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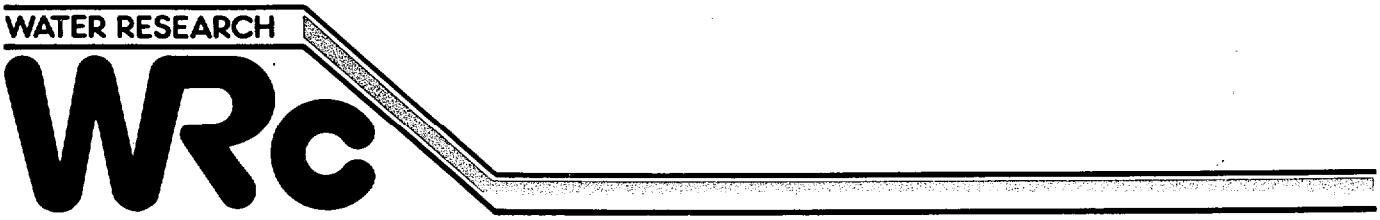

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SUMMARY

In 1979, as part of a contract funded by the Department of the Environment (Contamination of Water by Domestic Plumbing Fittings - H 280 CX), the Water Research Centre investigated the effect of water quality on the microbiological procedures of the then National Water Council (now the United Kingdom Water Fittings Byelaws Scheme) test for assessing the effect of materials on water quality. Previously this study had only been reported to DOE and the British Standards Institution (BSI EPC/44/7) but has now been made available for wider circulation.

During the investigation, a range of materials, including rubbers, plastics, jointing materials and gland packings were tested at eight locations in the UK. The study investigated (a) the effect of varying water quality, (b) the reproducibility of the method, and (c) the use of dissolved oxygen probe as a method of estimating microbiological activity.

CONTENTS

1. INTRODUCTION

The Model Water Byelaws[1] made under section 17 of the Water Act 1945 require that any equipment, fitting or accessory used in domestic plumbing systems may not 'cause or permit, or be likely to cause or permit, waste, undue consumption, misuse, erroneous measurements or contamination of water'.

There have been many instances where materials used in the construction of plumbing systems in contact with potable water have given rise to microbial growth. Houston as early as 1916 showed that leather tap washers could support the growth of coliform organisms[2]. Similar examples have been cited by Burman and Colbourne[3,4] and include microbial contamination derived from lubricating oil in pumps, materials used in hospital plumbing systems and drinks vending machines, and jointing materials for use in reservoirs. The subsequent deterioration of water quality caused by these materials can lead to consumer complaints, and flushing of plumbing systems to clear the offending turbidity or taste results in wastage of water.

The recognition that water quality can be affected in this way by materials used in plumbing installations and distribution systems has led to the development of a technique to assess the ability of these materials to support microbial growth[4]. The test procedure (the Burman Test) is designed to evaluate the suitability of materials for use in contact with potable water and involves the assessment of certain microbiological determinations over a period of 6 weeks (see Appendix A).

Although these procedures are generally reliable and are capable of consistently passing and failing materials, there are circumstances where these tests may not accurately assess microbial numbers resulting in inconsistencies in interpretation. This is especially so where there is a surface growth on the material or there are free-floating biological aggregates in the water. The need for a more reliable and quantitative technique has led to the consideration of alternative methods of assessing microbial activity including test material weight loss measurement and manometry[5,6]. However, most attention has been paid to three particular methods namely the determination of adenosine-5'-triphosphate (ATP),

total organic carbon (TOC) and dissolved oxygen (DO). These methods have been reviewed in some detail by Panel A of the British Standards Committee EPC/44/7 and it was considered that the DO method would be the most suitable. Although this method is no shorter in total analysis time it is simpler, less labour-intensive and provides a quantitive assessment of microbial growth allowing some flexibility in the acceptance criteria.

A limited number of comparative trials have been conducted at the Water Research Centre using TOC, DO and the conventional microbiological methods. The results indicate that the TOC assessment of microbial growth is a less reliable indication of the changes in the test vessels than DO, and this, coupled with the overall expense of instrumentation, may preclude the use of TOC and similiarly ATP. The use of ATP as a measure of microbial activity has also been considered although no trials have been undertaken. ATP is present in all living cells and the ATP photometer can be used to quantify the number of living cells in a sample of water. In practice there are difficulties in correlating ATP to viable bacterial numbers and its use is not considered practical for this type of test procedure.

A considerable number of developmental and comparative studies of the DO and the conventional microbiological methods have been conducted both at Thames Water Authority and WRC. Certain materials have been tested at both laboratories and results have shown a 100% agreement between methods for a number of materials.

The objectives of this study were threefold, to compare the microbiological method with the dissolved oxygen method, and to assess both the reproducibility of the tests and the effects of water quality on the performance of the procedures.

These parameters were considered particularly important if the procedures were to be applied with equal validity in laboratories using different waters. Also it was essential that any alternative method of testing should return a similar result to the current procedures.

2. MATERIALS AND METHODS

2.1. TEST MATERIALS

A total of 24 materials were tested at each location. A full listing of materials is given in Table 1. It includes materials commonly used in domestic

plumbing systems and a range of rubber samples and gland packings. The rubber samples were provided by the International Synthetic Rubber Company Ltd and the gland packings by Thames Water Authority. The remaining materials were purchased at local stores.

2.2. EXPERIMENTAL SITES AND WATER QUALITY

Eight locations were selected on the basis of tap water quality and source. A brief description of the waters is given in Table 2, each location being identified by a code letter. The inoculum at each site was taken from an impoundment or river source at or near the abstraction point used by the local water supply authority and fulfilled the requirements of the method. (See Appendix A).

Suitable laboratory accommodation was available at locations A,D,E and H, but a mobile laboratory was used at sites B,C and F. At location G a converted office had to be used. Throughout the whole exercise all equipment was supplied by WRC.

