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June 5, 1995

Dockets Management Branch (HFA-305)
Food and Drug Administration, rm 1-23
12420 Parklawn Drive
Rockville, MD 20857

Re: 21 CFR Parts 333 and 369
Docket 75N-183H.
Topical Antimicrobial Drug Products for Over-the-Counter Human Use: Tentative Final Monograph for Health-Care Antiseptic Drug Products.
59(116) Federal Register 31402-31452; June 17, 1994

To Whom It May Concern:

We would like to comment on points raised in the "tentative final monograph for health-care antiseptics drug products" published in the Federal Register of June 17, 1994.

In particular, our comments are directed to the following points:

1. The number of categories and the need for expansion of the number of categories.
2. The rational for specific performance criteria for the categories and how do these criteria relate to the benefits received from using these products?
3. The role of the carrier in test results.
4. The assertion that there is inadequate data to support the claims of broad spectrum activity for Chloroxylenol.
5. Finished product test methodology.

75N-183H

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I. THE NUMBER OF CATEGORIES AND THE NEED FOR EXPANSION OF THE NUMBER OF CATEGORIES

The inclusion of products focused at consumers and food handlers within the general category of antiseptic hand wash or health-care personnel hand wash appears unreasonable.

Health-care personnel are typically exposed to a variety of potentially virulent bacteria. As such, it is reasonable to expect that products designed for their use be effective over a broad spectrum of activity and to have properties that provide a significant degree of protection to the user.

In contrast, a consumer or food handler is not typically exposed to potentially wide spectrums of bacteria. Consequently, products designed for hygienic use or focused at specific bacteria, such as, salmonella or e-coli, should not be held to the same requirements as product applications requiring a broader spectrum activity.

It is suggested that two new categories of products be added. One for products considered to be consumer oriented antiseptic hand washes which are separate and distinct from the health-care personnel hand wash category. A second category should be added to address food handlers (including food processing, food preparation and restaurant personnel).

The agency has recognized that a health-care personnel hand-wash is different than surgical scrub. This recognition is apparent in the different proposed test methodology as well as the different standards that must be met. The addition of categories for products required to meet other requirements would be consistent with the direction the agency appears to be taking.

The establishment of the additional categories with attendant test methodologies will allow for a wider selection of products at the consumer level. These products would be required to meet standards that reflect the demands required by their application.

Likewise, the establishment of a category for food handlers and restaurant workers would enhance the selection of products for these applications. This category could build on the current E-2 authorization system which is currently administered by the USDA. This system has assured that products used for this application have worked effectively. With this system in place, we have not had outbreaks of infection that can be identified with bacteria commonly associated with human skin in the food processing and handling area.

2. WHAT IS THE RATIONAL FOR SPECIFIC PERFORMANCE CRITERIA FOR THE CATEGORIES AND HOW DO THESE CRITERIA RELATE TO THE BENEFITS RECEIVED FROM THE USE OF THESE PRODUCTS?

The agency has established performance criteria for the three proposed categories of antiseptic drug products. It is not clear that the criteria are in any way able to reflect the clinical benefits that is to be expected from the use of these products.

It is agreed that the use of a topical antiseptic drug product should reduce the risk of infection by reducing the bacterial population on the skin. But, it is not at all clear that this benefit is related to a one or two log reduction in recoverable bacteria from the skin.

Since the testing only measures recoverable bacteria, and not the bacteria population of the skin, it is a substantial leap to make value judgments related to clinical efficacy based on an arbitrary criteria related to reduction in recoverable bacteria. After all, we cannot determine the actual level of bacteria on the skin.

It appears that the criteria were established based on data submitted on some products that show a reduction in recoverable bacteria. But there is no evidence that the observed

reduction was adequate or even desirable. On the other hand there is no information to indicate what level of reduction is desirable.

The question of what population of bacteria is necessary to create an infection has not been established. Could the level be as low as 10 bacteria per square inch, or as high as 10^4 bacteria per square inch.

What is known is that bacteria can lead to infection. But what level of bacteria is necessary to create an infection is not known. The total bacterial population of the skin is judged by the bacteria that can be removed from the skin without any estimate of the percentage of the bacteria present the removed level may represent. With these uncertainties it is difficult to understand the basis for any numerical criteria for a products performance. All that can be stated is that an antimicrobial cleanser that is efficacious is preferred over a non-antimicrobial cleanser.

3. THE ROLE OF THE CARRIER IN TEST RESULTS

On page 31408 of the TFM there is the statement "Although the Category I active ingredients currently included in the tentative final monograph are broad spectrum independent of formulation,..." Whereas, on page 31438, there is a statement "The agency is adding to this amended tentative final monograph a definition of broad spectrum activity as follows: a properly formulated drug product, containing an ingredient included in the monograph."

On page 31438, the agency is acknowledging the role of a carrier in helping define the spectrum of activity of an ingredient. After all, the caveat, "properly formulated" does make clear that category I ingredients can be improperly formulated so that the resulting formulation is not broad spectrum. This is the reason that finished product testing is necessary.

It is understood that finished product testing is required and it is recognized that the formulation plays a very important role in the activity of a product. Consequently, the agencies position on the inherent activity of Chloroxylenol is puzzling. The activity of Chloroxylenol like iodine, is influenced by carriers. The agency did not characterize polyvinyl pyrillodone as a carrier for iodine, but that is exactly the role it plays. Yet the agency took a very different stance on the role of surfactants with other actives, such as, Chloroxylenol. Test result for iodine in the absence of carriers such as PVP or other surfactants in an aqueous media would be very different from the results obtained in their presence.

4. THE ASSERTION THAT THE "AVAILABLE DATA ARE INADEQUATE TO SHOW THE CONTRIBUTION OF CHLOROXYLENOL TO THE EFFECTIVENESS OF A FINISHED PRODUCT." (p. 31415)

A submission by NIPA Laboratory in 1992 (ref. 1) provided data on Chloroxylenol in a propylene glycol/water carrier with the vehicle tested concurrently, to demonstrate the contribution of chloroxylenol. Testing was conducted against three organisms. The data demonstrated that Chloroxylenol, at a concentration as low as 0.75% /120 or .00625% (62.5 ppm) is bacteriostatic against the selected organisms at 10 minute or less of contact time.

Data submitted in 1985 (ref. 2), demonstrated that Chloroxylenol, and only Chloroxylenol, is responsible for the activity of a Chloroxylenol/propylene glycol system. In this work the MIC's against candida albicans and tricophyton mentagrophytes was determined to be 125 ppm and 1000 ppm respectively. In other studies submitted in 1985 (ref. 3,4), the MIC's of Chloroxylenol were determined against a variety of organism (ref. 4). Of interest is that in ref. 3, the MIC of a Chloroxylenol/potassium ricinoleate formulation against candida albicans was determined to be 500 ppm. A dilution of Dettol (Chloroxylenol with other ingredients) yield an MIC for Chloroxylenol of 60 ppm. Reference 2 showed Chloroxylenol to have an MIC of 125 ppm against candida albicans. In one case a vehicle

control was used and in the other, no control was employed but the MIC are still of the same order of magnitude. This argues for the inherent activity of Chloroxylenol.

In yet another study (ref. 5), the MBC (minimum bacteriocidal concentration) of Chloroxylenol was determined to be 480 ppm against candida albicans. Even though this study employed Dettol, it is clear that the common ingredient in all of these studies was Chloroxylenol.

This is not to say that the vehicle can enhance or detract from the activity of an active. It certainly can have an effect. But, this does not detract from the inherent activity of a given active, such as Chloroxylenol.

In another study (ref. 6), the MIC's of Chloroxylenol in distilled water and in Dettol were determined. While there is a demonstrable influence of the Dettol vehicle, the activity of Chloroxylenol in distilled water is clearly evident against forty-one (41) different bacteria. Many of these bacteria are listed in 333.470(1)(iv) of the TFM. In this study, a technique for assessing if a bacteria can develop a resistance to Chloroxylenol was employed. The forty-one (41) bacteria types showed little , if any, evidence of developing a resistance to Chloroxylenol.

In two other studies found in the Hearing Clerks office (ref. 7), there is evidence that the addition of Chloroxylenol to various preparations increased the activity of these preparations in both in-vitro and in-vivo testing.

The data currently in the hands of the agency does support the following points:

a. Chloroxylenol does have a spectrum of activity that can be described as broad.
b. The data supporting Chloroxylenol activity represents the inherent activity of this action.
c. There is data on Chloroxylenol contained in a variety of carriers (water, propylene glycol/water, surfactant systems). In each and every case Chloroxylenol was seen to provide a significant and enhanced increase in

antimicrobial activity of the system. This increase, which is directly attributable to the presence of Chloroxylenol, speaks directly to the comment on page 31415 (third column) of the TFM - "conclusions regarding chloroxylenol's active contribution to the products efficacy cannot be supported." The data contradicts this assertion.

TESTING METHODOLOGY

There are several issues in connection with the testing methodology that should be addressed.

Of significant concern is the comment that appears on page 31431 (3rd column) of the TFM - "The agency recognizes that the list of organism to be tested may need updating to assure that it remains reflective of current trends in the microbial etiology of nosocomial infections. The agency intends to update the list as new information becomes available".

What is the meaning of this comment? Is it the intent of the agency to require the re-qualification of anti-microbial active ingredient at some future date? Does this mean that a category one ingredient may be withdrawn until it can demonstrate activity against a new, as yet undetermined, list of test organism.

It is understood that the regulatory status of an active or finished product can be changed when there is evidence that the data on the product cannot support its label claims. This can be done by improved technology focused at the original intent of the product (i.e. analytical methods, etc.). but how does the agency propose to treat actives that are effective against one set of standard organisms proposed by the agency but may show little activity against some future, as yet undetermined, set of proposed organisms? It is well established that virtually any antimicrobial active ingredient will fail to demonstrate activity against some selected bacteria.

Upon examination of proposed 333.470 several questions are raised.

a- In 333.470(1) there is the requirement that recently isolated strains or fresh clinical isolates be used in the in-vitro testing. What does the agency propose as a definition of "fresh" or "recent". Does this mean that different manufacturers may select different organisms to test their products against. If so, what would this mean to the effort to arrive at a consistent understanding of a given active ingredients spectrum of activity.

b- It is unclear why MIC's should be required in addition to time-kill studies. Proper time-kill studies can yield sufficient information on both rapidity of kill and when done at various dilution's the minimum cidal level of the active. This would yield more definitive information about the active as well as the product.

c- What does the time frame presented in 333.470(1)(iv)(D) represent. Are these contact times or sampling times? If contact times, they are not appropriate for the intended use of these products. If sampling times, are these times to sample after a given contact time or set of contact times?

d- The in-vivo testing requirements will often require more than 100 subjects. Currently the cost for a glove juice study is approximately $1000 per test subject. This increase in the number of subjects raises the cost for a study to the $100,000 range. This will effectively prohibit small regional companies from entering this market area. This is especially true if products for food handlers are included since this market is served by a large number of small to mid size regional manufacturers.

It is hoped that the agency will consider these comments in their deliberations on these complex issues. Thank you for your consideration.

