DATA QUALITY REPORT SERIES

MICROBIOLOGY QUALITY ASSURANCE PROGRAMS

1976

Ministry
of the
Environment

The Honourable
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Minister

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MICROBIOLOGY QUALITY ASSURANCE PROGRAMS

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December 1976

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1. INTRODUCTION

Setting up quality assurance programs in a media preparation laboratory presents some unique problems. Little information is available on the problems and pitfalls involved in the preparation and storage of media, and as a result few laboratories implement a strict quality control program. In contrast, such programs are in routine application in chemistry and haematology laboratories where the determinations are quantitative and can usually be analyzed statistically. Facilitating this is the fact that reference standards may be easily prepared or obtained commercially.

Major sources of error in a microbiology laboratory include improper preparation or storage of media, equipment malfunction, inadequate cleansing or sterilization of glassware and impure water supplies. A quality assurance program should strive to ensure reliability and reproducibility of results with minimal effort and expense by monitoring these problem areas.

To implement such a program entails first of all the preparation of a list of procedures, materials and equipment which are to be subject to periodic monitoring. Useful references on this subject are papers by Russell *et al.* (1969), Glasser *et al.* (1971) and Bartlett (1975b). A routine timetable can then be set up in accordance with the priorities specific to the laboratory involved. These priorities should reflect the likelihood of deficiencies based on previous observations, and the relative importance of the variable considered. All observations should be recorded in books or journals as described later in this report. Bartlett (1975b), in his detailed outline of a functional quality control program, has indicated that high priority areas in media surveillance should include sterility checks, pH, storage restrictions and testing with stock cultures. Of medium priority are depth of plates and reagent inventory. Under equipment maintenance, the items of highest priority include refrigerators, incubators, water baths, laminar air flow cabinets, autoclaves and hot air ovens. Bartlett also suggests that surveillance of microscopes, balances and glassware be given less emphasis, but stresses the high priority of maintaining a frequently updated methods manual.

Implementation of the programs must also be monitored. This is most efficiently accomplished by the preparation of a monthly surveillance report as suggested by Bartlett (1975b). Such a report should indicate whether or not surveillance has been conducted as scheduled, whether any deficiencies were observed and the necessary corrective action. Detail can be appended in a separate section or in a supplementary report as required.

Quality control must also extend to personnel, and frequent performance evaluations through the use of simulated water samples or proficiency test specimens are recommended (Cada, 1974; Gray and Lowe, 1976). It is planned that on a monthly basis in the Ministry of Environment Microbiology Section, well mixed water samples will be divided and distributed among the laboratories as part of the routine workload. By comparing the final results from several technicians, we have become aware in initial testing, of minor discrepancies in incubation temperatures, counting methods and dilution preparation. Proficiency testing should be conducted more frequently during the summer months when the sample load is heavier and the temporary personnel less trained.

Another aspect of quality control to be considered is laboratory safety. Employees must be made aware of occupational health and safety hazards involved in microbiology. Adequate precautions should always be taken to prevent the spread of microorganisms: such precautions should include swabbing of bench areas with a broad spectrum disinfectant, the use of plugged pipettes and effective decontamination of contaminated media and glassware. A safety checklist should be drawn up with quarterly inspections and a recording of findings and corrections. An example of a thorough checklist and suggestions for its administration is given in the paper by Blumberg (1975).

In cases where mobile laboratories play a large part in data gathering, quality control programs should be set up to monitor equipment, media sterility and water supplies. Glassware sterility and media performance could be checked beforehand in the central laboratory.

A quality assurance program should serve two primary purposes in any laboratory: first, it should monitor the reliability of the data accumulated or the results reported; secondly, it must control quality according to the reliability requirements. Ideally, all of the variables which may possibly affect the output of a laboratory should be controlled to some extent.

There is considerable disagreement over the extent of quality control measures necessary. Nagel and Kunz (1973) tested 907 lots of 46 different media and found only 17 to be unsatisfactory. They concluded that "... the employment of quality control methodology may even be detrimental to good practice by unnecessarily diverting limited resources of the laboratory to the testing of media previously certified by a responsible manufacturer." Nagel and Kunz (1973) also mentioned the great cost involved in terms of both money and man hours. On the other hand, Barlett (1975a) questioned the cost and found that this was offset by the 10 to 15 percent reduction in workload brought about by controls on overutilization and production of clinically irrelevant information. We have found during the past year in the Media-Taxonomy Laboratory that the time and expenditure involved in monitoring the materials and equipment used in the Microbiological Section have saved many hours spent in other years on test repetition and equipment repairs. We can also have considerably more confidence in the results and data reported.

The quality assurance programs applied in the Microbiology Section's Media and Taxonomy Laboratories are organized into four major areas: media, equipment, glassware and water quality. The following sections give detailed procedural instructions for each of these programs including recommendations for the recording and reviewing of quality control data.

2. MEDIA SURVEILLANCE

2.1 Dehydrated Media

One of the greatest advances in microbiology in the last five decades has been the development of dehydrated media. Replacing the rich "gravies" of the beginning of the century, commercial media combine enormous stability with relative ease of reconstitution. Nevertheless, these qualities depend on strictly controlled maintenance conditions.

Although most commercially prepared culture media have been subjected to quality control procedures by the manufacturers, it is wise to perform additional quality control checks in the laboratory. It is difficult to ensure that each supplier is enforcing strict quality assurance procedures on its products, and often a problem may be due to freezing or deterioration during transit.

In the Media Laboratory the following storage procedures are followed for all media. Bottles of dehydrated media and reagents are immediately dated and labeled on receipt, the label bearing the name and storage conditions. Each type of medium or reagent is assigned an identification number according to whether it is a broth, agar, chemical, stain or pH indicator. The labels also show this identification number and are colour coded according to the above categories. Storage shelves are organized into appropriate sections and the bottles arranged numerically. Media formulation cards list the identification numbers for each component in order to cut down on media problems caused by careless substitution of one component for another.

Most of the dehydrated products are stored at room temperature (approximately 22°C), in a slightly darkened room of low humidity. Certain media, such as Moeller Decarboxylase Broth Base (Difco) and Baird-Parker Medium (Oxoid) are kept at 4°C to prolong the life of heat-labile or highly sensitive substances. Bottles must be kept tightly closed to avoid any water loss which would result in increased concentration of ingredients. New bottles are stored behind older supplies and are not opened until the

previous lot is finished or no longer usable. In general, media can be stored for several years if kept closed in a cool, dry, darkened room. Carbohydrates should not be used if over two years old while most stains and indicators will keep for five years. Moisture uptake can alter the physical, chemical and bacteriological properties of a medium.

After labeling a newly received bottle of medium, it is then observed for colour and texture. This description is recorded in the "Dehydrated Media and Reagents Quality Control Record" (Table 1) along with any additional information on the use, chemical make-up or special precautions for the medium or reagent. Table 1 shows the method of data and supply recording used in the Media Laboratory for all dehydrated media, reagents, stains and pH indicators.

At least once every six months a visual check should be made on all the supplies of dehydrated products. Properties to be checked include hardening of free flowing powder, darkening or change in colour, moisture uptake or any other difference in appearance. All changes should be noted and, in most cases, the medium or chemical discarded. If this contradicts the shelf life estimate, a notation should be made so that future supplies can be monitored more frequently.

2.2 Rehydrated Media

Regardless of the measures taken to protect the dehydrated product, inaccurate weighing or errors in preparation can severely alter the colour, pH, solidity, productivity, selectivity or differential properties of the final prepared product. Therefore, the quality control procedures employed with rehydrated media are generally of a more thorough and time-consuming nature.

Table 1. Dehydrated Media and Reagents Quality Control Record.

