used in estimation of uncertainty in microbiological testing as there are no means to address them.

Non-homogenous distribution of analytes in food
Sample preparation and sample stability
Plate readings of less than 100 CFU / ml
Qualitative results [presence / absence]
Methods needing additional step for confirmation
Methods based on counting positive tubes / cells (MPN, micro-titer plates)

Factors that could be used in estimating measurement uncertainty in microbiological testing

The estimation of uncertainty in microbiological measurements is based mainly on the repeatability and reproducibility data generated by intra-laboratory and inter-laboratory testing. Proficiency testing makes a very important contribution to this by establishing the total bias of the results. The following table gives the routine analytical activities carried out in a microbiology laboratory in generating data to in quality management and their relationship to measurement uncertainty through repeatability and reproducibility data.

Analytical activity	**Parameters checked**
Replicates	Repeatability
Reference cultures	Accuracy (repeatability)
Blanks	Interference (detection limits)
Intra-laboratory testing	Reproducibility
Standard cultures	Accuracy, Repeatability, Linearity, Limits of detection
Spiked samples	Recovery, Interference, Repeatability, Range of applicability, Detection limits
Inter-laboratory tests	Reproducibility, Accuracy

Generation of information to establish measurement uncertainty

Information to establish measurement uncertainty could be generated partly along with testing and partly by special activities. Some of the more important information to work on is:

Information on intermediate precision generated continuously along with sample testing
Information from proficiency testing and inter-laboratory comparisons
Data related to accuracy and precision at the individual steps of testing
Data generated from quality control information during testing

The concept of intermediate precision is established in relation to two other levels of precision that may be observed in the performance of a laboratory, namely repeatability precision and reproducibility precision. In microbiological testing, comparison of test results for highly homogenous samples may be carried out by repeatability studies, where estimations are done under closely monitored conditions such as the same analyst working on the same day using the same equipment. This is described as repeatability precision. Reproducibility precision may be achieved when a similar set of samples is tested in different laboratories. These are the two possible and reasonable extremes encountered in testing. The term 'intermediate precision' is used to describe a set of test results generated within a laboratory by different analysts, on different days using different equipment for the same sample. The intermediate precision represents a more realistic analysis situation and the data could be used to form the basis for establishing measurement uncertainty in microbiology.

Uncertainty budget

The final measurement uncertainty in analysis is a combination of uncertainties arising from each step of the testing activities. An approach that could be taken here is to calculate the uncertainty budget for each step so that the values can be pooled and the distribution pattern of values in relation to each analytical activity taken into consideration. The results generated over a period during testing operations could be used to calculate the means and standard deviations in relation to each of the activities. The data could be used to understand the extent of uncertainty and develop practices to minimize its level. Some of the activities that the laboratory could get involved in are:

Cause of uncertainty	**Information to establish uncertainty**
Performance of the balance	Use the data already available from daily check weights for the balance
Inability to weigh exactly the quantity of matrix required for blending due to the nature of the matrix	Use the data already available on the samples weighed for testing
Volume variations due to the non-uniformity of the pipettes	Weigh the volume of water generated by each pipette of the same capacity as used in the laboratory
Volume variations due to techniques of transfer	Repeatedly transfer a set volume and measure its weight
Performance of dispenser used to prepare serial dilutions	Calibration information for the dispenser
Serial dilution techniques	Check repeatability and reproducibility
Plating techniques	Check repeatability and reproducibility
Counting colonies	Check repeatability and reproducibility, repeat counts at 24 and 48 hours

10.General guidelines for a microbiological laboratory of optimal capacity

The suggested space for a microbiological testing laboratory of optimal capacity 130 sq. m. is discussed here. A separate room in the vicinity, allowing public access needs to be identified for sample receipt and storage. It may be adjacent to the microbiology testing laboratory or away depending on the general layout plan of the institution and areas of public access. The sample receipt room may serve as a common facility to other testing laboratories (chemical etc). The spaces suggested for testing activities are as follows.