2.3. TECHNICAL ASSISTANCE

Assistance was needed at each location to change the water; twice weekly during the first 3 weeks and once a week for the rest of each test. At locations B,D,E and G the staff had no practical experience of this type of test whereas at location A,C,F and H they did. Staff at each location were briefed on the technique in general and the water changing procedure in particular and supplied with all necessary equipment. However, the performance at sites D and G may be criticised in that the procedure was not strictly adhered to. The final result at these sites was probably affected by this unsatisfactory work. The likely effects are considered fully in Sections 3.1. and 3.4. and Table 6.

2.4. TEST METHODS

Experimental procedures were based upon the microbiological and dissolved oxygen methods as amended (October 1978) by Panel A of the British Standards Institute Committee EPC/44/7. The full methodology and interpretation for both the microbiological method and the dissolved oxygen method are given in Appendices A and B, respectively but the test and pass criteria are listed here.

1. Microbiological method

Control and test vessels are examined during weeks 4, 5 and 6 for coliform organisms, *Pseudomonas aeruginosa*, fungi, visible growth and plate counts at 22 ^{o}C and 37 ^{o}C. A material is regarded as satisfactory if three consecutive counts of coliform organisms and *Pseudomonas aeruginosa* are <1 per 100 ml in the test and control flask, and the three consecutive counts of the remaining 3 groups and organisms are less than 10 times the corresponding count in the negative control flask. There should be no visible growth.

2. Dissolved oxygen method

Dissolved oxygen levels are measured in the 'control' and test vessels during weeks 4, 5 and 6. Analysis for coliform organisms and *Pseudomonas aeruginosa* are conducted in triplicate in week 6.

A material is regarded as satisfactory if the mean dissolved oxygen difference between the test vessel and the negative control is less than 2.0 mg O_2/l and the coliform organisms and *Pseudomonas aeruginosa* are <1 per 100 ml.

Some minor modifications of the standard test procedure were necessary to satisfy the experiment's objectives.

i) The same jars and samples were used for both methods. Therefore jar size, water volume and the initial and 'carry over' inocula were as specified by the dissolved oxygen method (see Appendix B). Dissolved oxygen was measured immediately on opening the jars and then samples were removed for the conventional microbiological examinations as stated in Appendix A.

ii) Paraffin wax was not included as a positive control in these tests as other materials whose likely behaviour was known had already been included e.g. leather tap washers.

iii) The dissolved oxygen method given in Appendix B allows for an extra 2 weeks analysis if after 6 weeks the Mean Dissolved Oxygen Difference (MDOD) falls between 2.0 and 2.5 mg/l. If after 8 weeks the MDOD is >2.0 mg/l then the material shall be deemed to have failed. The time scale of these exercises was such that a further 2 weeks could not be accommodated. To overcome this an unsatisfactory result was assumed if the MDOD was >2.0 mg/l after 6 weeks. As this was the same for every test vessel it was assumed that this would not materially alter the overall result.

iv) With increasing experience of the dissolved oxygen test methods, the following 4 modifications were made with a view to their being incorporated permanently into the method.

a) Between water changes the 20 litre aspirators and measuring cylinders are filled with chlorinated water (20 mg/l). This avoids microbial growth in the vessel which can be carried over from one week to the next. Care must be taken to thoroughly rinse the vessel before use with the test water, in order to remove any residual chlorine.

b) Aeration to achieve saturation of the change water has been identified as a possible source of contamination and aeration of the change water was discontinued. Sufficient saturation can be achieved by vigorously filling the aspirator from a suitable tap, thus removing any risk of contamination, which can be further reduced by careful cleaning, and sterilising of the tap by flaming before filling the aspirator.

c) The routine microbiological analysis of the change water is recommended to give an indication of any microbiological contamination which may affect the test vessels giving erroneous results.

d) A presence and absence test for coliform organisms was used throughout these experiments in an attempt to simplify the test procedure. The method involves the direct addition of 100 ml aliquot of sample to a sterile medical flat containing 25 mls of 5 × strength Minerals Modified Glutamate media (MMG) (Oxoid CM 289). Incubation is for 48 hours at 37 ^{o}C. Presumptive positive bottles (production of acid and gas) were confirmed by subculture into Brilliant Green Bile Broth (BGB) (Oxoid CM31) with incubation for 48 hours at 37 ^{o}C).

This method was compared directly with Most Probable Number (MPN) methods using MMG and the membrane filtration method using membrane Enriched Teepol Broth (ETB) (Oxoid MM 369) as described in Appendix A. The results of these exercises are given in 3.5.

e) The standard Kilner jar lid is provided with a butyl rubber seal which can support the growth of pseudomonads and so compromise the validity of the test. Replacement lids were made from soft PTFE sheet which had been shown not to support growth. The standard locking ring was retained.

3. RESULTS AND DISCUSSION

The testing of 24 materials at 8 locations has provided a substantial amount of information. It is impractical to present all the test data but a summary of the results is discussed covering the following aspects.

i. effects of water quality
ii. the reproducibility of the tests
iii. a comparision of the microbiological method with the dissolved oxygen method.

3.1. ASSESSMENT OF WATER QUALITY, ACCOMMODATION AND ASSISTANCE AT EACH LOCATION

An attempt has been made to rank the locations visited. This has been done in terms of factors which could affect the test result, namely water quality, accommodation and assistance. The measure of water quality was based upon a figure for TOC for the tap water and the 7 day, 22 ^{o}C plate count for the negative control vessel. Both these figures are mean values obtained over the 3 week period of analysis (Table 3). These values have been scored and ranked. The rank value has been used as the basis of further comparisons.

Consideration has been given to ranking based upon levels of nitrogen/22 ^{o}C plate count. The overall ranking of location is not substantially altered by this approach and as the effect of water quality appears not to affect the results obtained, the TOC/22 ^{o}C plate count ranking was maintained.