Example

Manu-facturer	Ident. No.	Product Information	Shelf Life		Storage	Ordered		Received		Lot No.
			Open	Closed		Date	Amt	Date	Am't	
Difco	208	EC Medium - fine, cream coloured powder - detection of *E. coli* at 44.5 °C either as primary medium or as secondary for confirmation.	1 yr	4-5 yr	22°C	10-5-76 14-9-76	100 g 100 g	14-5-76 20-9-76	100 g 100 g	623924
Difco	47	Inositol - $C_6H_{12}O_6$ - mol. wt. 180.16 - growth factor for microorganisms - anhydrous, non-hygroscopic crystals - m.p. 225-227°C - solubility 14,000 ml H_2O at 25°C.	1 yr	2 yr	22°C			on hand 1-11-75	100 g	472593
BDH	426	Phenol Red - unstable, should be dark reddish- brown in colour - check yellow at pH 6.8 periodically (red at pH 8.4)	1 yr	4-5 yr	22°C	19-1-77	30 g	24-1-77	30 g	9367601

All batches of rehydrated media are subjected to quality control on a daily basis. In general, four plates, tubes or bottles of each type of medium are set aside for the quality control check. If possible, these are taken from the centre of the autoclave load or the last poured of the batch of plates as such samples are more prone to contamination problems. These samples of media are first examined for physical appearance; this includes thickness (of plated agar), excess moisture, lumpiness, precipitates, colour, texture and depth (butt and slant of slanted media). Any problem noted that will or may affect results is discussed with the staff member responsible and the batch replaced if necessary.

Examples of such problems include improper dissolving (and therefore lumpiness of an agar medium), caramelization (browning) of skim milk media, and overly thin plated media. It is recommended that 20 ml of agar medium be dispensed into the round 100 x 15 mm plates, 25 ml into the square 100 x 15 mm plates and 4 ml into the round 50 x 9 or 12 mm plates using paraffin filled standards. The pH is also checked and should be ± 0.2 from that recommended by the manufacturer unless otherwise requested.

Two of the four media samples selected are used to check sterility, with one container of medium being incubated at 35°C and one at 20°C for at least three days. The media are examined daily for any sign of contamination or change in physical characteristics. No readings are taken on weekends; the incubation is simply extended, so that a batch prepared on a Thursday would be observed Friday and Monday. In the case of an occasional surface contaminant, no action is recommended; however, the presence of more than two surface colonies, whether bacterial or fungal, or any evidence of growth within the agar usually necessitates disposal of the batch.

Cloudy or milky media, those containing a precipitate, and dilution blanks or buffers all require special procedures as contamination can often not be checked by visual examination alone. After 48 hours of incubation, at 20°C and 35°C, media such as skim milk broth and Tetrathionate Broth (Difco) are spread-plated onto Trypticase Soy Agar (TSA) (Difco) using 0.1 ml aliquots. These plates are then incubated at the

two temperatures for 24 hours and observed for any sign of contamination.

The volume of dilution blanks is measured and should be within 2% of the required volume. Dilution blanks or buffers are tested on the day of preparation by adding 5 ml aliquots to 5 ml of double strength NIH Broth (Difco) which is incubated at 20°C and 35°C for three days. In each case the control medium is itself checked for sterility using sterile distilled water.

Double or triple strength broths are monitored by adding the appropriate volume of sterile distilled water on day one and incubating as usual, including a control with double strength NIH to check the distilled water.

To help avoid the danger of discovering a problem after the medium has already been used, each new batch is transferred immediately after cooling to a darkened storage room, and held at room temperature (approximately 22°C) for a 24-hour holding period. This provides enough of a delay to check most media for any gross contamination and to perform the usual 24-hour quality control check. It is also recommended that a minimum of two days' notice be requested for smaller media requisitions consisting of less than five types of media or fewer than eight litres, whichever is less and a minimum of five days for larger requisitions.

Quality control data on the rehydrated media are recorded in the format shown in Table 2, using facing pages of a ledger book. Any problems and corresponding action are recorded in red for easier backchecks. When applicable, results of the 24-hour holding period and a check mark or initials indicating delivery should be recorded also.

Although storage of rehydrated media is never completely satisfactory, it is usually unavoidable because of the impracticality of preparing fresh media daily. Very little published information is available on the storage of culture media and any limit on shelf life must be an arbitrary estimate based on experience. Many physical and

Table 2. Prepared Media Quality Control Record

Example

Date	Medium	Lot No.	Lab	pH	20°C			35°C			Spread		NIH		Controls		Comments Problems	Action
					24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	20°C	35°C	20°C	35°C	20°C	35°C		
					20-5	21-5	24-5	20-5	21-5	24-5								
19-5	Skim Milk Broth		Water	6.5	✔	✔		✔	✔		NG	NG						
	Nutrient Agar	362114	G. Lakes	7.3	NG	NG	G	NG	G	G							21-58 surface colonies at 35°C	Replace Batch
	Skim Milk Agar		G. Lakes	6.6	NG	NG	NG	NG	NG	NG							light brown - overheated	Replace Batch
					25-5	26-5	27-5	25-5	26-5	27-5								
24-5	Dilution Blanks (99 ml)		P. Waste	7.2									NG	NG	NG	NG	98, 98.5 ml	
	Tetrathionate	556014	River	7.8	✔	✔		✔	✔		G NG	G NG					< no Iodine < /w Iodine also NG at 41.5° C	No Action
	Trypticase Soy Agar	414223	Tax.	7.3	NG	NG	NG	NG	NG	NG							slightly thin (~18 ml round)	S.T. informed, media usable
					28-5	31-5	1-6	28-5	31-5	1-6								
27-5	Glucose OF	391321	Tax.	6.8	NG	NG	NG	NG	NG	NG							pH 6.8 should be 7.2	Replace Batch
	2 X Trypticase Soy Broth	944321	Tax.	7.4	NG	NG	NG	NG	NG	NG					NG	NG		

NG - no growth G - growth

Figures in hour columns represent dates

✔ - incubated, not possible to read growth by turbidity

chemical changes occur during storage, often due to gaseous exchange and dehydration (Neal, 1973). Frequently, changes begin to take place during the preparation or sterilization of the medium and proceed throughout its shelf life. Large molecule peptides in animal and vegetable protein extracts can form insoluble deposits or haze which may lead to clumping due to the electrostatic forces (Bridson and Brecker, 1970).

Other factors influencing a medium's useful life include its formulation, the storage container, processing treatment, storage temperature, relative humidity, exposure to light and presence of bacterial contamination. The presence of an even moderately stable antibiotic for instance, will drastically shorten the potential storage period. Similarly, plated media are far more subject to gaseous exchanges through the plastic than tubed or bottled media. The optimum storage temperature for most media is about 4°C and, in general, an increase in temperature will result in a proportionate decrease in shelf life. If storage containers are not effectively sealed, an atmosphere of low humidity will soon cause dehydration of the medium. Also, exposure to light may result in a rapid destruction of nutrients and indicators.

Waterworth (1969) has shown that direct sunlight can render plated media bactericidal within 15 minutes, probably due to peroxide production. Storage of chemicals in media refrigerators should be avoided where possible as "their volatility, coldroom humidity and gaseous exchange can cause considerable amounts to accumulate in adjacent stored media and affect their performance" (Neil, 1973). If a medium must be reheated before being used, it should be distributed initially in small amounts to reduce subsequent heating and should be heated only enough to produce a molten medium.

Generally speaking, tubed media may be melted once if they contain no heat labile or heat coagulable ingredients. Overheating carbohydrate media can cause hydrolysis or, in the presence of peptone, darkening due to a Maillard reaction (Martin, 1971). Carbohydrates which cannot withstand autoclaving include lactose, arabinose, sucrose, salicin, xylose, trehalose and maltose. These must be filter-sterilized and

added to the basal medium separately. Any media, if overautoclaved, can form precipitates, caramelize, depolymerize, or show a lowered pH. The medium may become more inhibitory or show a decrease in productivity or selectivity due to a change in dye content.

In general, it is recommended that plated media in plastic bags or sealed cakettes can be kept up to one month providing there is no evidence of contamination or chemical change in this time. Tubed and bottled media, if stored in the dark at 4°C, tightly capped, may be kept stored up to six months. However, after one month of storage, the media should be checked with suitable stock cultures (See Table 3) and also observed for any pH changes or dehydration that may have occurred in this time. Fluid media should be checked before use for any sign of precipitation, colour change or contamination. Tubed agar media should be observed for pH change due to alkali release from the glass, and for sufficient moisture at the slant base.

An equally important phase to the quality control of prepared media is the testing of each lot number or, preferably, each batch of media with stock cultures. The following chart (Table 3) gives general recommendations for the storage and testing of some of the commonly used dehydrated media. The testing with stock cultures should be repeated if the medium is plated and over two weeks old, or tubed and more than two months old (unless a shorter shelf life is indicated). Where possible, alternative test cultures have been suggested. The entire batch of medium should of course be checked for any visible changes whenever it is used.