Sincerely,

Gary R. Kramzar
Corporate Vice President

REFERENCES:

1- Nipa Laboratories, July 16, 1992 (75N0183); Testing of first aid antiseptic drug products.

2- NAMSA (75N0183 let 065); Determination of MIC of Chloroxylenol against Candida albicans and Trichophyton mentagrophytes.

3- Judis (75N0183 let 065); A comparison of in vitro activity of a 1% PCMX formulation.

4- Reckitt and Colman (75N0183 let 065); A comparison of the in vitro activity of Dettol.

5- Reckitt and Colman (75N0183 let 065); Scientific information on the 'in vitro' and 'in vivo' antimicrobial activity of Dettol.

6- Reckitt and Colman (75N0183 let 065); the bacteriostatic and bactericidal activity of Dettol against a range of recently isolated mesophilic strains.

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July 16, 1992

Dockets Management Branch
HFA-305 Food & Drug Administration
Room 1-23
12420 Parklawn Drive
Rockville, Maryland 20857

RE: DOCKET 75N0183 - CHLOROXYLENOL

Dear Sir:

In response to the July 22, 1991 FDA notice in the Federal Register regarding Topical Antimicrobial Drug Products for Over the Counter Human Use; Tentative Final Monograph for First Aid Antiseptic Drug Products, Proposed Rule (21 CFR 333 and 369).

I have enclosed efficacy data run substantially by the protocols published in 21 CFR 333.70. These data demonstrate the efficacy in the Bactericidal and Bacteriostatic assay at levels as low as 0.5-0.75% Chloroxylenol BP. We, therefore, request that the status of Chloroxylenol be changed in the final monograph from Category III to Category I for efficacy in these applications.

Please contact me if there are any comments or questions on this report.

Sincerely,

Gary R. Kramzar
Vice President

GRK/kb
Attachments

Nipa Laboratories Ltd.

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* 333.70 TESTING OF FIRST AID ANTISEPTIC

DRUG PRODUCTS

EVALUATION OF FIRST AID ANTISEPTIC ACTIVITY OF

NIPACIDE PX (Chloroxylenol BP Lot No. W218-1)

STUDY DIRECTOR

J.A. Parr, PhD
Business Director - Biocides

DATE: 26 MARCH 1992

LABORATORY PERSONNEL

C.A. Seldon, HNC
Laboratory Manager

P.M. Smith, HND
Product Development Microbiologist

REPORT ON CHLOROXYLENOL (NIPACIDE PX)

FDA TESTING

ORGANISATION

1. Summary and Conclusions

2. Discussion of Results, Protocol, Modifications.

3. Bactericidal/Bacteriostatic test results.

4. Methods including copy of FDA REG. and procedure used.

5. Neutraliser inactivation of antiseptic neutraliser effect on cell viability and test organism resistance results.

1. SUMMARY AND CONCLUSIONS

1. SUMMARY AND CONCLUSIONS

Test were carried out on Nipacide PX (BP grade Lot No. W218-1) in propylene glycol 60:40 water as per the test protocol of the Federal Register/Vol. 58. No. 140. July 1991 (Proposed Rules) for testing of first aid drug products.

Preliminary screening test of a wide range of concentrations of Nipacide PX i.e. 0.5%, 1.5%, 2.0%, 3.0% and 3.5% was followed by a final test of a narrow range of concentrations of Nipacide PX i.e. 0.5%, 0.75%, 1.0%, 1.25% and 1.5%.

The bactericidal and bacteriostatic test protocols do not specifically request the evaluation of the formulation without antiseptic.

The preliminary screening test did not include the carrier system (i.e. propylene glycol 60:40 water) without antiseptic.

The final test included the carrier system (i.e. propylene glycol 60:40 water) without antiseptic to ensure that there was no adverse effect of the carrier system on the test species.

BACTERICIDAL ASSAY (1.2)

Preliminary screening of Nipacide PX concentrations (0.5% to 3.5%) indicated that Nipacide PX at a minimum concentration of 1.5% passed the bactericidal test criteria, however 0.5% marginally failed.

Final testing of a narrow range of Nipacide PX concentrations (0.5% to 1.5%) indicated that Nipacide PX at a minimum concentration of 0.5% passed the bactericidal test criteria.

It was concluded that Nipacide PX at a minimum concentration of 0.5% to 0.75% passed the bactericidal test criteria.

BACTERIOSTATIC ASSAY (1.3)

Preliminary screening of Nipacide PX concentrations 0.5% to 3.5% indicated that Nipacide PX at a minimum concentration of 0.5% when diluted 1:120, passed the bacteriostatic test criteria.

Final testing of a narrow range of Nipacide PX concentrations 0.5% to 1.5% indicated that Nipacide PX at a minimum concentration of 0.75% when diluted 1:120 passed the bacteriostatic test criteria, however 0.5% Nipacide PX diluted 1:120 marginally failed.

It was concluded that Nipacide PX at a minimum concentration of 0.5% to 0.75% when diluted 1:120 passed the bacteriostatic test criteria.

2. DISCUSSION OF RESULTS, PROTOCOL, MODIFICATIONS

DISCUSSION OF RESULTS, PROTOCOL, MODIFICATIONS

The test protocol of the Federal Register/vol.58.No.140. Jul 1991. (Proposed Rules) for testing of first aid antiseptic dru products was followed in exact detail. (APPENDIX I)

The test protocol consists of five independent procedures, thre preliminary tests to ensure the efficacy of the neutralise inactivation of the antiseptic, the neutraliser effect on cel viability and test organism resistance, and two test procedure bactericidal and bacteriostatic.

Preliminary screening of Nipacide PX to the bactericidal tes procedure was carried out over a wide range of concentration i.e. 0.5%, 1.5%, 2.0%, 3.0% and 3.5% followed by final testing o a narrow range of Nipacide PX i.e. 0.5%, 0.75%, 1.0%, 1.25% an 1.5%.

The bacteriostatic test was carried out on a 1:120 dilution o the preliminary wide range of concentrations of Nipacide PX i.e 0.5%, 1.5%, 2.0%, 3.0% and 3.5% and the final testing range o 0.5%, 0.75%, 1.0%, 1.25% and 1.5%.

This test is valid for those antiseptics that are water solubl and/or miscible and that can be neutralised by one of the speci fied subculture media or that can be overcome by dilution.

Nipacide PX is sparingly soluble in water (0.03% w/v), therefor Nipacide PX was tested in a propylene glycol 60:40 water system.

MEDIA

Tryptone Soya Broth (Oxoid CM129) with and without neutralisers.

Tryptone Soya Agar (Oxoid CM131) with and without neutralisers.

5% w/v Phenol (B.D.H.10188)

Fetal Calf Serum (heat inactivated) (Sizma F-4135)

Sterile Water

Inactivators - added such that 5 g Soya Bean Lecithin (C.L.R), 40 ml Tween 80 ((I.C.I. Surfactants) neutralisers in 1000 cm^3).

TEST ORGANISMS

Staphylococcus aureus	ATCC 6538	(NCTC 8625)
Pseudomonas aeruginosa	ATCC 9027	(NCTC 8626)
Escherichia coli	ATCC 8739	(NCTC 8545)

MATERIALS

[illegible] (chloroxylenol BP)
Lot No. W218-1
Certificate of analysis attached [illegible]

3. BACTERICIDAL/BACTEROSTATIC TEST RESULTS

TEST PROCEDURE 1.2 : BACTERICIDAL TEST RESULTS

Preliminary Test Results

a. *Staphylococcus aureus* ATCC 6538
Initial inoculum level 2.0×10^7 colony forming units per ml

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.5% Nipacide PX	<10	<10	<10
1.5% Nipacide PX	<10	<10	<10
2.0% Nipacide PX	<10	<10	<10
3.0% Nipacide PX	<10	<10	<10
3.5% Nipacide PX	<10	<10	<10

b *Pseudomonas aeruginosa* ATCC 9027
Initial inoculum level 4.0×10^7 colony forming units per ml.

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.5% Nipacide PX	4.2×10^4	1.3×10^4	3.9×10^4
1.5% Nipacide PX	<10	<10	<10
2.0% Nipacide PX	<10	<10	<10
3.0% Nipacide PX	<10	<10	<10
3.5% Nipacide PX	<10	<10	<10

c. *Escherichia coli* ATCC 8739
Initial inoculum level 1.9×10^7 colony forming units per ml.

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.5% Nipacide PX	4.2×10^3	7.5×10^4	9.6×10^4
1.5% Nipacide PX	<10	<10	<10
2.0% Nipacide PX	<10	<10	<10
3.0% Nipacide PX	<10	<10	<10
3.5% Nipacide PX	<10	<10	<10

TEST PROCEDURE 1.2 : FINAL TEST RESULTS

a. Staphylococcus aureus ATCC 6538
Initial inoculum level = 1.4×10^7 Colony forming units per ml.

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.0% Nipacide PX	4.9×10^6	3.7×10^6	3.4×10^6
0.5% Nipacide PX	<10	<10	<10
0.75% Nipacide PX	<10	<10	<10
1.0% Nipacide PX	<10	<10	<10
1.25% Nipacide PX	<10	<10	<10
1.5% Nipacide PX	<10	<10	<10

b Pseudomonas aeruginosa ATCC 9027
Initial inoculum level = 2.2×10^7 colony forming units per ml.

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.0% Nipacide PX	1.5×10^6	3.1×10^6	1.9×10^6
0.5% Nipacide PX	<10	<10	<10
0.75% Nipacide PX	<10	<10	<10
1.0% Nipacide PX	<10	<10	<10
1.25% Nipacide PX	<10	<10	<10
1.5% Nipacide PX	<10	<10	<10

c. Escherichia coli ATCC 8739
Initial inoculum level = 1.3 x 10^7 colony forming units per ml.

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.0% Nipacide PX	$6.4x10^6$	$1.6x10^6$	$4.8x10^6$
0.5% Nipacide PX	<10	<10	<10
0.75% Nipacide PX	<10	<10	<10
1.0% Nipacide PX	<10	<10	<10
1.25% Nipacide PX	<10	<10	<10
1.5% Nipacide PX	<10	<10	<10

CONCLUSIONS AND OBSERVATIONS

Preliminary test results indicate that Nipacide PX at a minimu concentration of 1.5% passed the bactericidal test criteria, however, 0.5% Nipacide PX marginally failed.

Final test results indicate that Nipacide PX at a minimum concentration of 0.5% passed the bactericidal test criteria.

It was concluded that Nipacide PX at a minimum concentration o 0.5 - 0.75% passed the bactericidal test criteria.

Preliminary Test Results

a. Staphylococcus aureus ATCC 6538
Initial inoculum level 1.5×10^8 colony forming units per ml

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS Aliquot 1	Aliquot 2	Aliquot 3
0.5% Nipacide PX	- (<10)	- (<10)	- (<10)
1.5% Nipacide PX	- (<10)	- (<10)	- (<10)
2.0% Nipacide PX	- (<10)	- (<10)	- (<10)
3.0% Nipacide PX	- (<10)	- (<10)	- (<10)
3.5% Nipacide PX	- (<10)	- (<10)	- (<10)

b. Pseudomonas aeruginosa ATCC 9027
Initial inoculum level 2.3×10^8 colony forming units per ml.