The 22 ^{C}C plate count appears to be independent of the amount of TOC in each water, even though greater than 10 fold differences in the TOC were observed. These differences can be explained in terms of the quality of the TOC available to the microbial population. At locations C,D,E and H, which were all lowland eutrophic sources, the water would contain a considerable proportion of biodegradable organic carbon whereas at locations F and G the bulk of the TOC will be as refractory humic and fulvic acids and not necessarily biodegradable. Despite these differences, there is no great variation in the numbers of micro-organisms found in the negative control.

The accommodation and assistance for each location have been scored and ranked also (Table 4). The locations with highest score are considered to be less suitable as testing sites. In Table 4 the accommodation/assistance scores have been combined with those for water quality and an overall assessment for each location given.

3.2. COMPARISON OF THE MICROBIAL AND DISSOLVED OXYGEN METHODS

An examination of the results obtained for all materials at all locations show considerable agreement between the two methods employed. Out of 192 tests conducted only 5 show disagreement (Table 5).

There is a 97.4% agreement between methods. It has been argued that this is to be expected as the two methods are essentially very similar. However if one considers the variability in water quality, initial inoculum, accommodation and assistance then this figure becomes very important. Under these conditions a difference of 2.6% cannot be regarded as serious. The 5 tests showing disagreement all returned a pass result by the dissolved oxygen method and failed by the microbial method.

The 5 tests showing disagreement are spread between 5 different materials and would appear to be random, 3 failed due to visible growth and 2 for 37 ^{o}C plate counts.

When the agreement between methods is considered on a site basis, then 5 sites (A,B,D,E,H) have 100% agreement (i.e. 24 tests out of 24 at each site), two sites (C,F) have 1 disagreement (95.8%) while, at site G, 3 of the 5 disagreements were recorded. Location G had the worst accommodation/assistance ranking (Table 4).

3.3. EFFECT OF WATER QUALITY ON THE ACCEPTANCE/REJECTION OF A MATERIAL

The overall acceptance/rejection totals for each material are given in Table 5. Results for each of the methods have been separated, and tests showing disagreement, as discussed in Section 3.2., are marked.

It is difficult to identify or attribute a pattern of acceptance/rejection to varying water quality. Each material was tested in eight different waters for each method. For the conventional microbiological method 15 vessels gave a 100% (8 out of 8) acceptance or rejection. Of the remainder, 5 vessels show a 1 in 7 disagreement and 4 vessels namely 8,12,13 and 14 show >1 in 7 disagreement. For the dissolved oxygen method the results are similar. Here 14 vessels gave a 100% agreement, 6 gave a 1 in 7 disagreement and vessels 4,12,13 and 14 gave a >1 in 7 disagreement.

A 1 in 7 disagreement may be caused by chance and is to be expected in any exercise. However a greater than 1 in 7 disagreement, perhaps, represents those materials which are truly borderline cases. All 4 vessels in this category were rubber samples and it is not unlikely they would return a variable or borderline result however often tested. This would suggest that these results are a function of the material tested and not the water quality.

Clearly there are differences in results obtained in some locations, but it is difficult to put any significance on these disagreements.

3.4. ANOMALOUS RESULTS

In the dissolved oxygen method it has been shown that dissolved oxygen measurements will replace the need to assess fungi, visible growth and plate counts. Furthermore it is agreed that an MDOD of >2.0 mg O_2/1 will denote a failure in one or more of the above parameters and rejection of the material. Similarly an MDOD of <2.0 mg O_2/1 and the absence of coliform organisms and *Pseudomonas aeruginosa* will indicate acceptance of the material.

In these exercises there would appear to be a number of instances where the above hypothesis is not obeyed. These anomalous results are situations where there is an MDOD of >2.0 mg O_2/1 with no failures for fungi, visible growth or plate counts, or conversely an MDOD of <2.0 mg O_2/1 with failures in one or more of the microbial parameters.

Of the 192 tests there are 21 anomalous results (10.9%). Of these vessels, 14 show an MDOD of <2.0 mg/1 with obvious visible growth. This would suggest rejection of a material showing obvious growth irrespective of the dissolved oxygen result. However it would appear that the visible growth observed in weeks 4, 5 and 6 may represent non-viable surface growth formed during the initial weeks of the test. The presence of very obvious fresh surface growth in the later stages of the test would certainly affect the MDOD, and consequently a material should not be rejected because of visible growths if the MDOD is <2.0 mg/1.

The anomalous results obtained in these experiments only materially affect the overall result in 5 cases (2.6%) and are in fact accounted for in the disagreements between methods (see Section 3.2). Table 6 shows the distribution of these errors with locations. It is particularly interesting that locations D and G which are so markedly different to the remainder, were locations with high water quality/accommodation rankings. Similarly these two locations

returned the highest assistance scores. This would suggest that the satisfactory completion of these procedures depends heavily upon suitable laboratory accommodation and competent staff rather more than individual water quality. This does not mean that highly trained personnel with considerable experience in the test or specially equipped laboratories are required. It does mean that reasonable laboratory facilities and staff with basic laboratory skills and some knowledge of microbiology are essential. Their ability to follow instructions and a basic understanding of the reasons for them are important.

3.5. THE USE OF PRESENCE AND ABSENCE TESTING FOR COLIFORM ORGANISMS

A total of 150 vessels were examined using 5 × strength MMG, the standard MPN multiple tube method using double strength MMG and the membrane filtration method using ETB. Of these, 31 vessels were shown to contain presumptive coliform organisms by one or more of the above methods. Coliform organisms were confirmed in 26 vessels.

The 5 × strength MMG returned 20 vessels showing presumptive coliform organisms which were all confirmed, the double strength MMG also returned 20 vessels showing presumptive coliform organisms, of which 18 were confirmed and the ETB returned 19 presumptive coliform organisms of which 18 were confirmed. Of the vessels tested, 3 showed positive coliform organisms by the 5 × strength MMG method alone, 17 by the 5 × strength and double strength MMG and 11 by 5 × strength and ETB. Only 12 vessels returned positive coliform organisms by all three methods.