By following a daily quality assurance program, by ordering small quantities of media and by ensuring stock rotation, many of the more frequent media problems can be eliminated. In the long run this results in savings on both labour and expense. A diagrammatic representation of the various stages in quality control of media is presented in Figure 1.

Figure 1. Media Quality Assurance Program.

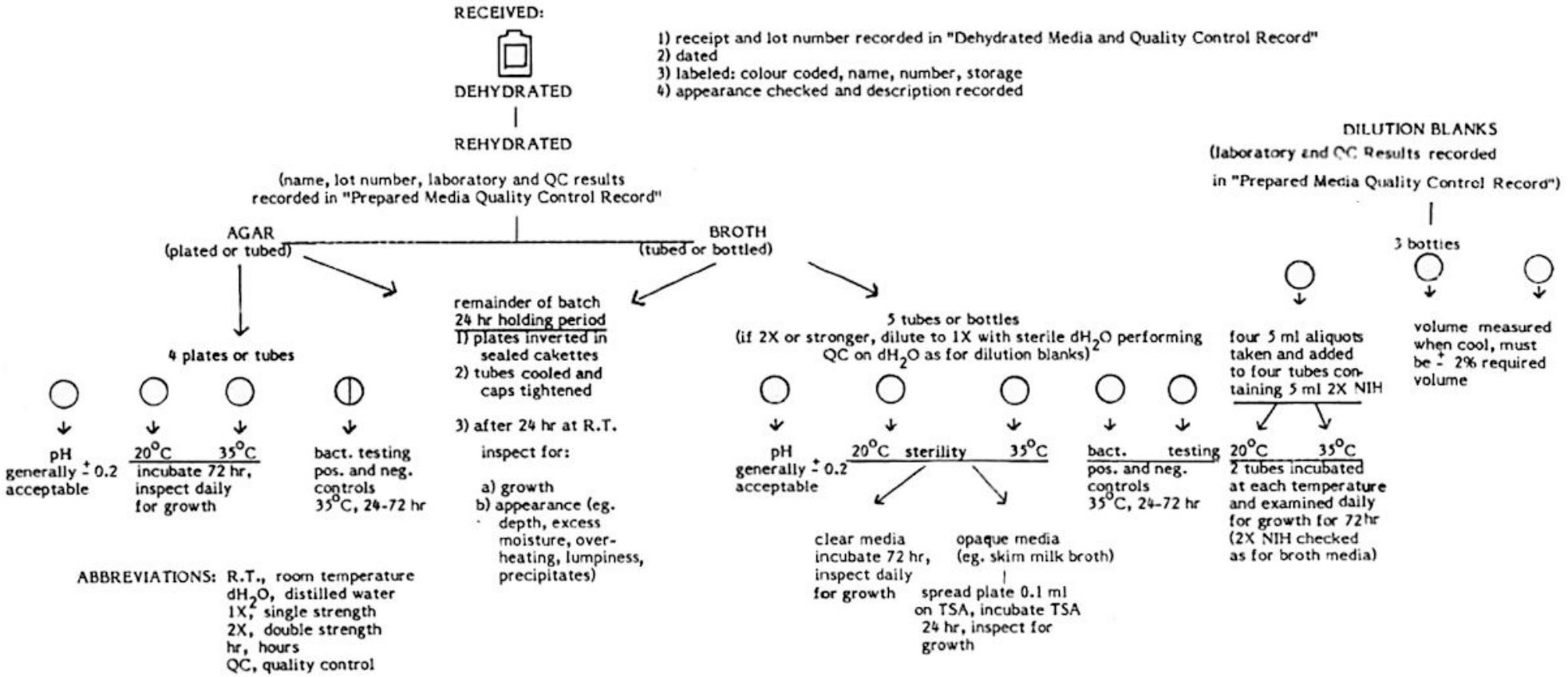


Table 3. Media Storage Recommendations and Stock Culture Testing.

Medium	Storage Recommendations [1] Dehydrated Open	Dehydrated Closed	Rehydrated [2] Tubes	Rehydrated [2] Plated	Testing with Stock Cultures [3] Control Organism	Incubation Temp.	Incubation Time	Acceptable Results	Comments/Precautions
Acetamide Agar			3 mo	1 mo	Ps. aeruginosa	35°C	24-48 h	+ utilization, red	
					E. coli	35	24-48	- no utilization and no growth	
Arginine Medium			3 mo		Strep. faecalis	35	72	+ hydrolysis, red	Ideal incubation 3-7 days
					Micrococcus sp.	35	72	- no colour change	Proteus sp. (alternative -)
Baird Parker Agar	1 mo (store at 2-8° C)	6 mo		see note	Staph. aureus	35	24	+ gray to black with zone of clearing	plated medium may be stored up to one month. Plates should be supplemented with pyruvate no more than two days prior to use
					Staph. epidermidis	35	24	- slight growth no clearing	
Blood Agar (BA)			2 mo	2-3 wk (glass)	Strep. pyogenes	35	24	+ B haemolysis	Sheep blood
					Strep. viridans	35	24	+ haemolysis	Strep. faecalis (alternative +)
					Salm. typhimurium	35	24	- no haemolysis good growth	Serr. marcescens (alternative -)
Brilliant Green Agar (BGA)	1 yr	4-5 yr		1 mo	Salm. typhimurium	41.5	24-48	+ pink growth surrounded by red	Check stored medium for hydrolysis
					E. coli	41.5	24-48	- inhibited or yellow	
					Staph. aureus	41.5	24-48	- no growth	
Christensen's Urea Agar	1 yr (store at 2-8°C)	2 yr	2 mo	2 wk	K. pneumoniae	35	24-48	+ hydrolysis, red to purple	Prot. vulgaris (alternative +)
					E. coli	35	24-48	- no hydrolysis, no colour change	
Decarboxylase Base Broths	1 yr (store	3 yr at 2-8°C)	2 mo		E. coli	35	48-72	A acid (yellow)	
					Alc. faecalis	35	48-72	NC no change, therefore test not applicable	Ideally should be incubated 4 days

Table 3. continued

Medium	Storage Recommendations Dehydrated Open	Dehydrated Closed	Rehydrated Tubes	Rehydrated Plated	Control Organism	Incubation Temp.	Incubation Time	Testing with Stock Cultures Acceptable Results	Comments/Precautions
+ Ornithine	1 yr	3 yr	2 mo		Salm. typhimurium	35°C	48-72 hr	+	Overlay with — 1 cm sterile
					Prot.vulgaris	35	48-72	-	paraffin or mineral oil
+Lysine	1 yr	3 yr	2 mo		Salm. typhimurium	35	48-72	+	Always inoculate a control (base)
					Prot. vulgaris	35	48-72	-	tube to check acid production
+Arginine	1 yr	3 yr	2 mo		Salm. typhimurium	35	48-72		
					Prot.vulgaris	35	48-72	-	
EC Broth	1 yr	4-5 yr	3 mo		E. coli	44.5	24	+ gas from lactose	
					Shigella sp.	44.5	24	- no gas production	
Eosin Methylene Blue Agar	1 yr	4-5 yr		I mo	E. coli	35	24	+ purple colonies with metallic sheen	
					Salm. typhimurium	35	24	- colourless to pink, no sheen	
					Staph. aureus	35	24	- no growth or pinpoint	
Endo Agar	1 yr	4-5 yr		96 hr	E. coli	35	24	+ good growth with green metallic sheen	Cultures may be streaked for isolated colonies
					Salm. typhimurium	35	24	- pink to dark red, no sheen	Protect prepared medium from light
					Staph. aureus	35	24	- no growth or pinpoint	
Enterococcus Agar	6 mo	2 yr		1 mo	Strep. faecalis	35	48	+ red colonies	Cultures should be filtered
					Staph. aureus	35	48	- no growth	as suspensions (FS Method)
Heterotroph Agar (CPS, Foot and Taylor)				2-3 wk	Alc. faecalis	35	48	+ typical growth	
Indole Broth					E. coli	35	24	+ red ring, indole prod'n	
					K. pneumoniae	35	24	- no colour reaction	

