A Safer Organism for the Rideal-Walker Test

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The Rideal-Walker (RW) test is a quantitative test by which the antimicrobial activity of the phenolic disinfectant is compared with that of phenol. [1–3]

The Rideal-Walker test is carried out:

— by manufacturers as a quality control procedure during production.

— is a tender specification for the phenolic disinfectant to be used in hospital and laboratories.

— to devise a practical use dilution for a particular brand of phenolic disinfectant.

— to test preliminary disinfection evaluation of new active phenolic agents.

Main problems in using this test

However the Rideal Walker test has two main disadvantages: [1–3]

— The test specify Salmonella typhi as the test organism, which is a very dangerous pathogenic organism responsible for the typhoid fever. This organism was the main concern of microbiologists during the discovery of the test, when typhoid was still fatal. The use of Salmonella typhi presents a problem for small scale manufacturers and laboratories because pure cultures of pathogenic organisms require high initial and running costs. The laboratory workers face serious hazards.

— It is a single organism test and so only the antimicrobial activity against Salmonella typhi can be studied. The use of a single test organism may produce incorrect results when comparing disinfectants for purchasing. This is because a certain disinfectant may possess a high Rideal-Walker coefficient against a certain test organism and a low Rideal-Walker coefficient when another test organism is utilized.

SPECTRUM OF ACTIVITY OF PHENOLICS

Phenolics have a wide range of bactericidal activity, including Pseudomonas, Tubercule bacilli. They have fungicidal activity but little viricidal activity. Bacterial spores are not sensitive to the phenolic disinfectant and are only moderately resistant to acid fast bacilli like Mycobacteria. The addition of sodium EDTA, pine oil and sodium castor oil soap enhance the antimicrobial activity of the phenolics.

Hard water and organic matter has a marked influence on their activity. Pus, blood, soil, faeces, milk etc. all reduce the effectiveness of a disinfectant.

Material such as fabrics, cork, plastics and rubber absorb and inactiviate them. So in the presence of interfering substances the concentration of the disinfectant must be increased[6].

Alterations to the test

Alterations to the Phenol coefficient tests were carried out by the British Standard institution in 1961[4] using Staphylococcus aureus instead of Salmonella typhi and also by the association of the official analytical Chemists[5] (AOAC) using Staphylococcus aureus and Pseudomonas aeroginosa. By using different species of microorganisms, a spectrum of activity of a certain disinfectant is given. However these microorganisms are still pathogenic and the problem can only be eliminated if non pathogenic organisms are used. **This study concerns the carrying out of the Rideal-Walker test using the non-pathogenic Escherichia coli.**

Rideal-Walker Test

The Rideal-Walker test, using the British Standard, 541:1985[8] Determination of the Rideal-Walker (RW) Coefficient of Disinfectants' was carried out substituting Salmonella typhi ATCC6539 for the non-pathogenic Escherichia coli. ATCC11229

1. The test culture using E.Coli is prepared in a specified way using Rideal-Walker broth (containing Oxoid nutrient broth (code CM 67)).

2. Serial dilutions of Phenol and of the disinfectant under test are prepared using sterile water.

3. 5ml volumes of the test disinfectant of phenol solution are inoculated with 0.2 mls of the culture at a temperature of 17-18°C.

4. At 2.5, 5, 7.5, and 10 minutes intervals a standard loopful of the contents is added to 5mls of the Rideal Walker broth to prepare a subculture of the surviving organisms.

5. The broths were then incubated at 37°C for 48-72 hrs and then tested for growth.

The pattern of growth in the test tubes is observed and those containing a turbid broth indicated that there was growth, whilst a clear broth indicate absence of growth.

6. The phenol coefficient was calculated by dividing the highest dilution of the test disinfectant showing growth after 5 mins but not after 7.5 mins, by the highest dilution of phenol giving the same result, e.g. Table 2, 3.

Higher RW numbers indicate better disinfectant performances.

Table 1.

Composition of the Disinfectant

The spicemen disinfectant is made up of:

Chorophenols
Choroxylenols
Pine oil
Sodium Castor Oil Soap
Alcohol
Sodium EDTA
Brown dye
ph 9.1
It is manufactured to BS 5197:1976[7].

Table 2. Rideal-Walker test results of the Phenol control.

Phenol Dilution	Exposure Time				Phenol Coefficient
	2.5 min	5.0 min	7.5 min	10.0 min	
1:95	–	–	–	–	
1:100	+	–	–	–	
1:105	+	+	–	–	1:105
1:110	+	+	+	–	
1:115	+	+	+	–	

Table 3. Rideal-Walker test results of the disinfectant at a dilution of 1:4.

Disinfection Dilution	Exposure Time				Rideal-Walker Coefficient
	2.5 min	5.0 min	7.5 min	10.0 min	
1:100	+	–	–	–	
1:150	+	–	–	–	200/105 = 1.90
1:200	+	+	–	–	
1:250	+	+	+	–	
1:300	+	+	+	–	

+ = growth. — = no growth.

Results

The Rideal-Walker test was performed on the sample disinfectant at different concentrations (1:1, 1:3, 1:5) using Escherichia Coli as the test organism. Table 4 gives the results of the Rideal-Walker coefficient at the different concentrations. Fig 1 represents the histogram of the test results.

Table 4. Results of the Rideal-Walker coefficient using E. Coli.