Throughout the field trials, presumptive organisms were observed in a total of 202 vessels when using 5 × strength MMG. Of these, 197 (98.5%) were confirmed as coliform organisms using BGB at 32 ^{o}C.

Although not an extensive study, the results indicate that the use of presence and absence testing using 5 × strength MMG is a suitable alternative for this test procedure. It compares favourably with MPN methods using MMG and returns a greater number of positive vessels than the ETB membrane filtration method. The method is less labour-intensive than the other recommended coliform techniques, both in the initial setting-up and confirmation and allows a greater number of samples to be handled. The acceptance of this method for coliform organisms and a similiar method for Pseudomonads developed by TWA should enable the dissolved oxygen method to be used with ease and accuracy by operators who are not trained microbiologists.

4. CONCLUSIONS

The completion of extensive studies of the performance of the test procedures currently used to assess the suitability of materials for use in potable water, did not detect any effect caused by differences in water quality. Trials of various plumbing materials in eight locations using waters varying from hard chalk borehole water to very soft oligotrophic upland impoundment have highlighted the difficulties of establishing a pattern for water quality effects. There are differences in results for the same materials at different locations but the relevance is not known. It seems that the cause is partially random, and in part, a function of certain materials giving rise to borderline results. The satisfactory completion of either the conventional microbiological methods or the dissolved oxygen method depends more upon suibable laboratory accommodation and competent staff than water quality.

The agreement observed between the methods is high (97.4%) and the discrepency of 2.6% includes the overall effects if varying water quality, inoculum, accommodati and the quality of assistance. There was however, a proportion of these test vessels which failed due to visible growth. This was found to be growth formed during the initial weeks of the test and should not be considered as grounds for rejection of the material if the MDOD is <2.0 mg/l.

As experience has been gained with the application and use of these methods it has become necessary to make certain modifications to the current procedures. These include the use of inert PTFE test vessel lids. The chlorination of equipment between water changing and the use of presence and absence testing for certain microbiological parameters.

This study has confirmed the overall comparability and reproducibility of both test methods and highlights the need for competent technical staff and suitable accommodation. The results indicate that a very high level of agreement can be achieved even when using waters of different quality and this coupled with the savings in labour and increased flexibility make the dissolved oxygen method a very suitable alternative to the current procedures.

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REFERENCES

1. ANON. *Model Water Byelaws*. London: HMSO, 1966.

2. HOUSTON, A. The growth of microbes on leather washers. 1916, *12th Research Report Metropolitan Water Board.*

3. BURMAN, N.P. and COLBOURNE, JENNIFER, S. Effect of non-metallic materials on Water Quality. *Journal of the Institution of Water Engineers and Scientists*, 1979, *33*, 11-18.

4. BURMAN, N.P. and COLBOURNE, JENNIFER, S. Techniques for the assessment of growth of micro-organisms on plumbing materials used in contact with potable water supplies. Journal of Applied Bacteriology, 1977, *43*, 137-144.

5. PANKHURST, E.S., DAVIS, M.J. and BLAKE, H.N. The ability of polymers or materials containing polymers to provide a source of carbon for selected micro-organisms. In: *Biodeterioration of Materials*. Ed. Walters, A.M. and Hueck-van der Plas, E.H. London, Applied Science Publications Ltd, 1972.

6. BURGESS, R. and DARBY, A.E. Two tests for the assessment of microbial activity of plastics. *British Plastics*, 1964, *37*, 32-37.

Table 1. Test Materials

Sample No	Material
2	Gland packing 656
3	Gland packing 715
4	Gland packing 788
5	Gland packing 828
6	Emulsion SBR 50 IRHD
7	Emulsion SBR 70 IRHD
8	Blend sol/emul SBR 45 IRHD
9	Solution SBR 70 IRHD
10	Polyisoprene 50 IRHD
11	Polybutadiene 50 IRHD
12	EP peroxide cure 60 IRHD
13	EP peroxide cure 70 IRHD
14	EPDM sulphur cure 50 IRHD
15	EPDM peroxide cure 50 IRHD
16	EPDM sulphur cure 70 IRHD
17	EPDM peroxide cure 70 IRHD
18	Leather tap washers
19	Rubber tap washers
20	Fibre tap washers
21	Polyethylene sheet
22	uPVC water tank
23	GRP water tank
24	Linseed oil jointing compound
25	Polyethylene water tank

Table 2. Test Waters

Location code	Description of water
A	hard, deep, chalk-borehole water
B	soft, unpolluted, upland-derived river water - direct abstraction.
C	moderately hard, lowland, eutrophic river water - direct abstraction.
D	moderately hard, lowland, eutrophic river water - direct abstraction.
E	moderately soft, lowland, eutrophic river water - direct abstraction.
F	very soft, upland impoundment, oligotrophic water with high colour.
G	very soft, upland impoundment, oligotrophic water with high colour.
H	moderately hard, lowland, eutrophic river water - pumped storage.

Table 3. Ranked values for TOC and 22 °C plate count for each location

Site	TOC mg C/l	TOC score (1 - 8)	22 °C plate count No/ml	22 °C plate count Score (1 - 8)	Total score	Rank No.
A	0.49	1	3.2×10^4	1	2	1
B	1.18	2	6.3×10^4	3	5	2
C	5.15	8	1.0×10^5	4	12	7
D	2.10	4	1.5×10^5	8	12	7
E	1.87	3	1.0×10^5	4	7	3
F	2.30	5	5.6×10^4	2	7	3
G	2.79	6	1.0×10^5	4	10	5
H	5.00	7	1.0×10^5	4	11	6

Table 4. Overall Ranking of locations in terms of accommodation, assistance and water quality