Table 3. continued

Medium	Storage Recommendations: Dehydrated Open	Dehydrated Closed	Rehydrated Tubes	Rehydrated Plated	Testing with Stock Cultures: Control Organism	Incubation Temp.	Incubation Time	Acceptable Results	Comments/Precautions
Kligler's Iron Agar(KIA)	1 yr	4-5 yr	1 mo (remelt before use)		E. coli	35°C	24 hr	A/AG(H_2 S-) lactose,glucose --> acid,gas	
					Salm. typhimurium	35	24	K/AG(H2S+) glucose --> acid, gas, H_2S	
					Prot. rettgeri	35	24	K/A(H_2S-) glucose --> acid	
					Alc. faecalis	35	24-	- or K/-(H_2S-) no acid production	
MacConkey Agar	1 yr	5 yr		1 mo	E. coli	35	24	+ lactose --> acid, red colonies	
					Shigella sp.	35	24	- colourless colonies	
					Staph. aureus	35	24	- no growth or pinpoint	
MacConkey Membrane Broth	6 mo	2 yr	1-2 wk (bottled)		E. coli	44.5	24	+ yellow colonies	◗ Acid from lactose
					Shigella sp.	44.5	24	- no growth	Cultures must be filtered as suspensions (FC Method)
Malonate Broth	1 yr	4-5 yr	3 mo		K. pneumoniae	35	24-48	+ blue, alk.,	◗ Malonate utilized
					E. coli	35	24-48	- no colour charge	
Mannitol Salt Agar (MSA)	1 yr	4-5 yr		3-4 wk	Staph. aureus	35	48	+ growth, yellow (acid)	
					E. coli	35	48	- no growth	
M - PA Agar				1 mo	Ps. aeruginosa	41.5	48	+ chocolate brown to black colonies	Cultures must be filtered as suspensions
					Aer. hydrophila	41.5	48	- no growth or atypical colouration	
MR-VP Broth	1 yr	4-5 yr	3 mo		E. coli	35	48	MR +, VP -	Preferred incubation 5 days 30°C
					K. pneumoniae	35	48	MR -, VP +	do not over-heat
					A. calcoaceticus	35	48	MR -, VP -	◗ no acid from glucose
Nitrate Broth			3 mo		Staph. aureus	35	72	+ red, nitrate --> nitrite	Add zinc dust to confirm negatives
					A. calcoaceticus	35	72	- no reduction	preferred incubation 5 days

Table 3. continued

Medium	Storage Recommendations: Dehydrated Open	Dehydrated Closed	Rehydrated Tubes	Rehydrated Plated	Control Organism	Testing with Stock Cultures Incubation Temp.	Incubation Time	Acceptable Results		Comments/Precautions
Nutrient Agar (NA)	1 yr	5 yr	3-4 mo	1 mo	E. coli	35°C	24 hr	+ good growth overnight, typical morphology		
					Strep. faecalis	35	24	+ fair growth overnight, typical morphology		
Nutrient Broth (NB)	1 yr	5 yr	3 mo		E. coli	35	*24*	+ good growth overnight		
					Strep. faecalis	35	24	+ fair growth overnight		
Nutrient Gelatin				1 mo (glass if acid indicator)	Ps. aeruginosa	35	24	+ liquefaction = clearing		Saturated $(NH_4)2SO_4$ as reagent
					E. coli	35	24	- growth but no clearing		
ONPG Broth			1 mo		E. coli	35	24	+ o-nitrophenol = yellow		Due to β-galactosidase protect from light
					Prot.mirabilis	35	24	- no β-galactosidase activity		
OF Medium with Glucose	1 yr	3 yr	2 mo		E. coli	35	48	F fermentation (anaerobic)		Ideally should be examined daily for I week
					Ps. aeruginosa	35	48	O oxidation (aerobic)		
					Alc. faecalis	35	48	- no acid production		
Phenol Red +Glucose Broths	1 yr	3 yr	*2* mo		E. coli	35	24	+ acid(yellow)		
					Alc. faecalis	35	24	- no acid production		
+ Lactose					E. coli	35	24	+	or	K. pneumoniae
					Alc. faecalis	35	24	-	or	Proteus sp.
+ Arabinose					E. coli	35	24	+	or	Citrobacter sp.
					Alc. faecalis	35	24	-	or	Proteussp.
+ Maltose					E. coli	35	24	+	or	Prot. vulgaris
					Alc. faecalis	35	24	-	or	Prot. mirabilis
Phenylalanine Agar	1 yr	3 yr	3 mo		Prot. vulgaris	35	24	+ greening with $FeCl_3$		
					E. coli	35	24	- no deamination		

Table 3. continued

Medium	Storage Recommendations				Testing with Stock Cultures				
	Dehydrated		Rehydrated		Control	Incubation		Acceptable Results	Comments/Precautions
	Open	Closed	Tubes	Plated	Organism	Temp.	Time		
Selenite Broth	1 yr	3 yr	72 hr		Salm. typhimurium	41.5°C	24 hr	+ good growth	
SIM Medium	1 yr	3 yr	2 mo		Prot. mirabilis	35	24-48	H_2S = +, Ind = -, Mot = +	
					K. pneumoniae	35	24-48	H_2S = -, Ind = +, Mot = +	
					Enterobacter cloacae	35	24-48	H_2S = -, Ind = -, Mot = +	
					A. calcoaceticus	35	24-48	H_2S = -, Ind = -, Mot = -	
Simmons Citrate	I yr	4 yr	3 mo	3-4 wk	K. pneumoniae	35	24	+ blue, aik., utilization	
					E. coli	35	24	- no growth	
Skim Milk Agar	1 yr	4 yr		1 mo	Ps. aeruginosa	35	24	+ caseinase clearing, pigment production, fluorescence	
					A. calcoaceticus	35	24	- good growth, no reaction or pigment	
Skim Milk Broth	1 yr	4 yr	1 mo		Cl. perfringens	35	24-48	+ acid, gas, clot	
					E. coli	35	24-48	- no visible reaction	
Tetrathionate Broth	6 mo	2 yr	(bottled) none		Salm. typhimurium	41.5	24	+ good growth (0.1 ml --> TSA)	Must be used same day. Testing of each lot number is only practical solution. Iodine sol'n added before incubation.
Trypticase Soy Agar	I yr	4-5 yr	3-4 mo	1 mu	E. coli	35	24	+ good growth, typical morphology	
(TSA)	(Store at 2-8°C)				Strep. faecalis	35	24	+ good growth, typical morphology	
Trypticase Soy	1 yr	4-5 yr	3 mo		E. coli	35	24	+ good growth	
Broth (TSB)					Strep. faecalis	35	24	+ good growth	

Table 3. continued

Medium	Storage Recommendations				Control Organism	Testing with Stock Cultures Incubation		Analytical Results	Comments / Precautions
	Dehydrated		Rehydrated						
	Open	Closed	Tubes	Plated		Temp.	Time		
Triple Sugar Iron Agar (TSI)	1 yr	4-5 yr	1 mo (remelt before use)		E. coli	35°C	24 hr	A/AG(H_2S-)	Lactose and/or sucrose, glucose —> acid, gas
					Salm. typhimurium	35	24	K/AG(H_2S+)	glucose --> acid, gas, H_2S
					Prot. rettgeri	35	24	K/A(H_2S)	glucose –> acid
					Alc. faecalis	35	24-	- or K/-(H^2S-)	no acid production from lactose, sucrose, glucose
XLD Agar	1 yr	3 yr		2 wk	Salm. typhimurium	35	24	+	black colonies or black centre
					Shig. flexneri	35	24	+	red colonies
					Staph. aureus	35	24	-	no growth or pinpoint

[1] These recommendations have been compiled from several useful reference papers and general experience. Literature which may be consulted for more details include those papers by Barry and Feeney (1967), Bridson (1969), Martin (1971) and Bartlett (1975b).

[2] Storage recommendations for the rehydrated media assume that tubes and bottles are tightly screw capped and that plated media are stored in cakettes or plastic bags. Tubed media with metal caps may be stored approximately half as long as screw capped media. Unwrapped plates have been observed to lose up to 7% of their moisture (by weight) per week at 4°C, while storage in polystyrene containers can reduce this loss to 0.5% per week (Bartlett, 1975a).