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS Aliquot 1	Aliquot 2	Aliquot 3
0.5% Nipacide PX	- (<10)	- (<10)	- (<10)
1.5% Nipacide PX	- (<10)	- (<10)	- (<10)
2.0% Nipacide PX	- (<10)	- (<10)	- (<10)
3.0% Nipacide PX	- (<10)	- (<10)	- (<10)
3.5% Nipacide PX	- (<10)	- (<10)	- (<10)

c. Escherichia coli ATCC 8739
Initial inoculum level 1.5×10^8 colony forming units per ml.

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS Aliquot 1	Aliquot 2	Aliquot 3
0.5% Nipacide PX	- (<10)	- (<10)	- (<10)
1.5% Nipacide PX	- (<10)	- (<10)	- (<10)
2.0% Nipacide PX	- (<10)	- (<10)	- (<10)
3.0% Nipacide PX	- (<10)	- (<10)	- (<10)
3.5% Nipacide PX	- (<10)	- (<10)	- (<10)

a. Staphylococcus aureus ATCC 6538
Initial inoculum level = 1.44×10^7 colony forming units per ml

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.0% Nipacide PX	+ (4.1×10^6)	+ (2.1×10^6)	+ (6.3×10^6)
0.5% Nipacide PX	- (<10)	- (<10)	- (<10)
0.75% Nipacide PX	- (<10)	- (<10)	- (<10)
1.0% Nipacide PX	- (<10)	- (<10)	- (<10)
1.25% Nipacide PX	- (<10)	- (<10)	- (<10)
1.5% Nipacide PX	- (<10)	- (<10)	- (<10)

b. Pseudomonas aeruginosa ATCC 9027
Initial inoculum level = 2.16×10^7 colony forming units per ml.

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.0% Nipacide PX	+ (2.4×10^6)	+ (2.9×10^6)	+ (1.0×10^6)
0.5% Nipacide PX	+ (1.3×10^4)	+ (2.8×10^4)	+ (2.1×10^4)
0.75% Nipacide PX	- (<10)	- (<10)	- (<10)
1.0% Nipacide PX	- (<10)	- (<10)	- (<10)
1.25% Nipacide PX	- (<10)	- (<10)	- (<10)
1.5% Nipacide PX	- (<10)	- (<10)	- (<10)

c. *Escherichia coli* ATCC 8739
Initial inoculum level = 1.32×10^7 colony forming units per ml

SAMPLE	COLONY FORMING UNITS PER ML AFTER 10 MINS		
	Aliquot 1	Aliquot 2	Aliquot 3
0.0% Nipacide PX	+ (5.0×10^6)	+ (3.4×10^6)	+ (5.1×10^6)
0.5% Nipacide PX	- (<10)	- (<10)	- (<10)
0.75% Nipacide PX	- (<10)	- (<10)	- (<10)
1.0% Nipacide PX	- (<10)	- (<10)	- (<10)
1.25% Nipacide PX	- (<10)	- (<10)	- (<10)
1.5% Nipacide PX	- (<10)	- (<10)	- (<10)

\+ growth (turbidity)

\- no growth

CONCLUSIONS

Preliminary test results indicate that at a minimum concentration of 0.5% Nipacide PX diluted 1:120 passed the bacteriostatic test criteria while final test results indicate that 0.5% Nipacide PX diluted 1:120 marginally failed, however, Nipacide PX 0.75% diluted 1:120 passed.

It was concluded that Nipacide PX at a minimum concentration of 0.5 - 0.75%, diluted 1:120 passed the bacteriostatic test criteria.

4. METHODS = INCLUDING COPY OF FDA REG. AND PROCEDURE USED

METHODS - INCLUDING COPY OF FDA REG AND PROCEDURE USED

1. NEUTRALISER INACTIVATION OF ANTISEPTIC

The efficacy of the neutraliser for the test antiseptic was evaluated by pipetting 0.8 ml of the antiseptic (0.8 ml sterile water for controls), 9 mls of culture medium containing an appropriate antiseptic neutraliser and 2 mls of test culture in 50% serum (all prewarmed at 32°C for 5 mins), into a sterile container, which were incubated at 32°C for 10 minutes. The numbers of survivors were then determined by serial dilution and plate counting, using diluting and plating media containing appropriate inactivators.

CRITERIA

Results differing by greater than 20% between test and control cultures indicate that the neutraliser was ineffective in inactivating the antiseptic.

2. NEUTRALISER EFFECT ON BACTERIA VIABILITY TEST

The effect of the neutraliser on cell viability was determined by diluting aliquots of each test organism in culture media, without neutraliser, (as specified in paragraph (b) (3) (i)) and in the appropriate neutralising media (as specified in paragraph (b)(4)).

The numbers of bacteria was determined by serial dilution and plate counting in growth agar media containing the same neutralisers.

CRITERIA

The neutraliser effect on the cell was determined by comparing the numbers of micro-organisms growing on the media with and without added neutralisers.

Results differing by greater than 20% indicate that at the concentration utilised the antiseptic neutraliser alters the determination of viable cells in the test cultures.

3. TEST ORGANISM ANTISEPTIC RESISTANCE TEST

The resistance of each test organism to phenol at 20°C (as per A.O.A.C. 1980 "Phenol Coefficient Method") was determined to ensure that their antiseptic resistance properties had not altered substantially.

A 5% stock solution of Phenol was used to prepare final dilutions of 1:60, 1:70, 1:80, 1:90 and 1:100.

5 mls of each dilution was inoculated with 0.5 ml culture, after 5, 10 and 15 minutes 1 x 4 mm loop full was transferred into subculture media (Nutrient Broth), these were then incubated at 32°C for 48 hours and any visible growth (turbidity) was recorded.

ACCEPTABLE RESISTANCE TO PHENOL

PHENOL DILUTION	GROWTH (TURBIDITY) AFTER 5 mins	10 mins	15 mins
S. aureus			
1:60 dilution	+ or 0	+ or 0	0
1:70 dilution	+ or 0	+	+
E. coli			
1:90 dilution	+ or 0	+ or 0	0
1:100 dilution	+	+	+ or 0
P. aeruginosa			
1:80 dilution	+ or 0	+ or 0	0
1:90 dilution	+	+	+

\+ growth (turbidity)

0 no growth

TEST PROCEDURES 1 METHOD 1

1. Method validation

This test is valid for those antiseptics that are water soluble, water soluble and/or miscible and that can be neutralised by one of the subculture media (specified in (b) (3) and (b) (4)) or that can be over come by dilution.

Nipacide PX is sparingly soluble in water (0.03% w/v) therefore Nipacide PX was tested in a propylene glycol 60:40 water system, (i.e. water soluble).

2. Bactericidal Assay Procedure

1ml of serum 1 ml of appropriate test culture and 8 mls of test antiseptic (all prewarmed at 37°C for 5 minutes) were transferred to sterile containers (plastic universals), mixed and incubated at 32°C for 10 minutes.

Triplicate 1 ml aliquots were removed and the numbers of surviving bacteria were determined by serial dilution, in media containing appropriate neutralisers, and plate counting using plating media containing appropriate neutralisers.

CRITERIA

A first aid antiseptic drug i.e. Nipacide PX must decrease the numbers of bacteria per ml by a factor of not less than 10^3 within 10 minutes of challenge at 32°C in the presence of 10% Fetal Calf Serum (inactivated).

3. Bacteriostatic Assay Procedure

1 ml of serum, 1 ml of appropriate bacterial test culture and 8 mls of test antiseptic at its recommended use concentration, (all prewarmed at 32°C for 5 minutes) were transferred to sterile containers (plastic universals) and mixed well.

Triplicate 1 ml aliquots of these test mixtures were transferred into 100 mls of media without neutralisers these were then mixed and incubated at 32° for 48 hours. The numbers of surviving bacteria were determined by serial dilution media containing appropriate neutralisers, and plate counting using plating media containing appropriate neutralisers.

CRITERIA

A 1:120 dilution of the formulated drug product in growth medium without neutralisers must prevent an increase in the numbers of organisms from an inoculum of 10^8 organisms of test culture when incubated at 32°C for 48 hours.

5. NEUTRALISER INACTIVATION OF ANTISEPTIC, NEUTRALISER EFFECT ON BACTERIA VIABILITY AND TEST ORGANISM RESISTANCE RESULTS

1 NEUTRALISER INACTIVATIONS OF ANTISEPTIC PRELIMINARY TEST RESULTS

a. 2% Nipacide PX in Propylene Glycol 60:40 water

TEST SPECIES	COLONY FORMING UNITS PER ML AT:- ZERO	10 MINS
S. aureus	4.3×10^6	4.0×10^6
E. coli	2.8×10^6	3.0×10^6
P. aeruginosa	6.1×10^6	6.8×10^6

b. Control 100% Sterile water

TEST SPECIES	COLONY FORMING UNITS PER ML AT:- ZERO	10 MINS
S. aureus	4.3×10^6	4.9×10^6
E. coli	2.8×10^6	3.2×10^6
P. aeruginosa	6.1×10^6	7.5×10^6

1 NEUTRALISER INACTIVATION OF ANTISEPTIC

FINAL TEST RESULTS

a. 1.5% Nipacide PX in Propylene Glycol 60:40 water (i.e. highest dilution tested)

TEST SPECIES	COLONY FORMING UNITS PER ML AT:- ZERO	10 MINS
S. aureus	3.9×10^6	3.6×10^6
E. coli	6.1×10^6	5.4×10^6
P. aeruginosa	4.5×10^6	4.0×10^6

b. Control 2:- 100 % Sterile Water

TEST SPECIES	COLONY FORMING UNITS PER ML AT:- ZERO	10 mins
S. aureus	4.3×10^6	4.5×10^6
E. coli	5.0×10^6	5.5×10^6
P.aeruginosa	4.9×10^6	4.3×10^6

c. Control 1:- 60 % Propylene Glycol : 40 % water only (no Nipacide PX)

TEST SPECIES	COLONY FORMING UNITS PER ML AT:- ZERO	10 MINS
S. aureus	4.1×10^6	4.5×10^6
E. coli	5.8×10^6	6.0×10^6
P. aeruginosa	4.0×10^6	3.7×10^6

CONCLUSIONS AND OBSERVATIONS

Test results differed by less than 20% between the antiseptic and the control indicating that the neutraliser was effective and inactivated the antiseptic.

2 NEUTRALISER EFFECTS ON BACTERIA VIABILITY

FINAL TEST RESULTS

TEST SPECIES	COLONY FORMING UNITS PER ML IN BROTH	BROTH & INACTIVATORS
S. aureus	4.6×10^6	3.9×10^6
E. coli	9.1×10^6	8.7×10^6
P. aeruginosa	2.5×10^6	2.6×10^6

CONCLUSIONS AND OBSERVATIONS

Test results differed by less than 20% indicating that the antiseptic neutraliser did not alter the determination of the viable cells in the test cultures.