Food testing laboratory	40 sq. m.
Reference culture room	10 sq. m.
Changing/entry room	10 sq. m.
Media preparation room	10 sq. m.
Washing/decontamination room	10 sq. m.
Office /td>	10 sq. m.
Head	14 sq. m.
Interior passages	16 sq. m.
TOTAL	120 sq. m. (approx)
Sample receipt room	10 sq. m.
GRAND TOTAL	130 sq. m.

Arrangements of the different areas to carryout functions preventing contamination are identified in the layout plan (Fig. 5). The plan identifies locations for the main testing laboratory, media preparation room, glassware washing and decontamination room, culture maintenance room, head's room, staff room/ office and other facilities. The required specifications in designing of the laboratory complex are given below in relation to Fig 5-8.

Set up partitions as in Fig 5, using aluminium and glass panelling. Put aluminium or cement brick up to 1.2 meters from the floor and then glass up to the ceiling. At ceiling level all the panels should fit tightly and be sealed with appropriate material to prevent air leaks that may lead to contaminations.

All doors to be of aluminium /glass, opening outward. The door marked A should have a width of 150 cm and the doors marked B a width of 130 cm to allow large equipment to be carried in. Other doors could be of standard size.

Fix the central workbench in the main laboratory as indicated in the layout diagram. (Fig. 5). There should be cupboards and drawers under the benches.

Construct workbenches along the wall in the glassware washing room, culture maintenance room, media preparation rooms and in the main laboratory. These should be 90 cm (height) x 90 cm (width) and suit the length to the walls as in Fig. 5. They may be

of concrete or wood with push-in cupboards and drawers underneath.
All bench and table tops in the testing laboratory, culture maintenance room, media preparation room and washing room need to be laid with granite, formica or a similar non-porous material.
The testing laboratory, the media preparation room and the culture maintenance room need to be air-conditioned to maintain a continuous temperature of 25 oC and RH (55 + 5)%.
In locations where an uninterrupted power supply is not available day and night, a backup generator with automatic switch gear needs to be used.
The floor of the testing laboratory, the media preparation room and culture maintenance room should be done with epoxy resin. In all other areas, use smooth floor tiles, preferably white and of dimension 30cm x 30 cm.
The locations for electricity outlets for 15 amps and 3-phase supply are indicated in Fig 6. Provide adequate lighting, using fluorescent or low energy bulbs to provide light intensity of 750 lux. The lights should be fixed at ceiling level and not hanging (to avoid collection and release of dust).
Locations of sinks are given in Fig 5. Stainless steel sinks are preferred. In the washing room a sink with a draining board will be more useful. It is preferable to fix two-way or three-way laboratory type taps for the sinks.
The water delivered to the laboratories should be at a minimum pressure of 2.0 bar (Where the pressure of the public supply is low and not continuous, install an overhead tank at 11metre height (equivalent of approximately the 4th floor of a building).
The gas lines should carry two-way or four-way taps. The supply tank should be located outside the laboratory with suitable safety valves and protection from mishandling.
All water exhaust down pipes from the sinks should be of 50 mm diameter leading down to 100 mm diameter horizontal pipes with no bends, and opening directly out of the laboratory so that they can be cleaned from outside.
All surfaces in the testing laboratory, culture room and media preparation room where dust could accumulate (window panes and sharp corners in the floor) need to be made at an angle and curved.
No pipelines and conduits should be laid on the surface of the floor or along the surface of walls in the laboratories as they permit dust and dirt to accumulate and are impossible to clean. They should be embedded.
Storage cupboards for glassware and chemicals should be put up at appropriate places above 1.6 meters on the walls in the testing laboratory, washing room, sampling room and media preparation room (height 100 cm x depth 45 cm).
Tint the glass windows in the outer walls to prevent direct sunlight entering the laboratory
There should be no ceiling fans in the testing laboratories and the ceilings should be smooth.
Extraction fans may be fixed in washing and media preparation room, but kept closed except during operation.
Enamel paints are preferable on the walls since they can be washed.

The proposed layout of equipment is given in Fig. 7.