[3] Many of the suggestions for stock culture testing are from Difco (1974).

2.3 Reagents and Chemicals

All chemicals are dated, numbered and stored as discussed in section 2.l. General information and stock records are kept in the "Media and Reagents Quality Control Record" described in section 2.1. Precautions and descriptions such as poison, caustic, volatile, carcinogen, etc. are also noted and the bottle labeled accordingly. In general, most stains and indicators can be kept for up to five years if kept cool, dry and protected from light; however, these and all chemicals should be inspected monthly and the appearance checked against the original description recorded. Some of the more commonly used chemicals with special precautions or storage recommendations are noted in Table 4.

Solutions of chemicals and stains should always be dated and initialed when prepared. Control cultures should be stained whenever the staining reagents are used or at least weekly. After six months, any remaining stock solutions should be discarded. Reagents such as ferric chloride and hydrogen peroxide, used with specific biochemical tests, should be dated and replaced monthly with a weekly inspection. All reagents should be checked with stock cultures before use, and the data recorded in the Prepared Media Quality Control Record.

2.4 Antibiotics

Antibiotics and antimicrobial agents are treated on a completely individual basis and few general recommendations can be made. A quality control record is maintained with a separate page for each of the 34 antimicrobial agents used in the Microbiology Section. Information entered includes the name and address of the manufacturer, date received, amount, lot number, expiry date and any comments or precautions. Most of the antibiotics are kept at 4°C in a sealed glass container with a desiccant. When the lot number is expired or depleted, a line is drawn through the entry. Information on the purity and any alternative names are also recorded. The appearance and texture of the antibiotic are recorded and checked with each use. An alphabetical index is kept at the front of the journal.

Table 4. Quality Control of Chemicals

Chemical	Storage and/or Precautions
Acetic acid, glacial	danger: avoid contact
N-Acetyl-l-cystein	store at 2-8°C
p-Aminodimethylaniline oxalate	keep dry
Barium chloride	dangerous to handle
Benzidine dihydrochloride	keeps 6 months prepared; carcinogen
Calcium chloride	keep cool and dry
Cysteine hydrochloride	keep cool and dry
p-Dimethylaminobenzaldehyde	protect from light
Ether	danger: do not inhale, volatile
Ethyl alcohol	keeps 3 months opened, keep dry
Ferric ammonium citrate	protect from light
Ferric chloride	protect from light
Glycerine	danger: avoid contact
Hydrochloric acid (conc.)	danger: avoid contact, do not inhale
Hydrogen peroxide (3%)	protect from light, keep cool, caustic
Magnesium sulfate	keep dry
Mercuric chloride	danger: poison
Naphthol	protect from light
Naphthylamine	danger: carcinogen
Oxgall	keeps 1 year only, keep cool and dry
Phenol (crystals)	keep dry, protect from light, avoid contact
Phenolphthalein diphosphate	danger: avoid contact
Potassium hydroxide	danger: caustic
Potassium permanganate	keep dry
Sodium carbonate	keep dry, protect from light, avoid contact, poison
Sodium hydroxide	danger: caustic
Sulfsalicylic acid	keep dry, protect from light
Tannic acid	protect from light
N,N,N,N,-Tetra methyl-p-phenylene diamine monohydrochloride	danger: poison, avoid contact
Zinc dust	danger: do not inhale

3. EQUIPMENT AND SUPPLIES QUALITY ASSURANCE

When choosing laboratory apparatus, one must look for reliability, quality, sensitivity and minimum servicing requirements. Operation manuals must be readily available for reference purposes and should be thoroughly read and understood by the personnel involved. A routine maintenance schedule should be set up for each piece of equipment according to the manufacturer's recommendations. Table 5 is meant as a general guide and should be adapted to the particular requirements of the individual manufacturers. Table 6 discusses pH maintenance and determination problems in greater detail as electrodes require daily maintenance.

The "Equipment Maintenance Journal" should list all items along with date of receipt, manufacturer and serial number. Weekly, monthly and yearly maintenance checks can be kept in chart form and the item checked off or initialed when completed. Any servicing or equipment failures should also be recorded and dated.

3.11 Membrane Filters

Unlike supplies of media, filters used in membrane filtration should be ordered in large quantities of a single lot number. Quality control procedures are generally on a lot number basis and are still being developed at the Ministry of the Environment. Presently, each lot number is checked for pH and chemical residuals by breaking and immersing filters in sterile distilled water at a ratio of one filter to four ml water. After one hour of soaking, the supernatant is tested in the same manner as the distilled and deionized water supplies to be discussed in Section 5. A control sample of sterile distilled water in a similar glass vessel is also tested. Filters are also checked for hydrophobic areas and any visible imperfections.

Data from the first six months of testing indicate that pH limits of 5.5 to 7.5 should be maintained, and an upper limit for conductivity of 250 µmhos/cm.

Table 5. Equipment Maintenance Schedule.

Equipment	Daily	Weekly	Monthly	Yearly	Comments
3.1 Water Baths	1) inspect time and temperature record, date daily	1) change recording chart	1) change water using distilled water		1) temperature variation should be kept at - 0.5°C. Some water baths may be capable of temperature control within ± 0.2°C
			2) if necessary due to contamination, slime or fungal growth, allow 24 hr contact with water containing 15 ml Javex per 4L water		2) use stainless steel or plastic coated racks to avoid metal corrosion
3.2 Drying Ovens	1) check temperature; should be 170 ± 5°C	1) clean interior and exterior with mild detergent			1) spore strips may be substituted as a sterility check
	2) record each run's contents, time in and out, initials				
3.3 Autoclaves	1) dated temperature and time records for each cycle	1) use Steam-Clox to monitor time and temperature	1) oil hinge pin with heavy machine oil (SAE 20 or 30 motor oil)	1) quarterly: grease door post grease fitting with high temperature white grease (e.g. Jet-lube AP-1W)	1) keep daily records for at least six months before discarding
	2) list of materials autoclaved with time in and out, technicians' initials	2) remove chamber, drain strainer and clean out lint	2) inspect door gasket, if brittle or cracked, replace		2) spore suspensions may be used instead of Steam-Clox
	3) heat sensitive tape on each basket, glass-ware item or apparatus	3) rinse drain with hot trisodium phosphate (13 g/L), flush with tap water five minutes later			3) with steam/gas sterilizer replace gas cylinder when necessary

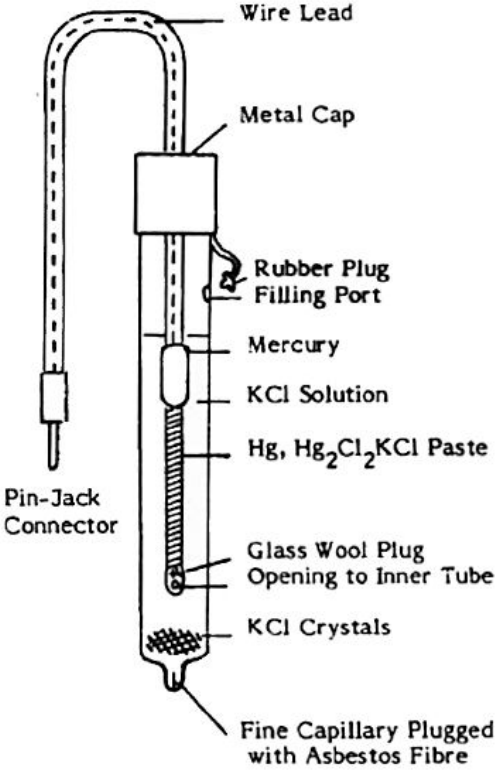
Table 5. continued

Equipment	Daily	Weekly	Monthly	Yearly	Comments
	4) check water level	4) wash inside of sterilizing chamber with a mild detergent, rinse and dry			4) if water backs up into chamber after rinsing and flushing, close door, set operating valve wheel to ster., at 10 psig advance to fast exhaust, when pressure =0, turn to off and open door; replace strainer
3.4 Balances	1) cleaning of exterior and pan	1) check that balance is level	1) calibration with weights	1) serviceman to adjust balance, clean and any necessary repair work	1) keep analytical balance free from dust, vibrations and wind currents
3.5 pH Meters	1) check that calomel electrode is filled with saturated potassium chloride 2) check and remove "KCl creep"	1) two or three buffer adjustment	1) soak glass electrode in 0.1 N HCl for 20 minutes		1) for detailed maintenance instructions and minor maintenance problems, see Table 6 on pH measurement problems and meter maintenance
3.6 Sonic Energy Cleaners		1) wash cabinet surfaces and chamber interior with a mild detergent, rinse and dry	1) inspect and clean air filter (using vacuum cleaner if possible) 2) flush out chamber drain strainer with warm water	1) quarterly: oil lid motor with SAE No. 10 motor oil	1) as needed, replace bottle of sonic detergent
3.7 Laminar Air Flow Cabinets			1) wipe out interior with germicidal cleaner 2) check sterility using 6 randomly placed TSA plates exposed for 2 hr, incubate 3 at 20°C, 3 at 35°C for 48-72 hr	1) serviceman to check: lighting air velocity main-tained velocity leakage noise level vibration level stability	