3 TEST ORGANISM RESISTANCE

PRELIMINARY/FINAL TEST RESULTS (identical)

PHENOL DILUTION	GROWTH (TURBIDITY) AFTER 5 mins	10 mins	15 mins
S. aureus			
1:60 dilution	O	O	O
1:70 dilution	+	+	+
E. coli			
1:90 dilution	+	0	O
1:100 dilution	+	+	+
P. aeruginosa			
1:80 dilution	+	+	O
1:90 dilution	+	+	+

0 no growth

\+ growth

CONCLUSIONS AND OBSERVATIONS

The resistance of each test organism to Phenol at 20°C was acceptable ensuring that their antiseptic resistance had not altered substantially.

NIPA LABORATORIES, INC.

3411 Silverside Road 104 Hagley Building Wilmington, DE 19810
Telephone (302) 478-1522 • FAX (302) 478-4097

July 16, 1992

Dockets Management Branch
HFA-305 Food & Drug Administration
Room 1-23
12420 Parklawn Drive
Rockville, Maryland 20857

RE: DOCKET 75N0183 - CHLOROXYLENOL

Dear Sir:

In response to the July 22, 1991 FDA notice in the Federal Register regarding Topical Antimicrobial Drug Products for Over the Counter Human Use; Tentative Final Monograph for First Aid Antiseptic Drug Products, Proposed Rule (21 CFR 333 and 369).

I have enclosed efficacy data run substantially by the protocols published in 21 CFR 333.70. These data demonstrate the efficacy in the Bactericidal and Bacteriostatic assay at levels as low as 0.5-0.75% Chloroxylenol BP. We, therefore, request that the status of Chloroxylenol be changed in the final monograph from Category III to Category I for efficacy in these applications.

Please contact me if there are any comments or questions on this report.

Sincerely,

Gary R. Kramzar
Vice President

GRK/kb
Attachments

Determination of Minimal Inhibitory Concentration (MIC) of chloroxylenol (PCMX) against Candida albicans and Trichophyton mentagrophytes.
Performed by NAMSA - North American Science Associates.
Submitted by Ottawa Chemical Company.

An MIC determination was made using PCMX solubilized in propylene glycol against Candida albicans and Trichophyton mentagrophytes. The results of the 2-fold tube dilution were Candida albicans - .125 mg/ml or 125 ppm and Trichyphyton mentagrophytes - 1.0 mg/ml or 1000 ppm.

EXIDE, INC.®

NORTH AMERICAN SCIENCE ASSOCIATES, INCORPORATED

61 Tracy Road • Northwood, Ohio 43619 • Phone 419/666-9455

OTC Vol. 070327

Ottawa Chemical Division
Ferro Corporation
700 North Wheeling Street
Toledo, OH 43605

Attn: Mr. J. Watkins

Lab No 79-35

Lot No - -

P O No 41331

Material(s) Parachorometaxylenol (PCMX) in Glycol Carrier Solution. Carrier Solution without PCMX. (MIC) MG31-01

Determination of the Minimum Inhibitory Concentration (MIC) of Parachlorometaxylenol (PCMX) against Candida albicans and Tricophyton mentagrophytes.

I. Materials

A. Test tubes with closures, 20 mm x 110 mm, sterile.
B. Test tubes with closures, 10 mm x 75 mm, sterile.
C. Petri dishes, 100 mm x 15 mm, plastic, sterile.
D. Propylene glycol, Harleco #41101.
E. Pipettes, 10 ml, serological, sterile.
F. Pipettes, 1 ml serological, sterile.
G. Balance, OHaus, triple beam.
H. Candida albicans, ATCC 10231.
I. Tricophyton mentagrophytes, ATCC 9553.
J. Soybean casein digest broth (SCD), sterile.
K. Plate count agar (PCA), sterile.
L. Peptone, 0.1%, 9 mls in test tubes, sterile.
M. Incubator, 35 ± 2°C.

II. Methods

A. Sample preparation:

Eight (8) grams of parachlorometaxylenol (PCMX) was added to 100 ml propylene glycol in a screw capped bottle. Sample was shaken until PCMX was dissolved.

B. Inoculum preparation:

1. Candida albicans, ATCC 10231. A loopful of organism from a slant culture was transferred to 10 ml of sterile SCD broth. The broth culture was incubated at 35 ± 2°C for 24 hours. Following incubation, a 1:1000 dilution was made in sterile water.

Completed 1-17-79 Tech BH Approved by

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Lab. No 79-35
Lot No - -
P O No 41331

Material(s) Parachorometaxylenol (PCMX) in Glycol Carrier Solution. Carrier Solution without PCMX. (MIC) MG31-01

B Inoculum preparation (cont'd)

2. Tricophyton mentagrophytes, ATCC 9553. Several loopfuls of organism from a slant culture were transferred to 10 mls SCD broth. The broth culture was incubated at 35 ± 2°C for 24 hours. Following incubation, a 1:1000 dilution was made in sterile water.

C. Range Finding MIC

1. Test Series:

Two series of 11 sterile tubes were prepared by adding 9 ml of sterile SCD broth to tube #1 and 0.9 ml to tubes #2 through #11 in each series. A 1.0 ml aliquot of the PCMX/glycol was added to tube #1. This tube was thoroughly mixed after which ten-fold serial dilutions were made using 0.1 ml transfers (to 0.9 ml SCD broth) through tube #10. Tube #11 in each series received no PCMX/glycol.

2. Control Series:

Two series of tubes were prepared as described above. However, in the control series, the PCMX/glycol was replaced by propylene glycol only.

3. Inoculation:

All tubes in one test series and in one control series were each inoculated with 1 ml of the 1:1000 dilution of C. albicans. All tubes in the second test series and second control series were each inoculated with 1 ml of the 1:1000 dilution of T. mentagrophytes.

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Attn: Mr. J. Watkins

Lab No 79-35

Lot No - -

P O. No 41331

Material(s) Parachorometaxylenol (PCMX) in Glycol Carrier Solution. Carrier Solution without PCMX. (MIC) MG31-01

D. Final MIC - Test sample concentrations based on range finding MIC resu

1. Test Series:

Two series of 11 sterile tubes were prepared by adding 9 ml of sterile SCD broth to tube #1 and 0.5 ml SCD broth to tubes #2 through #11 in each series. A 1.0 ml aliquot of the PCMX/glycol was added to each tube and the tubes were throughly mixed. Serial two-fold dilutions were made using 0.5 ml transfer through tubes #10. Tube #11 in each series received no PCMX/glycol.

All tubes in one test series were each inoculated with 0.5 ml of the 1:1000 silution of C. albicans. All tubes in the second test series were each inoculated with 0.5 ml of the 1:1000 dilution of T. mentagrophytes.

E. Inoculum Population Verification

1. The population of challenge organism present in each 1:1000 dilution used to inoculate all tube series was determined using standard pour plate count technique in PCA. Plates were incubated at 35 - 2°C for 48 hours.

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2061 Tracy Road • Northwood, Ohio 43619 • Phone 419/666-9455

Ottawa Chemical Division

Lab No 79-35 - -

Lot No. _____

P.O No 41331

Material(s): Parachorometaxylenol (PCMX) in Glycol Carrier Solution. Carrier Solution without PCMX. (MIC) MG31-01

RESULTS:

Tube	1	2	3	4	5	6	7	8	9	10	11	- Control
PCMX mg/ml	8	8×10^{-1}	8×10^{-2}	8×10^{-3}	8×10^{-4}	8×10^{-5}	8×10^{-6}	8×10^{-7}	8×10^{-8}	8×10^{-9}		0
C. albicans	-	-	+	+	+	+	+	+	+	+		+
T. mentagrophytes	-	+	+	+	+	+	+	+	+	+		+

Tube	1	2	3	4	5	6	7	8	9	10	11	- Control
Glycol %	10	1	.1	1×10^{-2}	1×10^{-3}	1×10^{-4}	1×10^{-5}	1×10^{-6}	1×10^{-7}	1×10^{-8}		0
C. albicans	+	+	+	+	+	+	+	+	+	+		+
T. mentagrophytes	+	+	+	+	+	+	+	+	+	+		+

+ = Growth

- = No Growth

Completed 1-17-79 Tech BH Approved by _____

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Ottawa Chemical Division

Lab No 79-35 - -

Lot No

P O No 41331

Material(s): Parachorometaxylenol (PCMX) in Glycol Carrier Solution. Carrier Solution without PCMX. (MIC) MG31-01

Tube	1	2	3	4	5	6	7	8	9	10	11 - Control
PCMX mg/ml	8	4	2	1	0.5	0.25	0.125	.06	.03	.015	0
C. albicans	-	-	-	-	-	-	-	+	+	+	+
T. mentagrophytes	-	-	-	-	+	+	+	+	+	+	+

+ = Growth

- = No Growth

Completed 1-17-79 Tech BH Approved by

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Ottawa Chemical Division

Lab No 79-35

Lot No - -

P.O. No 41331

Material(s) Parachorometaxylenol (PCMX) in Glycol Carrier Solution. Carrier Solution without PCMX. (MIC) MG31-01

Plate Count Results:

	Dilution for Total Plate Count				
C. albicans 1:1000 dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
	TNC	47	5	0	0

Thus, the 1:1000 dilution contains approximately 4,700 organisms/ml.

T. mentagrophytes 1:1000 dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
	202	14	2	0	0

Thus, the 1:1000 dilution contains approximately 2,020 organisms/ml.

DISCUSSION:

The above data indicate that parachlorometaxylenol in propylene glycol inhibited the growth of the test organisms at the following concentration:

Candida albicans - .125 mg/ml (125 ppm)

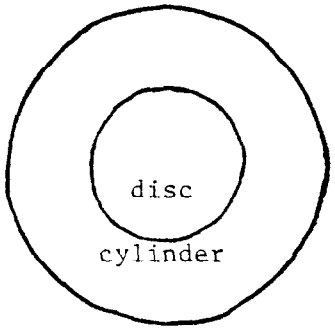
Tricophyton mentagrophytes - 1.0 mg/ml (1000 ppm)

Completed 1-17-79 Tech RH Approved by

Judis, J. 1958. From the University of Toledo for Ottawa Chemical Company.

A comparison of in vitro activity of a 1% PCMX formulation with Isodine when tested against Candida albicans and Trichomonas vaginalis.

Antibiotic Assay Cylinder Technique:



measurement recorded

Zone

The zone sizes recorded are measured in an old-fashioned way. They should at least be doubled. Cylinder width not known. This means that 5 ml at 17% PCMX solution gives at least a 22 minimum zone size.

Phenol coefficient-type values were determined for various dilutions of PCMX. Dilutions of PCMX in water or Isodine in water were tested for growth on subculture after a timed exposure of 5, 10 or 15 minutes.

The same procedures were used for the determination of phenol-coefficient type values for Trichomonas vaginalis.

Both PCMX and Isodine (Iodophor) had about the same activity against C. albicans, but only PCMX was active against T. vaginalis.

EXIDE, INC.®

Report of Investigation

27

UNIVERSITY OF TOLEDO

TOLEDO, OHIO 43605

COLLEGE OF PHARMACY

April 10, 1958

Report to: Dr. Sol Boyk
Ottawa Chemical Division
700 N. Wheeling Street
Toledo, Ohio 43605

Re: A comparison of the in vitro activities of PCMX (1% PCMX, 3.2% potassium ricinoleate in water) and Isodine (PVP - Iodine) against Candida albicans and Trichomonas vaginalis.