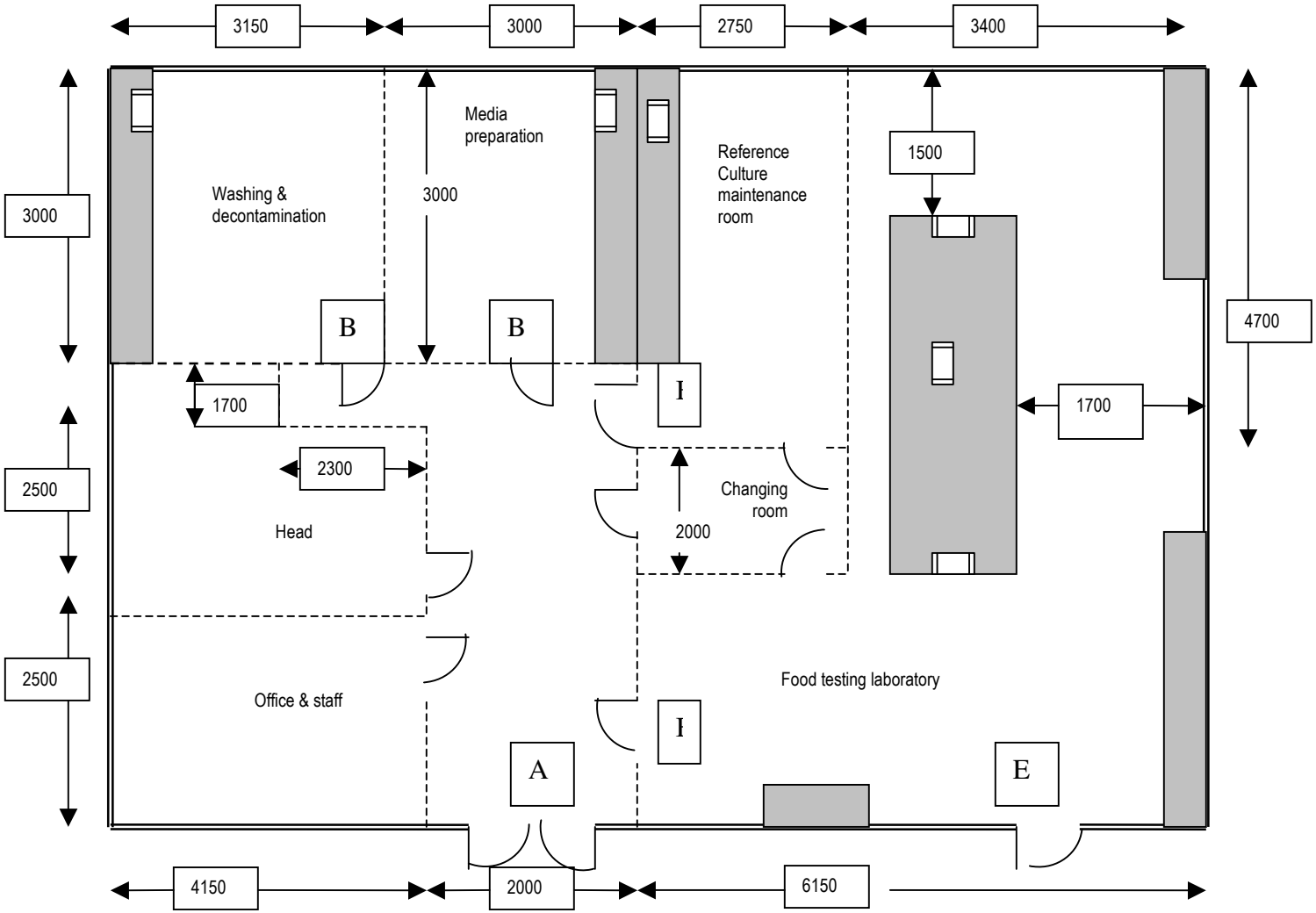


FIG 5- LAYOUT PLAN FOR A MICROBIOLOGICAL LABORATORY OF CAPACITY 130 sq. m

Door width A= 150 cm, B= 130 cm, E =Emergency door with glass panes)