1. Disinfectant of Conc. 1:1:	$RW_1 = 4.76$
	$RW_2 = 5.24$
	$RW_3 = 4.65$
Average RW Coefficient	= 4.88
2. Disinfectant of Conc. 1:3:	$RW_1 = 4.09$
	$RW_2 = 1.90$
	$RW_3 = 2.38$
	$RW_4 = 2.14$
Average RW Coefficient	= 2.63
3. Disinfectant of Conc. 1:5:	$RW_1 = 1.39$
	$RW_2 = 1.16$
Average RW Coefficient	= 1.27

From these results the mean Rideal-Walker coefficient of the undiluted disinfectant using E. Coli is 9.63. (Standard deviation 2.74; Coefficient of variation 28.74%).

The Rideal-Walker test was performed on the same batch of sample disinfectant using Salmonella typhi as the test organism. Table 5.

Table 5. Rideal-Walker test results using Salmonella typhi.

Disinfectant Dilution	Rideal-Walker coefficient
1:7	1.86
1:4	20.5

Average Rideal-Walker coefficient of the undiluted disinfectant is **10.64**.

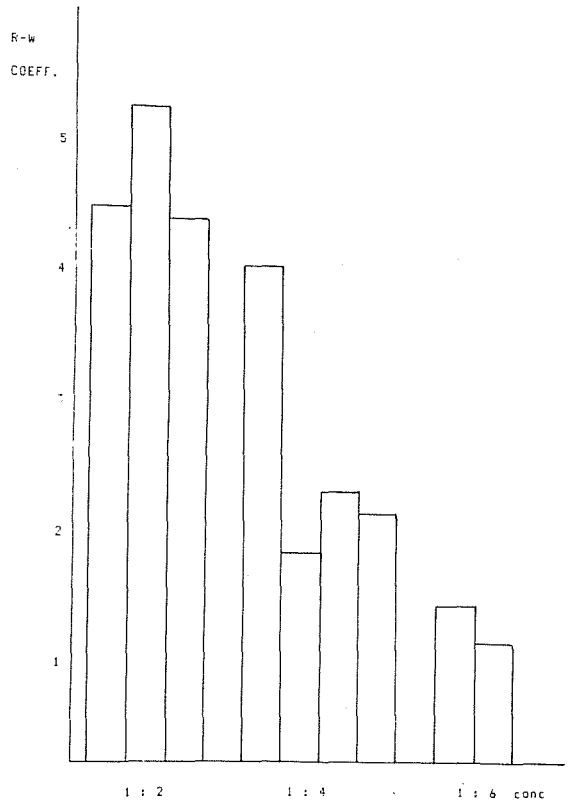

Fig 1. Histogram representing the test results.

Discussion

The statistical results show that the coefficient of variation is 28.74%. This result is in the same order as when a large number of tests were carried out by skilled operators.

But the high coefficient of variation can be due to a number of errors whilst performing the test.

In this study, not a large number of tests on the specimen disinfectant using different concentrations were carried. The test should be performed for a large number of times to reduce the significance of errors.

Errors can arise if the conditions of the laboratory vary, such as temperatures, apparatus, material and different batches of reagents. The analyst must be very careful so that the conditions vary as little as possible.

In the test results when E.Coli was used to evaluate the disinfectant at a concentration 1:3 the first result is much higher than the others. This could be because the microorganisms were damaged with time.

The test culture should be changed frequently as the microorganisms are damaged with time and so a lower concentration of disinfectant will be needed to kill them. The RW coefficient will be lower than it should actually be.

Long intervals between successive sampling lead to imprecision. Samples are removed at intervals of 2.5 minutes. The phenol and disinfectant dilutions should kill the organism in just over 5 or just under 7.5 min respectively and still give the same end-point.

In practice disinfection takes place at a variety of temperature and because bactericides have characteristic coefficients, their performance at the temperature of the test may fail to reflect their behaviour at other temperatures.

Sampling is done with a small inoculating loop and there can be considerable variation in the size of the sample.

Conclusion

When the test results using E. Coli are compared with those using S. Typhi, it can be concluded that the average Rideal-Walker coefficient do not differ so much from each other.

The antimicrobial activity of the specimen disinfectant against E.Coli is similar to that against S. typhi. However this can only be true for the same type of phenolic disinfectant as the specimen, as results can deviate when another phenolic disinfectant is tested.

It is recommended to consider the possibility of including E.Coli as a possible test organism in official RW tests to facilitate the carrying out of quality control of disinfectants. This change has the advantage of not using a highly pathogenic organism. In this way the initial and running cost for such a test are reduced. The test is still useful especially in small scale manufacturing and purchasing.

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REMINDER

In the last 15 years significant new problems in the use of disinfectants have arisen. The emergence of Hepatitis 'B' and more recently the AIDS virus has made it necessary to reconsider disinfectant policies.

It is worth noting that the following biocides do not have wide spectrum viricidal-activity:

Phenolics
Quaternary Ammonium Compounds
Chlorhexidine

THE CONTROL OF HEPATITIS 'B' AND AIDS VIRUS (HTLV III)

The U.K. Advisory Committee on Dangerous Pathogens produced interim guidelines in 1984. For the destruction of the virus they recommended Glutaraldehyde or Chlorine based Biocides.

There is substantial evidence to demonstrate that both 2% Alkaline Glutaraldehyde and Chlorine releasing biocides will effectively destroy both Hepatitis 'B' and AIDS virus. 1% inactiviated Glutaraldehyde was shown to be effective within minutes. Immersion in 2% Alkaline Glutaraldehyde for 10 minutes for clean instruments and if pre-cleaning is not possible for 1 hour, will effectively eliminate a key enzyme of the AIDS virus.

It was also demonstrated that 1000ppm available Chlorine resulted in a large loss in enzyme activity of the virus in 5 minutes. They go on to recommend the use of 2000 ppm available Chlorine for disinfecting clean floor and work surfaces.

The key to safety is the use of suitable biocides and the avoidance of sharp injury and contact with blood.