I ACTIVITY AGAINST CANDIDA ALBICANS

Test organism: The test organism used was Candida albicans ATCC 10231, reported as having been originally isolated from a clinical case of pneumonia. It was carried on Sabouraud's maltose agar and incubated at 37°C for growth.

Testing techniques and results:

1. Antibiotic assay cylinder technique. A preliminary survey of the ability of the two compounds to inhibit the growth of Candida albicans was done using glass antibiotic assay cylinders placed on seeded Sabouraud's maltose agar. The growth from a 24 hour slant of Candida albicans was washed off with 10 ml. of sterile distilled water and 1 ml. of the suspension used to seed 100 ml. of melted Sabouraud's maltose agar (brought to 48°C). Plates were poured with the seeded agar and when the latter hardened, sterile antibiotic assay cylinders were placed on the agar. Approximately 0.2 ml. of various dilutions of PCMX and Isodine were placed in the cylinders and the plates were incubated at 37°C for 48 hours before reading.

Results:

The results obtained are given in the table that follows. The size of the zone was determined as the distance from the edge of the cylinder to the adjacent edge of the zone.

Table 1. Activity of PCMX and Isodine against Candida albicans as determined by the antibiotic assay cylinder technique.

Dilution of PCMX		Size of zone	Dilution of ISODINE		Size of Zone
ml. of 1% PCMX	ml. of water		ml. of ISODINE	ml. of water	
5.0	0	11 mm.	5.0	0	2 mm.
2.0	3.0	0 mm.	2.0	3.0	0 mm.
1.0	4.0	0 mm.	1.0	4.0	0 mm.
0.67	4.33	0 mm.	0.67	4.33	0 mm.
0.50	4.50	0 mm.	0.50	4.50	0 mm.
0.25	4.75	0 mm.	0.25	4.75	0 mm.

Conclusions: This technique could not yield a satisfactory comparison because of the apparent inability of the compounds to diffuse through the agar.

2. Exposure of Candida albicans suspensions to various dilutions of the agents with subcultures to determine the presence of survivors.*

This method is similar to the official phenol coefficient method except that a single exposure time was used rather than subculturing at 5, 10 and 15 minutes as is done in the phenol coefficient method. Various dilutions of PCMX solution and Isodine were made in distilled water to give a final volume of 5 ml. and 0.5 ml. of a suspension of Candida albicans ATCC 10231 was added. The suspension consisted either of a 24-36 hour culture in glucose-peptone broth or the growth from a 24-30 hour old Sabouraud's maltose agar slant suspended in 10 ml. of sterile distilled water. The composition of the glucose-peptone broth was as follows: glucose, 8 gm., Bacto-peptone, 2 gm., distilled water, 200 ml., pH adjusted to 6.0 and 5 ml. were placed in 19 mm. by 150 mm. test tubes. Exactly 8 minutes after the Candida albicans suspension was added to the dilution of PCMX or Isodine, a loopful was removed and added to a sterile tube of glucose-peptone broth. The latter was incubated at 37°C to determine the presence or absence of growth, and thus, respectively, the presence or absence of survivors from the exposure to the particular dilution of PCMX or Isodine. Exposure to the monilicidal agents was done at room temperature, (23°C). In one series, Candida albicans was exposed to aqueous dilutions of the PCMX or Isodine while in a second series, human serum was added to the dilutions. Subcultures of the dilutions of monilicidal agents was done at 37°C.

* A similar method is described by Hesseltine, H., 1939. Experimental and clinical therapy of vulvovaginal mycoses. Am. J. Obstet, Gynecol., 34, 439.

Results:

Series A (without serum present)

ml. of 1% PCMX or ml. of Isodine	ml. of water	growth upon subculture when exposed to: PCMX	ISODINE
2.0	3.0	-	-
1.0	4.0	-	-
0.67	4.33	-	-
0.50	4.50	-	-
0.25	4.75	-	-
0.10	4.90	++++	-
0.067	4.93	++++	-
0.050	4.95	++++	-
0.010	4.99	++++	-
Control		++++	

Series B (C. albicans exposed to agents in the presence of 10% serum)

ml. of 1% PCMX or ml. of Isodine	ml. of water	ml. of serum	growth upon subculture when exposed to: PCMX	ISODINE
2.0	2.5	0.5	-	-
1.0	3.5	0.5	-	-
0.67	3.83	0.5	+	-
0.50	4.00	0.5	+	-
0.25	4.25	0.5	++	-
0.10	4.40	0.5	++++	++++
0.05	4.45	0.5	++++	++++
Control			++++	

Conclusions: Candida albicans, under the above test conditions, is killed by much higher dilutions of Isodine than PCMX (in terms of the solutions used here). However, under more physiological conditions, such as in the presence of serum, the monilicidal powers of Isodine are considerably reduced and might even be more significantly reduced in the presence of tissue or other body fluids, although this would have to be demonstrated.

3. The ability of Candida albicans to grow in the presence of various amounts of PCMX or Isodine.

Glucose-peptone broth was tubed in 5 ml. amounts and the indicated volume of 1% PCMX or Isodine solution was added to the tube of broth. The inoculum consisted of 0.1 ml. of a suspension of the growth on a 24 hour old Sabouraud's maltose agar slant culture (incubated at 37°C), in 10 ml. of sterile, distilled water. After inoculation, the broths were incubated at 37°C for 24 hours and re-examined for growth at the end of 48 hours. Longer incubation did not change the results.

II ACTIVITY AGAINST TRICHOMONAS VAGINALIS

Test Organism: The culture of Trichomonas vaginalis was the C strain isolated at Abbott Laboratories in 1951 and was bacteria-free. It was cultured in C. P.L.M. medium* and transferred tri-weekly. Incubation was at 37°C and the medium was supplemented with 10% sterile beef serum.

Method: Various amounts of 1% PCMX solution and Isodine were added to tubes of C.P.L.M. medium. The latter was tubed in 8.25 amounts in screw cap test tubes. After the addition of the PCMX or Isodine, the tubes were seeded with 0.1 m. of a 48 hour culture of Trichomonas vaginalis in the same medium. The tubes were examined for growth and mortility after 48 hours and 96 hours of incubation at 37°C.

Results:

Tube Number	ml. of undiluted Isodine or undiluted 1% PCMX	ml. of 1:10 Isodine or 1:10 dilution of 1% PCMX (diluted with water)	growth in the presence of: PCMX	ISODINE
1	0.083	-	-	++++
2	0.033	-	++	++++
3	0.016	-	++	++++
4	-	0.083	++++	++++
5	-	0.016	++++	++++
6	-	0.008	++++	++++
Control				++++

The tubes in which there was growth were examined microscopically for motility of the trichomonads and all tubes in which growth was ++++ had almost 100% normal motility. In the case, however, of tubes 2 and 3 containing PCMX, there was not only a diminution of growth, but also a large proportion of non-motile forms.

Conclusions: In the concentrations examined, the PCMX solution was capable of preventing the growth of Trichomonas vaginalis completely in a dilution representing 1:10,000 of the active ingredient, PCMX (i.e. a 1:100 dilution of a 1% solution) while the concentrations of Isodine had no effect on the growth of this protozoan. It is presumed that the large amount (10%) of serum in the medium reduced any potential trichomonostatic powers of Isodine but the absence of serum in any testing would not yield results of clinical significance.

Results:

ml. of 1% PCMX or Isodine per 5 ml. broth	growth in the presence of	
	PCMX	ISODINE
0.55	-	-
0.21	-	-
0.10	-	-
0.05	++++	++++
0.01	++++	++++
0.005	++++	++++

Control ++++

Conclusions: Under these test conditions, the abilities of 1% PCMX and Isodine solutions to prevent the growth of Candida albicans were the same.

The composition of C.P.L.M. medium is given by: Johnson, Garth and Trussell, 1943, experimental basis for the chemontherapy of Trichomonas vaginalis. Infestation I. Proc. Soc. Expt'l Biol. and Med. 54, 245 - 249.

Submitted by:

/s/ Joseph Judis
Joseph Judis, Ph. D.
Asst. Prof. Pharmacy

A comparison of the in vitro activity of Dettol, hexylresorcinol and benzalkonium chloride.
Submitted and performed by Reckitt and Colman.

This work is an in vitro comparison of the activity of these three antimicrobial chemicals. The rationale presented by the authors was to use benzalkonium chloride (BAC) and hexylresorcinol as a comparison to the activity of PCMX. They refer to the actions of the OTC Antimicrobial Panel in placing both BAC and hexylresorcinol in Category I as a skin wound cleanser.

These two chemicals then were selected because of their Category I status, which had not been given to any ingredients in the antimicrobial review for "antisepsis," or in fact, for use as any other specific antimicrobial product definition.

The concentration of the chemicals used were: Dettol, 4.8%; hexylresorcinol, 0.1% in 30% glycerol; and 1% aqueous BAC.

A variety of microorganisms, including fungi, were grown in appropriate media and prepared in suspension for testing. A fairly standard procedure using doubling dilutions of the antimicrobial chemical after drop inoculation was used to indicate activity by determining the tube with a level of antimicrobial which inhibited growth (7 days for bacteria; 21 for fungi).

Killing dilution of the chemical was determined by inoculation of dilutions. After 5 and 10 minutes, subculture aliquots were removed, neutralized and incubated.

Both 5% and 20% serum was added to the test diluent solutions for the determination of the killing dilution above.

The comparison in activities occurs as might be expected. Pseudomonas aeruginosa is a difficult organism for all of the chemicals. The fungi vary, but the activity of PCMX against the fungi is clear. PCMX is not inactivated as badly in the presence of 20% serum as the two other chemicals.

One should remember that the basic theory of MIC (minimal inhibitory concentration) as an indication of activity is that the MIC value is that concentration which inhibits growth (a hangover from antibiotic effectiveness and therapy because most antibiotics are bacteriostatic and are effective in this mode). When examining data on skin disinfection or degerming, the killing concentration or time-to-kill data are really more meaningful. Because BAC is essentially a bacteriostatic chemical, the MIC values are used comparatively. Furthermore, the inactivation of BAC with organic material is legendary and well documented.

From the data derived from this comparison, PCMX can certainly be regarded as an active antimicrobial, even in the presence of high organic loading (20% serum).

0522

Laboratory Report No. BL 73/23

A Comparison of the in-vitro activity of Dettol, Hexylresorcinol and Benzalkonium Chloride

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Summary

A comparison of the antimicrobial activity of Dettol, hexylresorcinol and benzalkonium chloride has been made. All three agents show good antimicrobial activity against a wide range of micro-organisms. However, the addition of organic matter considerably reduces the activity of benzalkonium chloride.

Dettol and hexylresorcinol are also inactivated by organic matter but not to such an extent as benzalkonium chloride.

Introduction:

So far very few compounds have been placed in Category 1 by the F.D.A. None have been approved for skin antisepsis but hexylresorcinol and benzalkonium chloride are two antimicrobial agents which have been approved for use as skin wound cleansers.

The F.D.A. require data comparing the submitted agent ie. PCMX with known antibacterial compounds. Hexylresorcinol and benzalkonium chloride seemed logical choices for comparative purposes.