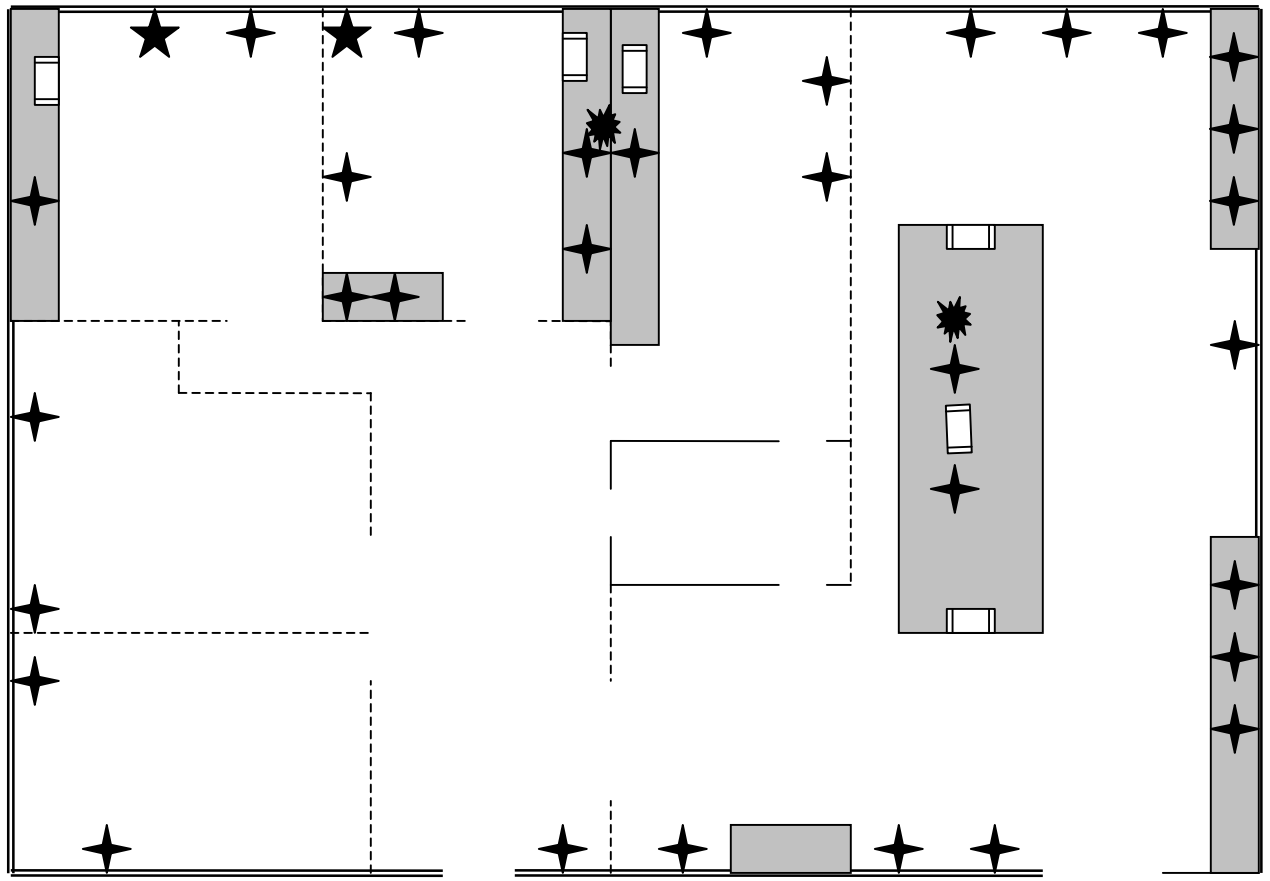


FIG 6 - SERVICE REQUIREMENTS FOR A MICROBIOLOGICAL LABORATORY OF CAPACITY 130 sq. m.