Site	Accommodation* (score 1 - 4)	Assistance† (score 1 - 4)	Water Quality Score (from Table 3)	Total score	Rank No.
A	1	1	1	3	1
B	2	2	2	6	2
C	2	1	7	10	6
D	2	4	7	13	8
E	2	2	3	7	4
F	2	1	3	6	2
G	3	4	5	12	7
H	1	1	6	8	5

* A score of 1 ≡ WRC/TWA: 4 ≡ unacceptable

† A score of 1 ≡ WRC/TWA: 4 ≡ very poor level of assistance

Table 5. Effect of water quality on the overall acceptance/rejection of a material for both the conventional microbiological and the dissolved oxygen methods

Sample No	Material	Acceptance/rejection using conventional microbiological method				Acceptance/rejection using dissolved oxygen method				
2	Gland packing 656	8	Reject	0	Accept	7	Reject	1	Accept*	
3	Gland packing 715	8	"	0	"	8	"	0	"	
4	Gland packing 788	8	"	0	"	8	"	0	"	
5	Gland packing 828	0	"	8	"	0	"	8	"	
6	Emulsion SBR 50 IRHD	8	"	0	"	8	"	0	"	
7	Emulsion SBR 70 IRHD	8	"	0	"	8	"	0	"	
8	Blend sol/emul SBR 45 IRHD	6	"	2	"	6	"	2	"	
9	Solution SBR 70 IRHD	8	"	0	"	7	"	1	"	*
10	Polyisoprene 50 IRHD	8	"	0	"	8	"	0	"	
11	Polybutadiene 50 IRHD	8	"	0	"	8	"	0	"	
12	EP peroxide cure 60 IRHD	6	"	2	"	5	"	3	"	*
13	EP peroxide cure 70 IRHD	5	"	3	"	4	"	4	"	*
14	EPDM sulphur cure 50 IRHD	6	"	2	"	6	"	2	"	
15	EPDM peroxide cure 50 IRHD	7	"	1	"	7	"	1	"	
16	EPDM sulphur cure 70 IRHD	7	"	1	"	7	"	1	"	
17	EPDM peroxide cure 70 IRHD	8	"	0	"	8	"	0	"	
18	Leather tap washers	8	"	0	"	8	"	0	"	
19	Rubber tap washers	7	"	1	"	7	"	1	"	
20	Fibre tap washers	7	"	1	"	7	"	1	"	
21	Polyethylene sheet	0	"	8	"	0	"	8	"	
22	uPVC water tank	0	"	8	"	0	"	8	"	
23	GRP water tank	0	"	8	"	0	"	8	"	
24	Linseed oil jointing compound	8	"	0	"	8	"	0	"	
25	Polyethylene water tank	1	"	7	"	0	"	8	"	*

* tests showing disagreement between the two methods

Table 6. Percentage of anomalous results at each location

Site	% errors	Water quality, accommodation assistance rank number	Assistance score
A	0	1	1
B	4.2	2	2
C	8.3	6	1
D	29.2	8	4
E	4.2	4	2
F	8.3	2	1
G	33.3	7	4
H	0	5	1

APPENDIX A

METHOD OF TEST FOR MICROBIOLOGICAL GROWTH AS AMENDED BY PANEL A OF B.S.I. EPC/44/7

A.1. PRINCIPLE OF METHOD

Dechlorinated tap water in contact with the material under test is inoculated with a mixture of micro-organisms as described in section A.4.4. The microbiological growth is compared with that in the controls. Coliform organisms are used in the assessment of growth because of their importance in Report 71 (The Bacteriological Examination of Water Supplies, Reports on Public Health and Medical Subjects No. 71, London HMSO 1970) and their international recognition for assessing water quality.

Pseudomonas aeruginosa is used as an indicator of the fluorescent pseudomonad group which utilises a wide variety of substrates. It is the most commonly used test organism for similar purposes, it is not likely to be suppressed by other organisms when growing in competition in mixed cultures and it dies out rapidly in the controls so that quantitative interpretation of results is relatively easy and reliable.

A.2. CONTROLS

It is recommended that the test be carried out in parallel with two control tests, one negative and one positive. It is suggested that glass with the same surface area as the test sample be the negative control although ideally the controls should have the same surface character as the test sample. Paraffin wax containing between 20 and 25 carbon atoms per molecule (melting point 52/54 ^{o}C) is a good positive control, although it may break up, producing fragments, and care is needed to avoid these fragments being interpreted as visible growth. Break-up of the wax may be prevented by melting it and forming in a mould to give a surface area 15 200 mm^2 ± 10%. This may be achieved by forming a cuboid of dimensions 130 mm × 45 mm × 10 mm. The negative control glass sample must be specially cleaned using a 3.5% (v/v) solution of a cleaning agent and steaming for 2 hours. Decon 90 and Lipsol are suitable cleaning agents. Chromic acid must not be used as a cleaning agent. When cool, the sample should be rinsed twice with tap water and finally with distilled water. The glass sample when dry should be placed in a polythene bag and treated in the same way as the test samples (see A.4.2.).

A.3. TEST PREMISES

Exercise care to ensure that these tests are carried out in premises free from the presence of any volatile organic chemicals in the atmosphere, as these can dissolve in exposed water surfaces in sufficient quantities to produce abundant microbial slimes which will mask similar growths due to the test sample. The volatile chemical most frequently responsible for such slimes is ethanol but many other volatiles, including natural gas, are used in industry and this requirement may be difficult to achieve on factory premises where volatile organic chemicals are freely used.

A.4. TEST PREPARATION

A.4.1. Nature of Samples

Wherever appropriate, samples and controls must be treated in the same way. Samples must be packaged in clean polythene bags, identified on the bag, not on the sample. Adhesive labels, ink or pencil marks must not be attached to the sample.

The surface area of the sample shall be 15 200 mm^2 ± 10%. This may be achieved by using a cuboid of dimensions 130 mm × 45 mm × 10 mm.

Where samples of coatings are to be tested, they must be applied to panels of the appropriate material of the size just described, including all appropriate undercoats and completely covering all surfaces and edges.