A programme of work was devised comparing the activity of PCMX, hexylresorcinol and benzalkonium chloride against a wide range of vegetative bacteria and fungi. The effect of organic matter on activity was also determined.

Materials and Methods

Materials

Dettol (4.8% PCMX)

Hexylresorcinol (0.1% in 30% glycerol)

Benzalkonium chloride (1% aqueous solution)

Test organisms

All the bacteria except Mycobacterium fortuitum were grown in suitable nutrient broth for 24 hours at 37°C. Mycobacterium fortuitum was incubated for 72 hours at 37°C. Candida albicans was cultured in malt extract broth for 24 hours at 37°C. The dermatophyte fungi were grown on Sabouraud dextrose agar for ten days at 27°C. Spore suspensions were then prepared by homogenizing the mycelial mat and filtering off the mycelial elements.

Measurement of bacteriostatic and fungistatic activity

Doubling dilutions of the antimicrobial agents were prepared in nutrient broth for the bacteria and malt extract broth for the fungi. Each tube was inoculated with one drop of the organism under test and incubated at 37°C for seven days for the bacteria and Candida albicans. The dermatophyte fungi were incubated at 27°C for 21 days. The minimum concentration of the antimicrobial agent which inhibited the growth of the organism was recorded. The results are shown in Table 1.

Measurement of bactericidal and fungicidal activity

Dilutions of the antimicrobial agent were made in distilled water and placed in a water bath at 20°C. 0.5ml broth culture or spore suspension was added to 5ml antiseptic dilution. After 5 and 10 minutes contact time loopfuls of the antiseptic/organism mixture were inoculated into either nutrient broth or malt extract broth containing 0.3% lecithin and 1.0% Tween 80 as neutralizer.

After incubation at 37°C for 5 days for the bacteria and Candida albicans and at 27°C for 10 days for the dermatophyte fungi the dilution of the antimicrobial agent killing at 10 minutes was recorded. The results are shown in Tables 2-4.

The effect of Organic Matter

Dilutions of the antimicrobial agents were made up in 5% serum in distilled water and in some cases in 20% serum. The bactericidal and fungicidal activity was then determined as in the previous section. The results are shown in Tables 2-4.

Table 1

1. Minimum Inhibitory Concentrations

Organism	(1gm in - ml distilled water) PCMX in "Dettol" Formulation	Hexyl-resorcinol	Benzalkonium chloride
B. cereus	25600	4000	64000
Strep. faecalis	8300	16000	600000
Staph. aureus	25000	32000	204800
Micrococcus luteus	25000	32000	512000
Myco. fortuitum	40000	64000	512000
Ps. aeruginosa	800	< 2000	2000
Proteus vulgaris	13000	16000	32000
E. coli	13000	8000	32000
Klebs. aerog.	13000	4000	64000
Candida albicans	16000	32000	128000
Trich. rubrum	48000	256000	128000
Microsporum canis	49000	256000	64000
Trich. interdigitale	49000	256000	64000

Killing Dilutions (1gm in - ml)

Table 2: Gram-positive bacteria

Organism	Conditions	Benzalkonium chloride	Hexyl-resorcinol	PCMX Dettol
Staphylococcus aureus NCTC 4163	Dist. H_2O	20,000	20,000	8000
	5% serum	14,000	3,500	1000
	20% serum	3,000	<1,000	830
Streptococcus faecalis NCTC 8213	Dist. H_2O	40,000	6,000	8000
	5% serum	18,000	5,000	2000
	20% serum	2,400	< 1,000	780
Bacillus cereus	Dist. H_2O	40,000	12,000	4000
	5% serum	12,000	4,000	8000
Micrococcus luteus NCTC 2665	Dist. H_2O	6,000	6,000	6400
	5% serum	800	1,500	3120
Mycobacterium fortuitum NCTC 8573	Dist. H_2O	10,000	12,000	4000
	5% serum	2,000	3,000	4000

Table 3 : Gram-negative bacteria

Organism	Conditions	Benzalkonium chloride	Hexyl-resorcinol	PCMX Dett
E. coli NCTC 86	Dist. H_2O	30,000	12,000	7,600
	5% serum	16,000	4,500	2,800
	20% serum	3,000	< 1,000	3,300
Proteus vulgaris NCTC 4636	Dist. H_2O	15,000	6,000	7,000
	5% serum	10,000	5,000	4,000
	20% serum	12,000	3,000	3,120
Klebsiella aerogenes NCTC 9172	Dist. H_2O	10,000	4,000	6,000
	5% serum	3,000	1,750	4,500
	20% serum	300	< 1,000	1,040
Pseudomonas aeruginosa NCTC 1999	Dist. H_2O	6,000	< 1,000	800
	5% serum	2,250	< 1,000	400
	20% serum	400	< 1,000	< 200

Table 4 : Fungi

Organism	Conditions	Benzalkonium chloride	Hexyl-resorcinol	PCMX : Dettol
Candida albicans	Dist. H_2O	128,000	32,000	2,700
	5% serum	5,000	7,000	4,000
	20% serum	300	3,000	1,040
Trichophyton interdigitale	Dist. H_2O	12,000	3,000	3,125
	5% serum	4,000	4,000	3,125
	20% serum	1,000	3,000	2,400
Trichophyton rubrum	Dist. H_2O	15,000	6,000	6,250
	5% serum	10,000	4,000	4,800
Trichophyton mentagrophytes	Dist. H_2O	1,500	6,000	3,125
	5% serum	< 1,000	2,000	< 800
Microsporum canis	Dist. H_2O	40,000	5,000	3,125
	5% serum	< 5,000	3,000	2,500

Table 5

% germicidal activity retained in the presence of organic matter *

Organism	% serum	Benzalkonium chloride	Hexyl-resorcinol	PCMX in Dettol
Staphylococcus aureus NCTC 4163	5	70	17.5	33.3
	20	15	< 5	27.7
Streptococcus faecalis NCTC 8213	5	45	83.3	25
	20	6	<17	9.8
Escherichia coli NCTC 86	5	53.3	37.5	36.8
	20	1	< 8.3	43.4
Proteus vulgaris NCTC 4636	5	66.7	83.3	57.1
	20	80	50	44.5
Klebsiella aerogenes NCTC 8172	5	30	43.8	75
	20	3	<25	17.3
Ps. aeruginosa NCTC 1999	5	37.5	-	50
	20	6.7	-	<25
Candida albicans	5	3.9	21.9	100
	20	0.23	9.4	32.5
Trichophyton interdigitale	5	33.3	100	100
	20	8.3	100	76.2

* Killing dilution in distilled water/killing dilution in serum x 100%.

(MPS 7/21/76)

Discussion and Conclusions

As might be expected benzalkonium chloride shows extremely high bacteriostatic activity expecially against Gram-positive organisms. However, minimum inhibitory concentrations are of little practical use as the killing power of an antimicrobial agent is the important feature.

Again in the absence of organic matter benzalkonium chloride has high bactericidal activity against Gram-positive organisms. However, the addition of organic matter to the test system shows that benzalkonium chloride loses most of its antibacterial activity in the presence of 20% serum.

Neither hexylresorcinol or PCMX are inactivated to such an extent against both Gram-cositive and Gram-negative organisms. PCMX is considerably superior against Candica albicans.

Scientific information on the 'in vitro' and 'in vivo' antimicrobial activity of Dettol as determined in the Bacteriological Laboratories of Reckitt and Colman.

Dettol, which contains 4.8% para-chloro-meta-xylenol, was tested, using a modified AOAC phenol-coefficient procedure, on 168 strains of organisms (70 gram positive and 90 gram negative).

The results are expressed in dilutions which kill the inoculum in 10 minutes but not in 5. For topically used products, this type of expression is better than the representation of the minimal inhibitory concentration which reflects only the concentration to inhibit. The values listed as the dilution effective in killing can be calculated to give a minimal bactericidal concentration.

The results show activity against a wide variety of microorganisms including gram negatives. The activity is maintained in the presence of hard water or organic matter.

Both clinical isolates and conventional laboratory strains were used to test. Tests were done:

1. Killing dilution in hard water with calcium chloride calculated on the HO scale.
2. Killing dilution with organic material using 5% horse serum.
3. Killing dilution at elevated temperature.
4. Rapidity of bacterial kill using 30-second exposure.
5. Activity against dried organisms.

Activity, though reduced, was maintained with organic material and hard water. Elevated temperature increased activity.

EXIDE, INC.

In vivo antibacterial activity:

An in vivo test was performed by applying broth cultures onto the skin of the forearm followed by the addition of Dettol dilutions and allowing it to dry for 5 minutes. Following contact time, (5 or 10 min) cultures were done using the velvet transfer technique onto an agar surface. Extended contact periods were later used (2 or 4 hours) to demonstrate the persistent activity of the PCMX in Dettol.

Neutralizers were used in these in vitro and in vivo tests.

The attached tables of results are self-explanatory, but a chart showing dilution killing values converted to Minimal Bactericidal Concentration (MBC) in parts per million is attached for interest.

DETTOL

Scientific Information on the 'in-vitro' and 'in-vivo' antimicrobial activity of Dettol as determined in the Bacteriological Laboratories of Reckitt and Colman, Hull.

Summary

Dettol has been evaluated against a total of 168 strains of micro-organisms using a modified A.O.A.C. phenol co-efficient technique. Of these organisms 70 were Gram-positive, 89 Gram-negative, 8 strains of fungi and one protozoam. Forty of the strains were obtained as standard cultures from various official culture collections while the remainder were from hospital sources of infection.

Dettol was found to be bactericidal against this wide range of micro-organisms achieving a complete kill of the inoculum within 10 minutes at dilutions in excess of the recommended use-dilution. This activity, although slightly reduced, is maintained at dilutions in excess of the use-dilution when Dettol is evaluated in the presence of hard water and organic matter.

Pseudomonas aeruginosa was the organism most resistant to the activity of Dettol, certain strains required concentrations of Dettol in excess of the use-dilution to achieve a complete kill. However, dried cultures of Pseudomonas aeruginosa were rapidly killed by a 1:100 dilution of Dettol and at 37°C Dettol possessed a killing dilution of 1:60 against this organism in an aqueous system.

Higher temperatures (37°C) increased the bactericidal activity of Dettol and dried organisms were particularly sensitive to treatment for all cultures evaluated. In addition, Dettol at its use-dilution was found to be rapidly bactericidal killing all nine culture organisms tested within a ½ minute.

In a simulated 'in-vivo' experiment Dettol was found to reduce the number of organisms which had been artificially applied to the skin of human volunteers by levels of between 79 and 100% when compared to water.

1. Introduction

This series of experiments was designed to evaluate the spectrum of antibacterial activity of Dettol under a variety of test conditions.

2. EXPERIMENTAL PROCEDURES

2.1. Product

The standard production formula of Dettol was used throughout these experiments.