12 Amp; 3 phase power supply; Sinks; Gas;

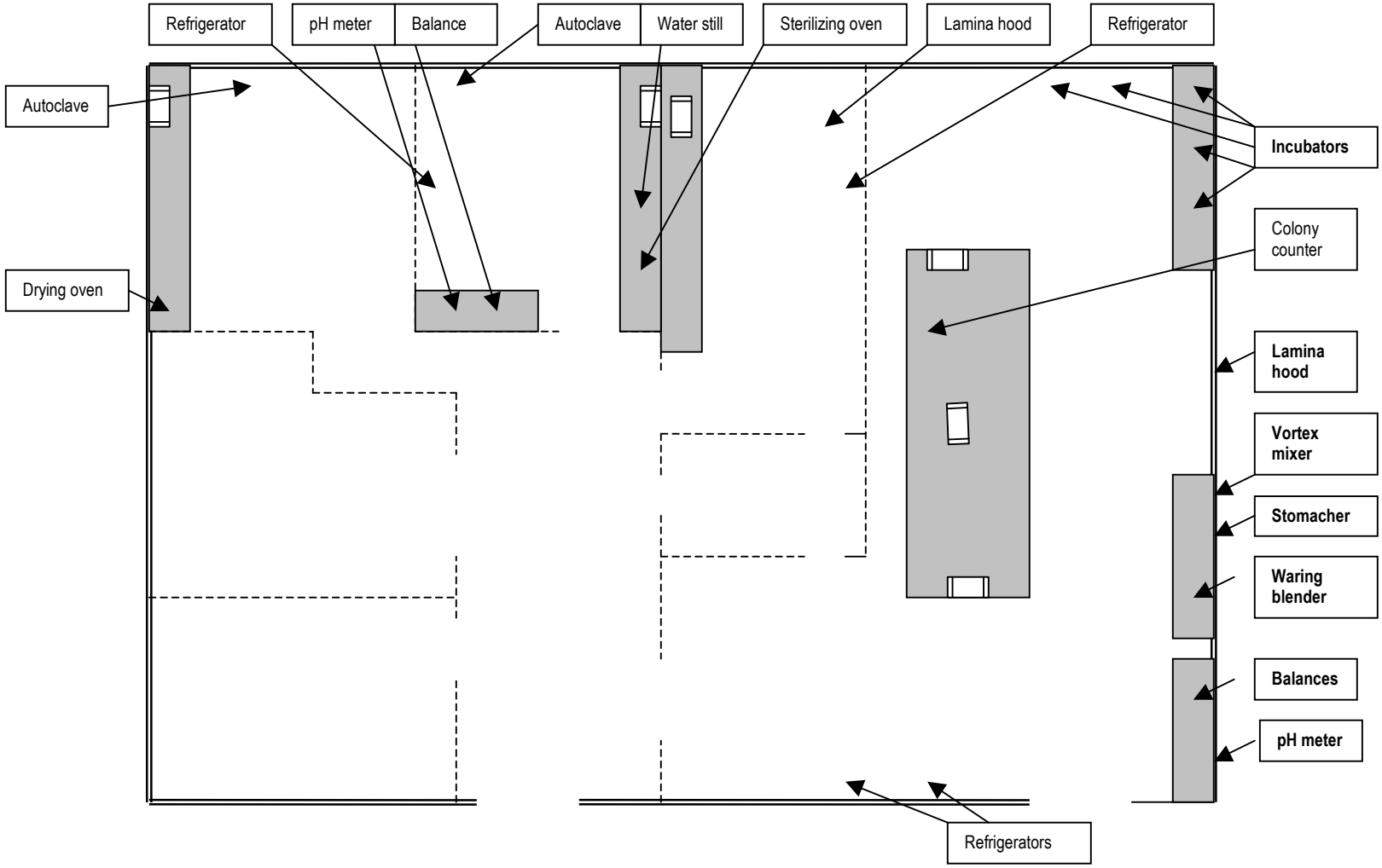


FIG 7- LAYOUT OF EQUIPMENT IN A MICROBIOLOGICAL LABORATORY OF CAPACITY 130 sq. m.