In many fittings, water comes into contact with only one surface. If such materials are not homogeneous and the outer surface is of a different material from the inner surface, a sample must be prepared with the same material on all surfaces and edges. It is important that samples specially prepared for testing must be subject to the same conditions of maturing or curing as would normally exist either in manufacture or on site.

A.4.2. Sample cleaning

During the period between arrival at the laboratory and commencement of testing, samples must be protected from contamination by dirt, oil, grease, dust, etc.

Ideally, materials to be tested must be cleaned by a method which does not remove any constituent which could support growth. In the absence of an ideal method, samples and controls must be cleaned by immersion in glass-distilled water in a 2-litre wide-necked glass flask for 24 hours, using a magnetic stirrer for agitation, at ambient temperatures. This is recommended at present, although the evidence available indicates that the effect of cleaning in this way is probably not significant.

A.4.3. Preparation of sample

Place the sample in a specially cleaned 2-litre wide-necked glass flask. Use similar flasks for the controls. Where the sample is in the form of a container, this will serve as the recipient for the water and inoculum, but such containers should be capable of holding not less than one litre of water, and it must be possible to cover the tops of such containers with aluminium foil. The water level in the control flask (or beaker) must be nominally the same as in the test flask (or beaker). The sample must be completely immersed, if necessary using glass weights.

N.B. Flasks must be specially cleaned in the same way as the glass negative control sample (see A.2.). After rinsing and draining, the flasks must be dried in a hot air cabinet and covered.

A.4.4. Test Procedure

Wherever appropriate, samples and controls must be treated in the same way. Using the method given later in section A.4.5.1. or a suitable commercially available alternative, standardise the content of coliform organisms and using the method given in A.4.5.3. standardise the *Pseudomonas aeruginosa* present in a water containing a suitable source of micro-organisms. This is most likely to be a river water which receives both sewage effluent and land drainage. Care should be taken to avoid rivers containing toxic wastes which might inhibit microbial growth. This can be done by ensuring that the river water is suitable for abstraction for treatment to produce potable water.

To the container in which the sample has been placed add a volume of this water sufficient to contain between 100 and 10 000 coliform organisms and between 1 and 500 *Pseudomonas aeruginosa* and make up to one litre with mains tap water. If the mains water contains residual chlorine

this must be removed using sodium thiosulphate as a 3% solution (18 mg/l sodium thiosulphate should neutralise up to 5 mg/l of residual chlorine).

A.4.4.1. Incubation and growth assessment procedures

Cover each flask with aluminium foil, (N.B. not cotton wool), and incubate at 30 ± 1 °C in the dark.

Twice weekly, remove all the water except 10 ml from each flask and refill the flask to one litre with fresh mains tap water if necessary, dechlorinated as above. For practical reasons, Mondays and Thursdays are the most suitable days of the week for this to be done.

At weekly intervals, beginning four weeks from the initial inoculation, withdraw samples of water from each flask after shaking, just prior to the water being changed. Examine these samples quantitatively for coliform organisms, bacteria capable of growth at 37 °C and 22 °C, *Pseudomonas aeruginosa* and fungi. Also examine each flask weekly for visible growth.

Treat the control containers similarly.

A.4.5. ENUMERATION TECHNIQUES

A.4.5.1. Coliform organisms

Count coliform organisms by the standard membrane filtration technique or the standard multiple tube method, including confirmation procedures, described in 'The Bacteriological Examination of Water Supplies', Reports on Public Health and Medical Subjects No. 71, London HMSO 1970.

A.4.5.2. Colony counts at 37 °C and 22 °C

Count by the standard agar plate count procedure using yeast extract agar described in 'The Bacteriological Examination of Water Supplies', Reports on Public Health and Medical Subjects No. 71, London HMSO 1970.

A.4.5.3. *Pseudomonas aeruginosa*

Aseptically filter a 100 ml volume (or less if numbers are high) of the test water through a sterile, standard, bacterial membrane of pore size 0.45µ. Place the membrane face upwards on a sterile Whatman No.17 Filter pad (or equivalent) saturated with sterile King's A broth.

Incubate the membranes at 37 °C ± 1 °C for 48 hours in a closed container to prevent drying out.

Count the colonies arising that are green and/or fluorescent under an ultra-violet lamp of 350 nm wavelength to obtain a presumptive count of *Ps. aeruginosa*. Colonies shall be confirmed by subculture from the membrane on to milk agar, or other suitable medium, incubated at 41.5 °C ± 0.5 °C for 24 hours. Growth at this temperature, coupled with production of a green and/or fluorescent pigment and casein hydrolysis, is confirmation of the organism.

King's A broth

Bacto-peptone		20 g
Ethanol		20 g (25 ml)
Anhydrous potassium sulphate		10 g
Anhydrous magnesium chloride		1.4 g
(or $MgCl_2.6H_2O$)		2.9 g)
Hexadecyltrimethylammoniumbromide (Cetrimide)		0.5 g
Distilled water	to	1000 ml

Sterilise at 121 °C for 15 minutes in a small autoclave not exceeding 6 litres, remove the bottles and cool as rapidly as possible to prevent loss of the ethanol.

Milk Agar

Skim milk powder		100 g
Yeast extract broth*		250 ml
Agar		15 g
Cetrimide		0.3 g
Distilled water	to	1000 ml

* Yeast extract broth make up in the % w/v given below

Bacteriological yeast extract	0.3 g
Peptone (Evans)	1.0 g
Sodium chloride	0.5 g

To a 2 litre flask add the yeast extract broth (pre-sterilised at 121 °C and 10 Kpa for 20 minutes), Cetrimide and agar. Swill contents to disperse the agar and steam to dissolve.

To a second 2-litre flask add the distilled water and milk powder. Disperse by shaking. Autoclave both flasks at 121 °C and 10 Kpa for 5 minutes and reduce the pressure rapidly to avoid caramelisation of the milk. When cooled to 55 °C mix aseptically by pouring the milk into the agar, mix well and pour plates. Allow to set, then dry.