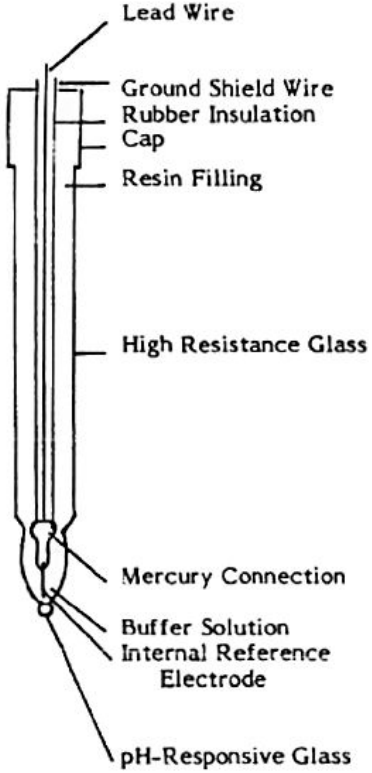
Table 5. continued

Equipment	Daily	Weekly	Monthly	Yearly	Comments
3.8 Refrigerators	1) inspect time and temperature record, date daily	1) change recording chart	1) check automatic alarm system	1) preventative maintenance by annual inspection by qualified serviceman	1) keep records for at least 6 months before discarding 2) temperature variation should be kept ± 0.5°C
3.9 Incubators	1) inspect time and temperature record, date daily	1) change recording chart		1) preventative maintenance by annual inspection by qualified serviceman	1) keep records for at least 6 months before discarding 2) temperature variation should generally be ± 0.5°C 3) keep ambient room temperature as stable as possible (within 18 to 27°C) 4) humidity should be maintained at approximately 75-85%
3.10 Microscopes	1) clean off immersion oil lenses immediately after use	1) general inspection and cleaning		1) qualified serviceman to check and clean all lenses and inspect microscopes	

Table 6a. pH Meter Maintenance and pH Determinations.



FIBRE TYPE CALOMEL ELECTRODE



CUT-AWAY DRAWING OF A TYPICAL GLASS ELECTRODE

pH Measurement:

1. Check that calomel electrode is filled with potassium chloride solution and that crystals are visible.

2. A two-buffer adjustment must be made at least once per week, more often when protein solutions are being measured, and always with new electrodes.
   a) Bring buffers to same temperature as solution to be measured and adjust acid buffer's reading with buffer control knob.
   b) Adjust reading of second (basic) buffer using "Temperature Control" knob (this is actually a bias control and changes the slope of the electrode).
   c) Remeasure first buffer; it should give correct reading. Ideally, a third buffer (near neutral) should also be checked.

3. If necessary, set screw may be used to adjust rest position.

4. Rinse electrodes with distilled water between measurements.

Electrode Maintenance:

1. New electrodes should be soaked 4 hours in 0.1 HCl followed by 30 minutes in the buffer solution that will be used for adjustments.

2. Electrodes must be soaked in expired buffer between measurements, not in water.

3. The KCl solution tends to creep and encrust the outside of the electrode with salt. This should be removed with dH_2O and can be avoided by applying silicone grease to the filling port.

4. Store the electrode with rubber plug in place and the rubber cap over the electrode tip.

5. Level of KCl solution in electrode should be higher than level of sample being measured, during pH measurement.

6. Avoid wiping electrodes, blot if necessary, rinsing should be sufficient.

Table 6b. pH MEASUREMENT PROBLEMS.

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. CONSTANT READING (Needle stays in one position)	a) Powerline, fuse or battery malfunction or batteries if needle stays at rest position	Try different power line, replace old fuse or batteries.
	b) Broken glass membrane if reading stays at pH 4-5	Replace electrode.
	c) pH meter malfunction	Using short circuit strap or copper wire, attach one end to the outlet for the calomel electrode and the other to glass electrode outlet; adjust to pH 7.0. If this cannot be accomplished, meter is at fault and a service-man should be consulted.
2. pH DRIFT (Reading is unstable)	a) Protein-coated glass electrode (may be visible as a yellow film)	Soak electrode in 0.1 N HCl 20 minutes, wash in dH_2O; soak in expired buffer for 30 minutes.
	b) Unsaturated calomel electrode	Refill with saturated KCl solution, ensuring that some crystals are visible.
	c) Rubber plug is inserted into filling port	Remove this plug as otherwise a vacuum will be created preventing flow of KCl through the capillary. This may cause dilution of the KCl solution and possible contamination.
3. ERRATIC READING (Reading fluctuates)	a) pH meter malfunction	See 1 c)
	b) Air bubbles in calomel electrode	Expel by shaking and fill with saturated KCl.
	c) Static electricity	Use glass vessels rather than plastic, if pH meter has plastic face, replace or use antistatic spray.
	d) Scratched glass membrane	Replace electrode.
4. NEEDLE DEFLECTS TO END-SCALE POSITION	a) Air bubbles in calomel electrode	See 3 b)
	b) Potassium chloride crystals form a cluster, blocking the hollow stem	Heat electrode to approximately 60°C in a water bath to dissolve crystals. Pour out, refill with cold saturated potassium chloride, heat and repeat process until the crystal lump is dissolved.

4. QUALITY ASSURANCE OF CULTURE APPARATUS

4.1 Preparation of Laboratory Materials

Microbiological results are always dependent to a certain extent on the freedom from chemical and microbial contamination of the glassware and plastics used. For this reason quality assurance in this area is mainly concerned with the cleanliness and sterility of the materials used.

4.1.1 Cleaning of Materials

4.1.1.1 Glassware

Glass is still the most widely used material in both chemical and microbiological laboratories. Chemically, glass is a supercooled liquid consisting of a network of molecules made up mainly from silicon and elements of group VI of the periodic table: oxygen, selenium and tellurium. It behaves like a mixed bed ion exchange resin of low capacity. A detailed chemical synopsis of the properties of glass and other laboratory materials is beyond the scope of this report; however, Brookes (1969) gives an excellent discussion on chemical properties affecting microbiological work.

Of the three major types of glass, borosilicate (e.g. trade names "Pyrex", "Kimax") is preferred for laboratory glassware over silica glass or soda-lime glass. Borosilicate glass has a characteristically low thermal expansion coefficient and is chemically almost completely inert.

Generally, the most practical and satisfactory method of cleaning glassware in a large laboratory involves the use of mechanical glassware washing equipment. High pressure directional jet streams break up and remove most microbiological growth films, autoclaved proteins, agar, sediments, chemical residuals and wax markings. Wash, drain and rinse cycles are followed in automatic sequence and the final rinse in distilled water should result in sparkling clean glassware free from acidity, alkalinity and toxic residues. (Wilson and Cauffman, 1968). All glassware should be inspected

after washing, and any chipped or cracked items discarded. Water should spread evenly over the surface of clean glassware without pulling away to form patches and drops.

Often, however, glassware is left with rings and residues which are difficult to remove, especially if they have been allowed to dry onto the glass. Dichromate cleaning solution (chromic acid) is one of the most powerful cleaning agents for such purposes. There are many versions of this solution; that recommended by the Analytical Quality Control Laboratory Environmental Protection Agency (1972) is prepared by adding one litre of concentrated sulfuric acid slowly to 35 ml saturated sodium dichromate solution. Extreme care must be employed in the preparation or use of this mixture. A warm solution of 2% ethanolic or aqueous sodium hydroxide is also useful with any greases or oils coating the glass. In either case, the item should be immersed for approximately 15 minutes, then rinsed with tap water, followed by successive rinses with distilled water. Any new glassware should be soaked in 1% hydrochloric acid overnight to neutralize any free alkali and then washed before using (Elliott and Georgala, 1969).