11. Documentation in the microbiology laboratory seeking accreditation

It is essential to establish a quality management system in a laboratory seeking accreditation against the ISO 17025:2005 International Standard. The quality management system brings the top management of the laboratory and its technical activities together through a documentation process and a series of practices. It provides information for the continuous improvement of the laboratory's activities and identifies the responsibilities of individual staff members. The basic procedures behind a good quality management system are:

1) Documentation of methods for all management and technical operations

2) Practice of all operations as documented with no deviations

3) Documentation of the activities in the laboratory during performance

Strict adherence to these three procedures provides the basis for traceability, corrective actions and improvements in the performance of a testing laboratory. Some of the steps that a microbiological testing laboratory needs to take to become accredited are reviewed here. The laboratory is expected to prepare a quality manual and related technical documents (a technical manual) following the guidelines of the International standard. The quality manual needs to clearly identify the policies and procedures needed to implement each element in the management of the laboratory discussed below. For the convenience of the reader, this document uses the same sequenced and numbering system as the international standard.

4.0 Management Requirements

The International Standard 17025:2005 expects laboratories to establish a management system that confirms the commitment of top management and documents procedures that will ensure that the laboratory operates, and continues to improve, according to internationally accepted norms, so that its test results are scientifically valid and universally acceptable. The key elements under management requirements are discussed below.

4.1 Organization

This section of the quality manual is expected to document the legal responsibility of the microbiology laboratory and its role of in relation to other functions of the organization. An organogram will be very helpful. It should document the responsibilities of the staff, including those of the quality manager and the technical manager and indicate how the laboratory will maintain its independence in making technical decisions.

4.2 Quality system

A laboratory quality system consists of documented policies, management systems, programs, tests and other related procedures and instructions for those who manage the delivery of quality test results. The document needs to highlight the chief executive's quality policy on the following:

Management's commitment to good professional practice and quality of testing in the service of clients.
A statement of the standard of service provided by the laboratory.
Objectives of the quality system.
A requirement that all the laboratory staff should familiarize themselves with quality documentation and implement the policies.
A statement that all testing shall use the methods in the manuals and meet the requirements of the clients.
The commitment of the laboratory management to comply with ISO 17025:2005.
The quality manual should have cross-references to the sections in the technical manual.

4.3 Document control

The quality manual, the technical manual, the standard operating procedures, and the formats and forms for keeping records constitute the controlled documents. They have a standard header and are approved by the top manager for use in the laboratory. They cannot be changed without his/her approval. A limited number of the manuals carrying the top manager's stamp are made available to specified persons, and are never photocopied. The manuals provide the guidelines for all the laboratory's managerial and technical procedures and decisions.

4.4 Review of requests, tenders, or contracts

This section address the documented policy and procedures to be used in handling, reviewing and meeting contractual agreements related to clients who obtain services from or provide them to the laboratory. It is meant to eliminate conflicts of interest. Records of

all decisions are retained for future reference and action.

4.5 Subcontracting of tests

A laboratory needs to document its policy and procedures on subcontracting its tests, the conditions of entry into subcontracts and the use of test results from subcontractors in issuing test certificates. It should, in general, subcontract to laboratories of the same accreditation status or similar work standards. Test reports generated from subcontracting should be submitted to clients as separate documents.

4.6 Purchasing services and supplies

The laboratory needs to document its policy and procedures for evaluating the suppliers of services, equipment and consumables in order to maintain its quality standards and ensure the validity of its test results. This includes identifying the warranties, quality assurance certificates, calibration certificates, installation services and periodic checks requested from suppliers as part of purchase agreements, and getting technical clearance before payment is made.

4.7 Services to clients

Accredited laboratories are expected to keep up regular communication with their clients, pay prompt attention to their needs and maintain a good relationship with them. The policy and procedures for providing these services, without infringing on the interests of other clients, need to be documented under this section. The laboratory should make use of individual feedback from its clients, or from regular surveys to improve the performance.