A.4.5.4. Fungi

Filter a volume of 100 ml of the test water (or less if the numbers are high) through a sterile standard bacterial membrane of pore size 0.45 µ which is then placed face-upwards on a previously poured Martin's rose bengal agar plate containing Kanamycin base (100 µg/ml).

Incubate at 22 °C for seven days and count the number of fungi which develop. These could have developed either from fungal spores or mycelial fragments. Other organisms which occasionally grow on this medium are aerobic sporing bacilli which appear as dry, wrinkled colonies. These can be confirmed by microscopic examination. *Pseudomonas aeruginosa* may also grow but these colonies are orange in colour and are readily distinguished by flourescence under an ultra-violet lamp of 350 nm wavelength.

Rose Bengal Agar

Oxoid mycological peptone	5 g
Magnesium Sulphate ($MgSO4.7H_2O$)	0.5 g
Glucose	10 g
Oxoid agar (1%)	10 g
Rose bengal (1% solution)	3.5 mg
Kanamycin acid sulphate, BP 1973 (10^4 units/ml. solution - 937 500 I. units ≡ 1 g Kanamycin base)	10 ml
Potassium Phosphate (KH_2PO_4) (1% solution)	100 ml
Distilled water to	1000 ml

Basal Medium

Steam the peptone, agar and magnesium sulphate to dissolve, add glucose, adjust pH to 5.4, add rose bengal, distribute in 90 ml volumes into screw cap bottles and sterilise at 110 °C for ten minutes.

To each 90 ml volume of basal medium at 55 °C add 10 ml of potassium phosphate solution pre-sterilised at 110 °C for 20 minutes and 1 ml Kanamycin solution aseptically. Mix well and pour six plates.

A.5. MICROBIAL GROWTH

A.5.1. Negative control

Coliform organisms, bacteria capable of multiplication at 37 °C and 22 °C, *Ps. aeruginosa* and fungi shall be counted by the methods in Appendix A during the 4th week and continuing once per week for as long as the test flasks are being counted. Three consecutive counts of coliform bacteria and *Ps. aeruginosa* from the 4th, 5th and 6th weeks shall be <1 per 100 ml. If these described counts are not obtained the test must be considered as void and the tests unsatisfactorily carried out. There shall be no film of growth on the glass panel or flakes of growth suspended in the water readily visible to the naked eye.

The bacterial and fungal counts in the control flask will vary according to the type of inoculum, tap water and counting method used.

A.5.2. Positive Control

Microbial growth shall be visible to the naked eye on the surface of the control sample and in the test water. This growth shall be confirmed as being microbial in origin by microscopic examination. If no visible growth is observed the test must be considered as void and unsatisfactorily carried out.

A.5.3. Microbiological growth in the test flask

Coliform organisms, bacteria capable of growth at 37 °C and 22 °C, *Pseudomonas aeruginosa* and fungi shall be counted by the methods described in Appendix A, beginning during the 4th week after the initial inoculation and continuing a further 2 weeks.

The three consecutive counts of coliform organisms and *Pseudomonas aeruginosa* shall be <1 per 100 ml both in the test and negative control flasks.

The three consecutive counts of the remaining 3 groups of organisms, i.e. bacteria capable of growth at 37 °C and 22 °C and fungi, shall be less than 10 times the corresponding count in the negative control flask.

If the counts in the negative control flask are very low, a tenfold increase in the test flask could still be acceptable; therefore notwithstanding the tenfold requirement, the counts in the test flask should not be considered significant if they are less than the maximum limits given in Table 1.

Table A1

MAXIMUM LIMITS BELOW WHICH

COUNT IS ACCEPTABLE

37 °C	Colony count	100 per ml
22 °C	Colony count	10 000 per ml
Fungi		100 per ml

Note : The counts of fungi obtained by the method given will include spores and fragments of mycelium which become detached from sessile growth on the material.

If all three of the counts of each group of micro-organisms are within the prescibed requirement, the test shall cease and the sample be deemed not to have supported a significant microbial activity.

If all three of the counts of each group of micro-organisms fall outside the prescribed requirement, the test shall cease and the sample be deemed to have supported a significant microbial activity.

If one (or two) of the three counts on any group of micro-organisms are within the prescribed requirement, the test shall continue and two (or one) further counts of those groups of micro-organisms made. If three consecutive counts within the prescribed requirement are then obtained for each group of micro-organisms, the sample shall be deemed not to have supported a significant microbial activity. Any sample not giving three satisfactory consecutive counts on each group of micro-organisms within eight weeks from the beginning of the test shall be deemed to have supported a significant microbial activity.

In addition, there shall be no film of growth on the sample or suspended in the water which is readily visible to the naked eye and

which is confirmed as microbial growth on microscopic examination. Assessment of this growth is subjective and slight surface blooms should be ignored pending the introduction of more quantitative methods of assessment currently under development.

If counts in the test flask are less than 10% of those in the control flask after the 4th, 5th and 6th week of testing, this indicates the presence of leachable biocidal material and makes the test procedure described in Appendix A unsuitable for the assessment of the material under test.

APPENDIX B

ALTERNATIVE METHOD OF TEST FOR DETERMINING TOTAL MICROBIOLOGICAL GROWTH BY MEANS OF DISSOLVED OXYGEN USAGE

B.1. PRINCIPLE OF THE METHOD

The method differs from that described in Appendix A only in that the total microbiological growth is determined by the difference between the amount of dissolved oxygen used in supporting the growth. The growth of coliform organisms and *Pseudomonas aeruginosa* is determined in the same way as described in Appendix A.

B.2. CONTROL TESTS - See A.2.

B.3. TEST PREMISES

The containers used for the test are sealed during incubation and are therefore unaffected by the presence of volatile organic chemicals in the atmosphere of the test premises. It is, however, important to ensure that no organic chemicals of any kind are introduced into the water during the aeration procedure.