It is also good practice to rewash or at least re-rinse any glassware which has been stored for a long period of time, as fresh cations will have migrated to the surface.

Sintered glass and glass wool should be cleaned with dichromate cleaning solution and nitric acid.

Fogging and etching of glassware are problems caused by corrosive reagents, culture by-products, aging of glass material (especially disposable flint or soft glass items), excessive concentrations of alkaline detergents or repeated abrasions (Geldreich, 1975).

4.1.1.2 Plastic ware

In general, the plastic ware used in the Ministry of the Environment is disposable and is simply discarded after autoclaving if contaminated. Certain temperature resistant plastics can be autoclaved and, as they are more expensive, are used for special applications such as centrifuge tubes and sealing gaskets for small fermenters. Plastic should never be treated with dichromate solution or any oxidizing agent as these will tend to produce free carboxyl groups on the walls of the vessel.

Various laboratories have attempted to reuse disposable plastic culture dishes. Contaminated or old media is removed and autoclaved and the top and bottom sections of the petri dish are soaked in 70 percent ethanol for 30 minutes (Geldreich, 1975). Any remaining material may be removed by hand using a mild household detergent. After rinsing in several distilled water washes and drying, the plastics can be sterilized in 12 percent ethylene oxide at 50 to 55°C for a four-hour period, or rinsed in one percent hydrochloric acid and dried overnight in a 60°C oven.

4.1.1.3 Other Materials

Cleaning of items such as dialysis tubing, membranes, and metal or rubber materials require special treatment which is described well by Brookes (1969) and is rarely necessary in routine Ministry of the Environment Microbiology Laboratories.

4.1.2 Sterilization of Materials

Sterilization is the total inactivation of all forms of microbial life in terms of their ability to reproduce (Sykes, 1969). It can be achieved by moist heat (autoclave), dry heat, irradiation, certain gases or vapours (ethylene oxide), certain chemicals in solution and filtration. Table 7 lists general recommendations and precautions involving the use of autoclave and dry heat sterilization; the remaining methods will be discussed separately.

Table 7. Sterilization Procedures for Laboratory Materials.

Laboratory Material	Autoclave	Dry Heat	Method of Choice
Small individual glassware	121°C - 20 min.	170°C - 1 hour	autoclave or dry heat
flasks tubes bottles dishes	final drying is necessary	may not be suitable if wool plugs or paper used - not suitable with plastic caps	
Glassware in metal containers	121°C - 20 min.	170°C - 2 hours	dry heat or autoclave
petri dishes pipettes	final drying is necessary	may not be suitable if wool plugs or paper used	
Syringes, close fitting joints	carefully control heat and cooling rates	unacceptable due to heat	autoclave
Rubber materials gloves tubing plugs	repeated sterilization will cause misshaping and denaturation	unsuitable except with silicone rubber	ethylene oxide
Plastics (heat sensitive)	unsuitable due to heat sensitivity	unsuitable due to heat sensitivity	irradiation or ethylene oxide
Plastics (autoclavable) polypropylene polycarbonate polyallomer	121°C - 20 min. do not screw caps tightly		autoclave
MF funnels and membrane holders	121°C - 20 min.		autoclave

4.1.2 Irradiation

The radiations most commonly used for sterilization purposes are the electromagnetic x- and γ-rays, and the particulate cathode rays. However, the main field of application for these are in pharmacy and medicine. Ultraviolet radiation cannot strictly be classed as a sterilizing agent because of its low energy and lack of penetration into solids. Even penetration into liquids is only slight, with over 50 percent of the energy lost at a depth of less than two inches (5.08 cm) in clear water (Sykes, 1969). The main applications of ultraviolet radiation used in microbiological laboratories are in the field of air disinfection, and for the disinfection of enclosed spaces and certain equipment.

The most effective lethal wavelength using ultraviolet radiation is in the range of 240 to 280 nm with equal effect on both Gram positive and Gram negative bacteria. Lethal doses are in the range 1,000 - 6,000 microwatts per second per square centimeter for vegetative cells, up to ten times this amount for bacterial spores, and up to 50 times this dose for mold spores. Opinions concerning the susceptibility of viruses vary.

4.1.2.3 Ethylene Oxide

Ethylene oxide is useful for sterilization of heat sensitive materials and, although slow acting, is almost as active against spores as against vegetative cells, and is also a virucide. The minimum time of exposure necessary at a fixed temperature varies inversely with gas concentration.

4.1.2.4 Chemicals

Disposable pipettes used in culture work should be immediately immersed in a disinfecting chemical solution for several hours. There are many such disinfectants marketed but only those most commonly used in the Ministry of the Environment will be discussed.

"Dettol" (Reckitt and Colman, Canada, Limited) is usually used as a surface disinfectant and is relatively nontoxic. It has fairly low activity and is readily inactivated by organic matter. Dettol contains 4.8 percent chloroxylenol which is relatively inactive against *Pseudomonas* species and can actually be incorporated into a selective medium for the isolation of pseudomonads (Sykes, 1969).

"Wescodyne" (West Chemical Products), an iodophor, is also readily inactivated by organic matter but is rapidly effective against vegetative organisms including fungi. It is slowly sporicidal and can be used as a powerful skin disinfectant.

It is recommended that bench areas in the media preparation and pouring rooms be swabbed down with a 1/30 solution of "Wescodyne" alternated with a 1/5 solution of "Dettol" prior to the preparation of media. For decontamination of pipettes: "Wescodyne" is the preferred disinfectant.

4.1.2.5 Filtration

Filtration is frequently used in the sterilization of antibiotic and carbohydrate solutions which are to be added to media. In this way the danger of heat damage or chemical contamination is minimized. Available filters include Seitz filters, sintered glass filters and cellulose acetate filters, the latter being the filter of choice with a pore size of 0.22µ.

4.2 Cleanliness Monitoring

Chemical residuals in microbiological glassware should be checked on a bimonthly basis or at least whenever there is a change in the wash-up procedure. By filling and soaking representative glassware items with sterile distilled water for 24 hours at room temperature, toxic or nutritive residuals will be extracted. The rinse water can then be analyzed for pH and selected chemical parameters using fresh sterile distilled water as control. A fluctuation in pH and/or chemical residuals may indicate inefficient washing procedures or insufficient rinsing, and the cause should be

investigated. At least once a year, or if a change in chemical results occurs, a suitability test should be performed as suggested in Standard Methods of the American Public Health Association (1975). Nutritive extractables will produce a significant growth response while toxic residuals will cause a reduction in the bacterial recovery compared with that of the control.

4.3 Sterility Monitoring

The glassware sterility monitoring program must be adapted to the individual laboratory's media use and the relative proportions of each type of glassware used. The following chart (Table 8) details the program in use at the MOE Central Regional Microbiology Laboratory where sterile graduated cylinders and pipettes are in greatest demand. The timetable is organized into four-week cycles with testing of the appropriate items on the Tuesday and any sign of growth (turbidity) checked daily until Friday of each week. In each case NIH Broth (Difco) is recommended for checking the glassware with negative controls included, using a known sterile container to confirm sterility of the NIH Broth. Other items may have to be added according to use. All quality control testing is performed under a laminar flow hood where possible, using very careful sterile technique. A typical four-week plan follows:

Week 1	graduated cylinders
	flasks
	disposable pipettes
Week 2	graduated cylinders
	glass pipettes
Week 3	graduated cylinders
	beakers tubes
Week 4	graduated cylinders
	glass pipettes.

Where possible, glassware is chosen from the centre of the autoclave load or basket where sterility problems are most likely to occur.

Table 8. Glassware Quality Assurance Timetable.