4.8 Complaints

The laboratory needs to prepare an action policy and procedures to record, investigate and act on complaints from its clients or other parties regarding its test results. These procedures need to be documented with forms designed for logging and following up complaints.

4.9 Control of Nonconforming Testing

Evidence of nonconforming testing may appear from monitoring the trends in test results, performance checks on instruments, quality control activities, visual examination of microbiological media, observations of staff during supervision, checking of test certificates, internal or external audits, f inter-laboratory comparisons, proficiency testing, and in many other ways. They may be related to the technical operations or to the operation of the quality system. Procedures for handling such situations should be documented in the quality manual and should include:

Identification of management personnel responsible for taking action when non-conforming work is recognised.
Evaluating the significance of nonconforming work related to test results.
Deciding on the acceptability of nonconforming work.
Taking corrective actions, where necessary.
Recalling certificates and immediately notifying clients, where necessary.
Responsibility for authorizing resumption of work if it was stopped.
Follow up actions to ensure that the nonconformity does not recur.

4.10 Corrective actions

Corrective actions will be required when there is a departure from testing procedures, nonconforming work or weaknesses in the quality system. The policy and procedures to be documented for corrective action include:

Analyse the cause [Start actions to investigate the origin of the problem]
Decide on corrective action [identify and document the proposed corrective actions]
Implement corrective action
Monitor the outcome of the corrective action [Monitor the results to ensure that the corrective action has been effective]
Conduct additional audits [if the nonconformity was serious enough to affect the test results, an additional audit may be carried out to ensure that the system is now operating]

4.11 Preventive actions

Preventive action is a pro-active measure taken when potential problems are identified. The need for them may be recognized when operational procedures are reviewed, or after changes in services purchased, repeatability and reproducibility experiments, equipment performance checks, daily temperature records of equipment, analysis of data for trends, and proficiency test results. Policy and procedures to identify and handle preventive actions need to be documented.

4.12 Control of records

The laboratory is expected to keep coded and filed records of matters related to management and technical requirements. Records related to management requirements include:

Quality records.
Reports from internal audits.
Minutes of management review meetings.
Records of complaints.
Records of corrective actions
Records of preventive actions

Records related to technical activities need to show all the original data, calculations, checking of the calculations, derived data and the format in which the test reports or certificates were produced. The information should be written legibly, with any corrections done by crossing off the original entry and writing the new entry alongside it. The person making the correction should initial it. Most of the entries in the technical records are made during working and not later. They should be identifiable to the specific tasks, persons and test. Records should include:

Workbench record books /sheets
Check sheets
Quality control charts
Certificates
Calibration certificates
Temperature, humidity record charts
Test protocols
Sampling protocols
Media purchase documents with quality assurance certificates
Contract and tender documents
Cleaning records
Equipment files
Staff files

All these records should be coded and kept in the custody of management, especially when individuals resign from the laboratory, so that historical evidence can be provided of the laboratory's performance.

4.13 Internal audits

A laboratory is expected to carry out planned internal audits on a regular basis as a means of identifying areas that need strengthening and corrective actions that need to be taken. These initiatives will generate the continuous improvement that is viewed positively in

the accreditation process.

4.14 Management reviews

Annual management reviews are a proactive source of improvement in a laboratory. The review should include the following in its agenda:

The suitability of policies and procedures documented in the quality and operating manuals
New developments in testing that need to be incorporated into the system
Reports from managerial and supervisory personnel
The outcome of the recent internal audits
Corrective and preventive actions taken
Assessments carried out by external bodies
The results of inter-laboratory comparisons and proficiency testing
Changes in the volume and type of work
Client feedback
Complaints
Future needs of equipment and consumables
Staffing requirements and further training
lEfficiency of the quality control system
It is important that all these items should be included in the review agenda, discussed and decisions and responses recorded.

5.0 Technical Requirements

5.1 General

This section identifies the technical requirements that should be met in a microbiological testing laboratory in order to minimize uncertainties arising during technical activities and to provide traceability. Strict adherence to these requirements contribute to the credibility of the laboratory.