2.2. Micro-organisms and method of culture

(a) Standard Bacterial Cultures:

Except where stated, all standard bacterial cultures from official culture collections were grown in Nutrient Broth No. 2 (Oxoid Ltd) for three subsequent generations at 37°C for 18 hours prior to utilization in the product evaluation scheme. The exceptions were;

(1) Staphylococcus aureus: F.D.A. culture broth used of the following composition:-

Oxoid L37 Peptone	10 gms
Sodium Chloride	5 gms
Lab Lemco Powder	4 gms
Distilled water	to 1,000 mls
pH 6.8	

(2) Pseudomonas aeruginosa: D.A. culture broth used of the following composition:-

Eupeptone No. 1	10.0 gms
Sodium chloride	5.0 gms
Lab-Lemco buffer	5.0 gms
Distilled water	to 1,000 mls

(3) Streptococcus anaerobicans: Thioglycollate medium (U.S.P.) used as the growth medium, and 48 hours used as the generation time.

(4) Corynebacterium acnes: Thioglycollate medium (U.S.P.) used as the growth medium and 48 hours used as the generation time.

(5) Streptococcus pyogenes: 48 hours used as the generation time.

(6) Mycobacterium tuberculosis: Growth was achieved in Dubos medium with a generation time of seven days.

(7) Salmonella typhi: growth was achieved in Rideal Walker Broth (British Standard BS 541).

(b) Standard Fungal Cultures:

Except where stated all standard fungal cultures from official culture collections were grown on Malt Extract Agar for 10 days at 27°C. The resulting mycelium was scraped from the surface of the plate, homogenised in 10ml of Ringers solution and filtered through a sintered glass filter to obtain the spore suspension. This spore suspension was utilized for product evaluation.

The exceptions were;

(1) Candida albicans: grown for three successive generations in Malt Extract broth at 37°C for 18 hours.

(2) Pityrosporum ovale: grown on agar of the following composition for 5 days at 37°C:-

Malt Extract Agar	60 g
Ox-bile	20 g
Tween 40	10 ml
Glycerol mono-oleate	2.5 g
Distilled water	1,000 ml

The growth was scraped off, filtered to remove agar lumps and resuspended in 10 ml of Ringers solution.

(c) Hospital Isolates

Dettol has also been evaluated against a wide range of micro-organisms isolated from outbreaks of infection in hospitals. Except where stated all hospital isolates were cultured in the same medium used for the standard organisms for one generation of 18 hours at 37°C. The exceptions were;

(1) Haemolytic Streptococci. These organisms were cultured direct into cooked meat for 18 hours at 37°C and then transferred to Horse-flesh broth for 18 hours at 37°C.

The identification of all organisms tested was confirmed using the procedure of Cowan and Steele (Identification of Medical Bacteria, Cambridge University Press 1965)

2.3. Evaluation of in-vitro antibacterial activity

(a) Killing Dilution in distilled water

The killing dilution of the product is defined as the extent by which the product may be diluted and yet still kill the test organism within 10, but not 5, minutes. The method follows that of the A.O.A.C.

phenol co-efficient and has been modified only in so far that other organisms in addition to those normally recommended have also been tested.

A series of five dilutions of Dettol, each of five mls were made in distilled water in sterile containers and placed in a water bath at 20°C. 0.5 ml of test culture was added to each dilution. The mixture was agitated and loopfuls removed after 5 and 10 minutes contact time and transferred into a suitable sub-culture medium containing 0.3% Lecithin and 3% Tween 80 (LCTW 20) as neutralizer.

Bacterial subcultures were incubated at 37°C for 48 hours except for Mycobacterium tuberculosis where the incubation period was 21 days. Fungal sub-cultures were incubated at 27°C for ten days.

(b) Killing Dilution in Hard Water

The method follows the procedure described above except that dilutions of Dettol were prepared in hard water equivalent to 300 ppm calcium chloride calculated on the World Health Organisation Scale.

(c) Killing Dilution in the presence of organic material

The method follows the procedure described above except that dilutions of Dettol were prepared in 5% serum (Horse Serum, Oxoid Ltd.)

(d) Killing Dilution at elevated temperature

The method follows the procedure described above except that the product dilutions are maintained at 37°C.

(e) Rapidity of Bactericidal Activity

5 ml quantities of the specified Dettol dilution, diluted with either soft water, 5% serum or 2.5% blood, were maintained at 20°C in a water bath. 0.5 ml of a third generation broth culture of the organism under test is added to the 5 ml of product, mixed, and subcultures taken with a platinum loop at 30 second intervals into subculture broth containing neutralizer. The subculture broth was incubated at 37°C for 3 days.

(f) Bactericidal activity against dried organisms

Third generation cultures of Staphylococcus aureus (NCTC 4163) and Pseudomonas aeruginosa (NCTC 1903) were grown in the appropriate nutrient broth and applied to the flat bottoms of a series of glass tubes. After removing the excess fluid with a Pasteur pipette, the tubes were

dried for 3 hours at room temperature, then the flat bottoms of the tubes were immersed in either 1:40 or 1:100 Dettol dilutions. At intervals of ½, 2½, 5, 10 and 15 minutes the tubes were removed and washed for 15 seconds in tap water. The tubes were then pressed onto nutrient agar plates for 10 seconds and removed. After incubation for 48 hours at 37°C the number of colonies on the agar plate were counted.

A similar experiment was also conducted where the culture organism was mixed with milk (final concentration 20% v/v) before applying to the flat bottoms of the series of glass tubes.

2.4. Evaluation of 'in-vivo' antibacterial activity

Four equal sizedzones (1.5" diameter) were marked out on the forearms of human volunteer subjects. These areas were infected with one drop (0.02 ml) of a nutrient broth culture of the test organism which was spread evenly over the test zone. The areas were allowed to dry for five minutes. The dilution of Dettol under test (two drops, 0.04 ml) was applied to the zones and spread evenly over the area with a sterile glass rod. Water was applied to the control areas.

After 5 or 10 minutes contact time, a sterile velvet pad was used to transfer the remaining micro-organisms from the skin to the surface of an agar plate containing 0.3% Lecithin and 3% Tween 80 as neutralizer. The number of colonies which developed on the plate after incubation for 48 hours at 37°C was counted, and the percentage reduction in the number of viable organisms was calculated by comparison with control areas treated with water.

In order to illustrate the persistant effect of Dettol on the skin the above method was modified. The marked out areas on the forearm were subject to treatment with Dettol (or water in the control situation) for 2 or 4 hours before infection of the sites with the test culture. The percentage reduction after a 5 minutes contact time was determined by the velvet pad transfer technique, as in the previous experiment.

2.5. Evaluation of 'in-vitro' Trichomonacidal Activity

A series of dilutions of Dettol were prepared in distilled water and 1 ml amounts placed in sterile tubes plugged with cotton wool. Each tube was inoculated with 1 ml of Trichomonas vaginalis containing c. 400,000 flagellates per ml. Five strains of Trichomonas were used and the inoculums contained large numbers of healthy trichomonads. This peak of growth occurred at two days incubation in all strains except strains X. Strains X grew more slowly and the inoculum used in this instance was three days old.

The tubes of Dettol and culture were mixed thoroughly and left at room temperature for 1 hour. After intervals of 2 minutes, 10 minutes and 1 hour a sample of the contents of each tube was examined microscopically to assess the condition of the trichomonads. Control tubes, one containing 1 ml of distilled water and 1 ml of inoculum and one containing 1 ml of inoculum alone were included in each experiment.

2.6. Neutralization of Dettol

In order to nullify the antibacterial effect of Dettol it is necessary to incorporate a neutralizer into the subculture medium.

Two minimum inhibitory concentrations were evaluated for Dettol alone and Dettol combined with 0.3% Lecithin and 3% Tween, using the culture broth for appropriate standard organisms as the growth medium. Incubation was achieved for 4 days at 37°C.

3. Results

3.1. Neutralization of Dettol in subculture media

Minimum inhibitory concentrations were evaluated for Dettol, with and without the presence of 0.3% Lecithin and 3% Tween 80, against Staphylococcus aureus (NCTC 4163). The following M.I.C. figures were obtained;

	Minimum Inhibitory Conc. of Dettol	
	Without Neutralizer	With Neutralizer
Staphylococcus aureus	1:800	< 1:50
Escherichia coli	1:400	< 1:50
Salmonella typhi	1:800	< 1:50

The results show that the activity of Dettol is greatly reduced in the presence of Lecithin Tween. In the majority of experiments, particularly those determining the killing dilution of Dettol against the test organism, 1 loopful of Dettol/bacteria suspension is transferred to 5 ml of subculture broth. Thus a dilution factor of 1 to 500 is involved and such final concentrations of Dettol are more than adequately neutralized in the subculture broth by the presence of 0.3% Lecithin and 3% Tween 80.

3.2. Killing Dilutions of Dettol in distilled water against a range of Standard Bacterial Cultures

The results are shown in Table 1.

Dettol is active against a wide range of Gram-positive and Gram-negative bacteria at concentrations which are more dilute than the recommended use-dilution. This activity achieves a complete kill within a 10 minute contact time. The inoculum of bacterial suspension to the Dettol dilution contained a minimum of 1×10^6 viable organisms per ml in all cases.

3.3. Killing Dilutions of Dettol in distilled water against a range of Standard Fungal Cultures

The results are shown in Table 2.

Dettol is active at dilutions greater than the use-dilution against the dermatophytes and the two species of yeast examined. This activity achieves a complete kill within the 10 minute contact time. The inoculum of fungal spore suspension or yeast cell suspension used for evaluation contained a minimum of 1×10^6 viable spores (cells)/1 ml.

All fungal cultures were obtained from the Institute of Hygiene and Tropical Medicine, London, except Penicillium notatum and Aspergillus fumigatus. These were obtained from the Commonwealth Mycological Institute.

3.4. Killing dilutions of Dettol, in hard water, against a range of Standard Bacterial Cultures

Previous results have shown that Dettol, diluted in distilled water, is active against a wide range of bacteria. In practice, of course, disinfectants and antiseptics are usually diluted with tap water which may affect the performance of the product. In this experiment Dettol was diluted with hard water (equivalent to 300 ppm calcium chloride) which is equivalent to tap water which may be found in a particularly severe hard water area.

The results are shown in Table 3.

The activity of Dettol is only slightly reduced in the presence of hard water, and bactericidal activity is maintained at reasonable levels.

3.5. Killing Dilutions of Dettol, in the presence of organic matter, against a range of Standard Bacterial Cultures

Antiseptic and disinfectant products are often required to exert their antibacterial activity in the presence of body fluids, notably, urine, serum or blood. This experiment was designed to show the effectiveness of Dettol, in the presence of 5% serum, against a range of micro-organisms.

The results are shown in Table 3A.

As with most products, organic material does decrease the activity of Dettol. As with hard water, this decrease in activity is marginal and Dettol is still capable of exerting a complete kill against the organisms under test.

3.6. Killing Dilutions of Dettol, diluted in distilled water against a range of Standard Bacterial Cultures at 37°C.

Antiseptics, when applied to the skin, encounter a temperature somewhat higher than that (20°C) used in the standard killing dilution test procedures. With this in mind the killing dilutions of Dettol against a range of bacteria were assessed at 37°C.

The results are shown in Table 4.

Dettol possesses an improved antibacterial performance with increasing temperature. Thus, the results obtained from evaluation at

20°C are likely to underestimate the true performance of the product at skin temperature.