Glassware/Plasticware	Freq.	Size	Number	Method	Incubation °C, hr.	Comments
Graduated cylinders	weekly	50 ml	1	add 20 ml NIH,	20, 72	incubate NIH in
			1	swirl high on sides,	35, 72	original container
		10 ml	1	incubate in grad. cylinder	20, 72	as NIH control
			1		35, 72	
Flasks	monthly	250 ml	1	as above, using 50 ml NIH	20, 72	"
			1		35, 72	
Beakers	monthly	250 ml	1	as above, using 50 ml NIH	20, 72	"
			1		35, 72	
Pipettes : glass	biweekly	2 ml	2	draw up 2 ml NIH, release	20, 72	use 10 ml NIH
			2	and draw up 5 times, incubate	35, 72	in tube as NIH control
		1 ml	2	as above using 10 ml NIH	20, 72	"
: disposable	monthly	1ml	2	as above, using I ml NIH	35, 72	
			2		20 , 72	
					35, 72	
		10 ml	2	as above, using 10 ml NIH	20, 72	
			2		35, 72	
Tubes	monthly	13 x I00 mm	2	aseptically pour 3 ml	20, 72	"
			2	into tubes, swirl, incubate	35, 72	
		16 x 150 mm	2	as above, using 6 ml	20, 72	choose older baskets
			2		35, 72	
		other	2	according to size	20, 72	choose according to use
			2		35, 72	

5. WATER QUALITY ASSURANCE

5.1 Distilled Water

Theoretically distilled water should contain only H_20; practically, distilled water used for the preparation of media and glassware should be "... free of inorganic and organic substances, either toxic or nutritive, that could influence survival or growth of bacteria and viruses ...." (Geldreich and Clark, 1965). Factors affecting the water quality include (a) design of the distillation apparatus, (b) source of raw water, (c) use of a carbon filter, (d) storage chamber for reserve supply, and (e) temperature of the stored supply and length of storage before use (Geldreich, 1975). Such factors affect the extent of chemical and bacterial contamination, and often the pH. A quality assurance program should consider all these effects.

Distilled water supplies are monitored in the Media Laboratory in three phases: conductivity on a daily basis, and chemical and bacteriological purity on a monthly basis. Conductivity is considered important enough to check daily as it is an immediate indicator of many changes in the chemical purity of the water. Water purity standards set out in 1972 by the Analytical Quality Control Laboratory of the U.S. Environmental Protection Agency are as follows:

Degree of Purity	Max. Conductivity (micromhos/cm)	Approx. Concentration of Electrolyte (mg/L)
Pure	10	2 - 5
Very Pure	1	0.2 - 0.5
Ultrapure	0.1	0.01 - 0.02
Theoretically Pure	0.055	0.00

For microbiological purposes, an upper limit of 3.0 micromhos/cm (μmhos/cm) is recommended. In practice, six months of almost daily monitoring of the distilled water conductivity has revealed a tight range of 0.72 to 1.2 μmhos/cm. For this reason, conductivity is now being checked weekly with an arrangement to be notified by the Chemistry Section of any increase to greater than 2.0 μmhos/cm.

After checking the chemical purity of distilled water running for 0, 5, 10, 15, 20 and 30 minutes before sampling, it was determined that a 15-minute period was adequate to flush out any residual sludge or debris. Samples are collected from the most commonly used taps in sterile sample bottles which have been thoroughly rinsed in the sample water just prior to sample collection. Parameters determined and typical results follow (mg/L):

Nitrogen as N	Free Ammonia	0.02
	Total Kjeldahl	0.02
	Nitrite	0.001
	Nitrate	< 0.005
	Total Phosphorus as P	0.002
	Dissolved Reactive Phosphorus as P	0.002
	Total Organic Carbon	0
	Total Inorganic Carbon	1
	Sodium as Na	1.0
	Potassium as K	0.1
	Calcium as Ca	1
	Magnesium as Mg	1
	Hardness as $CaCO_3$	1
	Copper as Cu	0.01
	pH	6.64
	Conductivity (μmhos/cm)	1.1

The pH generally ranges from 6.4 to 7.2 which is slightly higher than found in the literature. Geldreich and Clark (1965) reported a range of 5.8 to 6.8 in various geographical regions, while Chambers et al (1962) reported an average of 6.2.

Microbiological analysis of the distilled water supply is also conducted monthly. A 15-minute water sample collected in the manner previously described is filtered and plated in triplicate within one hour of sampling. A total plate count (TPC) is determined using Total Plate Count Broth plus 1.5 percent agar (Difco) and incubated at 35°C for 24 hours. Similarly, tests are conducted for total coliform (TC) count on LES m-Endo Agar (Difco), fecal coliform (FC) count on MacConkey Membrane Broth, fecal streptococcus (FS) count on M-Enterococcus agar (Difco) and *Pseudomonas aeruginosa* count on m-PA (Levin and Cabelli, 1972). In nine months of testing we have had zero counts for the TC, FC, FS and m-PA counts. The total plate count has fluctuated considerably, ranging from 327 colony-forming units (CFU) per ml to 1,870 CFU per ml. This variability has been noted by other workers (DeRoos *et al*, 1976) and is well within the expected range.

The effect of the bacterial quality is more due to the type than the number of microorganisms per ml. Isolates are usually Gram negative and include members of the genera *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, *Chromobacter*, *Aeromonas*, *Serratia*, *Moraxella*, *Erwinia* (Favero *et al*, 1975), *Xanthomonas*, *Cytophaga* (Herman, 1976) and *Caulobacter* (Hamilton and Myoda, 1974). Generally speaking, the most common genera are *Pseudomonas* and *Alcaligenes*. These organisms are capable of survival and growth in the presence of minimal concentrations of nutrients. When *Pseudomonas aeruginosa* begins to multiply in a distilled water system, its antagonistic action toward other organisms such as *Escherichia coli*, *Salmonella typhosa*, *Serratia marcescens* and *Vibrio comma* is a source of concern and action should be taken. For these reasons, the major contaminant should be identified. In the Media Laboratory, the organism most frequently isolated from the distilled water is *Pseudomonas alcaligenes.*

On an annual basis, the distilled water supply should also be tested for "suitability" according to the Standard Methods (1975) procedures. Additional suitability testing should follow any major line alterations or repairs to the system.

Geldreich (1975) describes the best distilled water system as preferably stainless steel, quartz or Pyrex, with connecting plumbing and storage tanks of the same materials. Polyvinyl chloride is a major contaminant in such water systems and must not be used for the connecting plumbing.

5.2 Deionized Water

The deionized water supply in the media room is provided by a Millipore Super-Q System (Millipore Corp., Bedford, Mass., U.S.A.) and is used when the distilled water supply is not functioning, or as requested for special media. The conductivity is monitored before each use, and chemical and bacterial parameters monitored monthly, again using 15-minute samples. Suitability testing should be performed annually. In spite of the 0.45 μ filter, deionized water supplies are usually reported more heavily bacterially contaminated than distilled water (DeRoos *et al*, 1976), due to multiplication in the resin bed. It has been suggested (Saunders, 1954) that the organisms actually thrive on organic matter filtered from the feed water, rather than on the resin itself. Frequent regeneration and treatment with a 0.25% formaldehyde solution followed by a thorough flushing is recommended.

In nine months of comparing the chemical parameters previously mentioned for both the distilled and deionized water supplies, we have noted very little difference. The pH of the deionized water is generally 6.8 to 7.3, the conductivity 0.8 to 2.0 μmhos/cm, and the other parameters at a similar level in most cases.

Bacteriologically speaking, the TPC is lower in deionized water, ranging from 24 to 159 CFU per ml, while the TC, FC, FS and mPA counts are all zero. The contaminant showing up most frequently in the TPC is *Alcaligenes faecalis*, which also shows up as background in the TC count.

There is still considerable difference of opinion among scientists as to the significance of bacteria in water supplies. In interviewing 25 scientists at the National Institutes of Health, DeRoos *et al* (1975) found a range of opinions on the suggested limit of bacterial CFU per ml, ranging from zero to no limit. Reasons for these limits also varied widely. In testing several high purity water systems, DeRoos *et al* (1975) observed a range of CFU per ml of zero to 3,600 in the deionized water supplies.

It is hoped that this report will provide sufficient guidelines to allow the scientist to institute or improve a quality control program designed to prevent and correct any factors which may affect the validity of the microbiological data generated. As progress is made in areas such as proficiency testing and laboratory intercomparisons, this report will be updated and sections added.

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