5.2 Personnel

General guidance on the qualifications and job descriptions of staff in a microbiology laboratory is given elsewhere in this document. Employees of a microbiology testing are expected to be medically cleared for color blindness and any characteristics that would

make them carriers of pathogenic microorganisms. Each employee is expected to train continuously so that they will be competent in new microbiology testing methods and concepts. Such training should be planned, documented and recorded.

5.3 Accommodation and environmental conditions

Microbiology testing laboratories are expected to provide physically separate space for testing, media preparation and decontamination functions as minimum preventives of cross contaminations during testing. The laboratory should be designed and activities organized in a way that will minimize the movement and presence of staff in aseptically maintained testing and media preparation areas. The air quality needs to be maintained under constant conditions (25-27 oC and around 60% RH) with regular monitoring on the microbial quality of the air. Records should show that these conditions are being maintained.

5.4 Test methods and method validation

When a laboratory seeks accreditation to carry out a given set of tests that it performs frequently it must demonstrate staff competency in performing them. The laboratory is expected to select test methods which are validated, published and internationally accepted. If modification of these methods is to be acceptable at accreditation, their validation must be shown. In using validated methods, the analysts are expected to demonstrate, at the time of seeking accreditation, competency gained through continuous practice of the methods. Guidance on the sources of microbiological test methods and validation are discussed elsewhere in this document.

5.5 Equipment

A laboratory is expected to contain only calibrated equipment in working order. It should provide an identification number for each item of equipment and maintain a file under this identification number. The file should provide information about the equipment: when it was received, its continuous calibration status, any problems observed, ay repairs carried out and the instructions followed to operate and maintain it.

5.6 Traceability of Measurement

A microbiology testing laboratory is expected to maintain traceability to the international system through calibration of autoclaves, balances, ovens, incubators, water baths,

blenders, volumetric apparatus and thermometers. In addition, intermediate checks should be carried out on the performance of the measuring equipment and documented to provide evidence of continuous traceability.

In microbiological testing standard reference cultures need to be used to test the microbiological media for accuracy of performance, and to establish identity of microorganisms tested through comparison of colour reactions and other colony characteristics. Acquiring quality assurance certificates from the producers at the point of purchase of media helps to avoid detailed testing of media to prove their reliability using positive and negative cultures.

5.7 Sampling

The growth of microorganisms on solid food surfaces is not uniform. The acceptance – rejection criteria for foods contaminated with microorganisms require testing of several samples drawn according to sampling plans. The laboratory needs to maintain documented sampling procedures, persons trained in sampling and evidence of adherence to sampling practices under aseptic conditions if it is to seek accreditation in this area.

5.8 Handling test items

Handling foods for microbiological testing requires special attention if the validity of the sample is to be retained up to the point of testing. This requires using stipulated storage temperatures, testing within 36 hours of sampling and finally disposing of all the test material only after it has been autoclaved and incinerated to avoid public health hazards. A laboratory is expected to establish and document handling procedures to be used throughout the process and to provide documentary evidence of strict adherence to these procedures.

5.9 Assuring quality of test results

The laboratory needs to establish its own quality control procedures to monitor the validity of tests aimed at detecting trends, carry out statistical analysis of test results and compare different test results. Trends should be examined along with observations of the following and any other relevant criteria.

Air quality and water quality monitoring
Results of interlaboratory comparisons and proficiency test results

Results of experiments used to establish repeatability and reproducibility
Use of standard and reference cultures
Performance checks on equipment
Equipment calibrations
Quality of microbiological media used
Any other relevant information

Any information derived from these activities should be used to take preventive and corrective actions, with appropriate records.

5.10 Reporting test results

The laboratory documentation system should be able to provide evidence of traceability from the test reports backward to the origin of the data at the workbench, in the preparation of reagents and media, or in sample handling, although the test report contains only derived data. The test report may contain interpretations requested by the client and signed by the technical manager.

To maintain its accreditation status, a laboratory must adhere strictly to the guidelines of the International Standard, maintaining proper work records and demonstrating its commitment to improvement.