B.4. TEST PREPARATION

B.4.1. Nature of samples

Wherever appropriate, samples and controls shall be treated in the same way. Samples shall be packaged in clean polythene bags, identified on the bag, not on the sample. Adhesive labels, ink or pencil marks shall not be attached to the sample.

The surface area of the sample shall be 11 400 mm^2 ± 10%. This may be achieved by using a cuboid of dimensions 100 mm × 43 mm × 10 mm.

If samples of coatings are to be tested, they shall be applied to panels of the appropriate materials of the size described above, including all appropriate undercoats and completely covering all surfaces and edges.

In many fittings, water comes into contact with only one surface. If such materials are not homogeneous and the outer surface is of a different material from the inner surface, a sample shall be prepared with the same material on

all surfaces and edges. It is important that samples specially prepared for testing shall be subject to the same conditions of maturing or curing as would normally exist either in manufacture or on site.

B.4.2. Sample cleaning - See A.4.2.

B.4.3. Bottle cleaning - See A.4.3.

B.4.4. Preparation of sample

Place the sample in a specially cleaned 2 lb preserving jar made from soda or borosilicate glass or other similar wide-mouthed jars capable of being sealed in such a way as to minimize the amount of air in the jar. Use similar bottles for the controls.

Note The dissolved oxygen method is not suitable for samples in the form of a container.

B.4.5. Preparation of control samples - See A.2.

B.5. TEST PROCEDURE

B.5.1. Test inoculum - See A.4.4.

B.5.2. Aerated tap water

Fill a 20-litre glass aspirator with fresh mains tap water and bubble clean air through the water by means of a silicone rubber and/or glass tube for between five and ten minutes.

B.5.3. Incubation and growth assessment

To the jar in which the sample has been placed add a volume of the test inoculum sufficient to contain between 100 and 10 000 coliform organisms and between 1 and 500 *Pseudomonas aeruginosa* and make up to 750 ml with aerated tap water.

Seal each jar and incubate at 30 °C ± 1 °C in the dark.

Twice weekly, remove all the water except 10 ml from each jar and refill the jar to 750 ml with fresh, aerated tap water. Reseal and reincubate the jars after each water change. A minimum of three days should be left between each water change.

At weekly intervals, beginning during the fourth week after inoculation, measure the dissolved oxygen concentration in the water in each jar, just prior to the water being changed. On the sixth week of the test, after recording the dissolved oxygen concentration before changing the water, samples should be withdrawn aseptically from each jar and examined quantitatively for colifrom organisms and *Pseudomonas aeruginosa*. These tests should be performed in triplicate. Treat the control containers similarly.

B.5.4. Determination of mean dissolved oxygen difference

B.5.4.1. Measurement of dissolved oxygen

Measure the dissolved oxygen concentration of the water in the jars using a dissolved oxygen probe and meter. Most models can be used but the probe should be provided with a stirrer and it is preferable that the meter has a dissolved oxygen range that is automatically temperature compensated for solubility of oxygen in water. The latter requirement removes the need to use conversion tables. The meter and the probe shall be maintained according to the manufacturer's handbook and the meter shall be calibrated before each set of readings is obtained. The method of calibration varies according to the sensitivity of the model, and it will usually be necessary to know the atmospheric pressure (mm Hg) in the test premises.

Remove each jar from the incubator, taking care not to disturb the water inside. Remove the seal from one jar and position the probe in the centre of the volume of water with the aid of a clamp. Switch on the stirrer and allow time for the probe to stabilise to the sample temperature and to the dissolved oxygen content. The stabilisation period will vary according to the meter used but it should not normally exceed one minute. Remove the probe and reseal the jar.

B.5.4.2. Calculation

Calculate the means of the dissolved oxygen readings obtained from the test container, in the 4th, 5th and 6th weeks and similarly the controls. Calculate the means dissolved oxygen differences (MDOD) by subtracting the mean of the negative control from the mean of the test container. Subtract the means of the negative control from the mean of the positive control to obtain the positive control MDOD.

B.5.4.3. Non-biological oxygen demand procedure

If the sample fails the test and contains chemical compounds likely to produce a non-biological oxygen demand in the test procedure, a separate sample should be tested by the following procedure and the chemical oxygen demand circulated. The sample and the controls should be prepared as decribed above except that the jars should be pre-sterilised by autoclaving at 121 ^{o}C and 10.5 KPa for 15 minutes. The sample should not be inoculated with micro-organisms. The procedures should be carried out except that aerated tap water should be replaced by glass distilled water pre-sterilised by autoclaving in the same way as the jars. Enumerations of coliforms and *Ps. aeruginosa* are not required. Measure the dissolved oxygen concentrations as described above. Calculate the mean of the three dissolved oxygen readings obtained from each test container and from the controls. Calculate the chemical oxygen demand by subtracting the mean of the negative control from the mean of the test container.

Materials likely to produce a non-biological oxygen demand include samples containing metal components and some peroxide cured rubbers.

B.5.5. Enumeration techniques

B.5.5.1. Coliform organisms - See A.4.5.1.

B.5.5.2. *Pseudomonas aeruginosa* - See A.4.5.3.

B.6. INTERPRETATION OF RESULTS USING THE DISSOLVED OXYGEN METHOD

If the non-biological oxygen demand for the material was measured and exceeded 0 mg/l subtract the result obtained from the MDOD. The resultant figure should then be the MDOD used to assess the microbial activity of the material.

The MDOD for the positive control should exceed 2.0 mg/l and if it is less the test should be considered as void and incorrectly carried out.

If the MDOD obtained for a test container <2.0 mg/l the test shall cease and the sample be deemed to be incapable of supporting a significant microbial activity in the test procedure. If the MDOD obtained for a test container $\geqslant$ 2.5 mg/l the test shall cease and the sample be deemed to be capable of supporting a significant microbial activity in the test container.

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