3.7. The rapidity of the bactericidal activity of Dettol

The effectiveness of Dettol at its recommended concentration can be judged by the length of time required for the dilution to kill a test inoculation of approximately 2×10^8 organisms when added to Dettol diluted with either soft water, 5% serum or 2.5% blood.

The results are shown in Table 5.

At its use-dilution, Dettol achieves a rapid kill of all organisms tested. In most cases a complete kill of the test inoculum is achieved within a ½ minute period. This occurs even in the presence of 5% serum or 2.5% blood.

3.8. The bactericidal activity of Dettol when acting on dried organisms

Most of the experiments described utilized micro-organisms in an aqueous environment. Very often, antiseptics and disinfectants are required to kill organisms in a dried state. Occasionally such organisms may even be embedded in films of organic matter. In attempts to represent these conditions in the laboratory, tests have been carried out in which dried films of organisms were formed on a glass surface and subjected to Dettol action.

The results are shown in Table 6.

The average time taken to kill the dried cultures was below 5 minutes in every case. Since Staphylococcus aureus appeared to be the most resistant organism in the dried state, further experiments, using a film of this organism embedded in milk, were performed.

The results are shown in Table 7.

The average time for Dettol to achieve a complete kill was increased to 21 minutes.

3.9. Killing Dilutions of Dettol against strains of haemolytic Streptococci isolated from hospital patients

Twenty three isolates of haemolytic streptococci were obtained from eight hospitals. The dilutions of Dettol required to kill the organisms were determined by the killing dilutions technique previously described.

The results are shown in Table 8 which also includes the source of the culture and the site of isolation.

All strains of haemolytic Streptococci were very sensitive to treatment by Dettol.

3.10. Killing Dilutions of Dettol against strains of Pseudomonas aeruginosa isolated from hospital patients

Thirty three isolates of Pseudomonas aeruginosa were obtained from fourteen hospitals. The cultures, as soon as they were received, were sub-cultured into broth, and after 18 hours, the cultures used to carry out bactericidal tests by the standard technique.

The results are shown in Table 9.

It is clear from these results that Pseudomonas aeruginosa is the organism which is most resistant to bactericidal activity of Dettol. Of the thirty three isolates tested, seventeen were killed by a 1:40 Dettol within a ten minute contact time, the remaining sixteen strains required higher concentrations of Dettol to achieve a complete kill of the inoculum.

3.11. Killing Dilutions of Dettol against strains of Escherichia coli isolated from hospital patients

Nine strains of E. coli were obtained from six hospitals. The strains were immediately subcultured into nutrient broth and evaluated against Dettol using the standard killing dilution technique.

The results are shown in Table 10.

All strains of E. coli were sensitive to dilutionsof Dettol in excess of the use-dilution.

3.12. Killing Dilutions of Dettol against strains of Staphylococcus aureus isolated from hospital patients

Thirty freshly isolated strains of Staphylococcus aureus were obtained from the pathological laboratories of seventeen hospitals. All original cultures were received on nutrient agar slopes and inoculum were placed immediately into culture broth.

The results are shown in Table 11.

A study of the table shows the fairly wide variation encountered in the resistance of staphylococci to Dettol. The variation may be due in part to the genetical differences between staphylococci from various sources, and in part to minor variations in the test. However, it is clear that a 1% solution of Dettol is capable of killing all but a few resistant strains in 10 minutes at 20°C and that even the most resistant are killed by a 2% solution in this time.

3.13. Killing Dilutions of Dettol against strains of Proteus vulgaris isolated from hospital patients

Cultures were collected over a period of six months from a number of hospitals throughout the country. In all, seventeen strains were collected from eleven different hospitals. As each culture arrived it was checked for purity and subcultured into nutrient broth. In addition each culture was subcultured monthly for six months and at the end of this time a broth culture initiated as before and the resistance of the strain to Dettol determined by the killing dilution technique. In this way each strain was tested immediately following receipt and after six months laboratory culture.

The results are shown in Table 12.

The resistance of these strains was determined with Dettol diluted in both distilled water and hard water (300 ppm). There is little difference between the results in hard and distilled water and variation between strains is probably not outside the limits of experimental error. It is apparent that Dettol is very effective against Proteus vulgaris and that this micro-organism is easily killed by the recommended dilutions for the product.

3.14. Killing Dilutions of Dettol against strains of Shigella sonnei isolated from the hospital environment

Eleven strains of Shigella sonnei were obtained from various hospitals.

The results are shown in Table 13, together with a direct comparison with phenol.

The results show Dettol to be effective in killing all strains of Shigella sonnei tested at concentrations far below the use-dilution. Compared with phenol, Dettol is approximately three times more effective.

4.1. The in-vivo bactericidal activity of Dettol acting on artificially contaminated skin

So far the data provided has related to 'in-vitro' tests. Whilst these have a place in establishing both the extent and range of antibacterial activity they may be criticised because in many cases they fail to represent conditions that apply in practice. In skin disinfection for example, the product is applied to surfaces at a pH somewhat removed from that normally used for 'in-vitro' tests; furthermore the product is required to work in the presence of skin secretions which are normally not incorporated into in-vitro' systems. The following experiment, the method is detailed in the experimental procedures, is designed to illustrate the bactericidal activity of products on the skin environment. Skin artificially inoculated with specific test organisms is used to obtain results which are more easily interpretable than those obtained from natural flora experiments.

The results are shown in Table 14.

Dettol achieves high reductions of the infected skin flora both at 5 and 10 minutes following application.

5.1. The 'in-vitro' Trichomonacidal activity of Dettol

The results are shown in Table 15.

A dilution of 1:400 Dettol was effective in killing all six strains of Trichomonas vaginalis'. Both water controls and inoculum controls showed healthy, active trichomonads present.

TABLE 1 The Bactericidal activity of Dettol, diluted in distilled water, against Standard Bacterial Cultures assessed by the Killing dilution technique

Organism	Culture broth	Sub culture broth	Killing dilution of Dettol
Staph. aureus NCTC 3750	FDA	Nutrient broth + LCTW 80	1:225
Staphylococcus aureus NCTC 4163	FDA	Nutrient broth + LCTW 80	1:160
Streptococcus viridans NCTC 3165	Nutrient broth	Nutrient broth + LCTW 80	1:425
Streptococcus pyogenes NCTC 8191	Nutrient broth	Nutrient broth + LCTW 80	1:500
Streptococcus pyogenes NCTC 8326	Nutrient broth	Nutrient broth + LCTW 80	1:200
Streptococcus faecalis NCTC 6213	Nutrient broth	Nutrient broth + LCTW 80	1:230
B. Subtilis (veg. form) (Lab. strain)	Nutrient broth	Nutrient broth + LCTW 80	1:675
B. cereus (veg. form) (Lab. strain)	Nutrient broth	Nutrient broth + LCTW 80	1:900
Streptococcus anaerobicans NCTC 9906	Thioglycollate Medium USP	Thioglycollate Medium USP	1:960
Corynebacterium acnes NCTC 737	Thioglycollate Medium USP	Thioglycollate Medium USP	1:480
Salmonella typhi NCTC 786	RW	Nutrient broth + LCTW 80	1:400
Salmonella gallinarum NCTC 5775	Nutrient broth	Nutrient broth + LCTW 80	1:250
Shigella sonnei NCTC 8290	Nutrient broth	Nutrient broth + LCTW 80	1:250

TABLE 1 Continued

Organism	Culture broth	Sub culture broth	Killing dilution of Detol
Vibrio cholerae NCTC 7254	Nutrient broth	Nutrient broth + LCTW 80	1:1100
Vibrio cholerae NCTC 8021	Nutrient broth	Nutrient broth + LCTW 80	1:1100
Vibrio eltor NCTC 3661	Nutrient broth	Nutrient broth + LCTW 80	1:1200
Vibrio fetus NCTC 10354	Nutrient broth	Nutrient broth + LCTW 80	1:500
Erwinia aroidae NCIB	Nutrient broth	Nutrient broth + LCTW 80	1:500

TABLE 2 The Fungicidal activity of Dettol, diluted in distilled water, against Standard Fungal Cultures assessed by the killing dilution technique

Organism	Culture broth	Sub culture Broth	Killing dilution of Dettol
Candida albicans	Malt Extract broth	Malt Extract broth	1:125
Trichophyton rubrum	Malt Extract Agar	Malt Extract Agar	1:300
Trichophyton mentagrophytes	Malt Extract Agar	Malt Extract Agar	1:160
Epidermophyton floccosum	Malt Extract Agar	Malt Extract Agar	1:200
Microsporum canis	Malt Extract Agar	Malt Extract Agar	1:100
Pityrosporum ovale	P. ovale Agar	P. ovale Agar	1:240
Penicillium notatum	Malt Extract Agar	Malt Extract	1:150
Aspergillus fumigatus	Malt Extract Agar	Malt Extract	1:40

TABLE 3 The Bactericidal Activity of Dettol, diluted in 300 ppm hard water, against Standard Bacterial Cultures assessed by the killing dilution technique

Organism	Culture Broth	Sub culture Broth	Killing dilution of Dettol
Staphylococcus aureus NCTC 4163	FDA	Nutrient broth + LCTW 80	1:65
Streptococcus faecalis NCTC 8213	Nutrient broth	Nutrient broth + LCTW 80	1:170
Streptococcus pyogenes NCTC 8191	Nutrient broth	Nutrient broth + LCTW 80	1:340
Escherichia coli NCTC 86	Nutrient broth	Nutrient broth + LCTW 80	1:360
Proteus mirabilis NCTC 5867	Nutrient broth	Nutrient broth + LCTW 80	1:300
Proteus rettgeri NCTC 7475	Nutrient broth,	Nutrient broth + LCTW 80	1:280
Proteus vulgaris NCTC 4635	Nutrient broth	Nutrient broth + LCTW 80	1:250
Pseudomonas aeruginosa NCTC 1999	DA	Nutrient broth + LCTW 80	1:20
Salmonella gallinarum NCTC 5775	Nutrient broth	Nutrient broth + LCTW 80	1:210
Salmonella typhi NCTC 786	R.W.	Nutrient broth + LCTW 80	1:220
Shigella flexneri NCTC 6516	Nutrient broth	Nutrient broth + LCTW 80	1:280
Shigella shigae NCTC 4637	Nutrient broth	Nutrient broth + LCTW 80	1:280
Shigella sonnei NCTC 2041	Nutrient broth	Nutrient broth + LCTW 80	1:320

TABLE 3A — The Bactericidal Activity of Dettol, in the presence of 5% serum, against Standard Bacterial Cultures assessed by the killing dilution technique

Organism	Culture Broth	Sub-culture Broth	Killing dilution of Dettol
Staphylococcus aureus NCTC 4163	F.D.A.	Nutrient broth + LCTW 80	1:50
Streptococcus faecalis NCTC 8213	Nutrient broth	Nutrient broth + LCTW 80	1:100
Streptococcus pyogenes NCTC 8191	Nutrient broth	Nutrient broth + LCTW 80	1:250
Escherichia coli NCTC 86	Nutrient broth	Nutrient broth + LCTW 80	1:140
Proteus mirabilis NCTC 5387	Nutrient broth	Nutrient broth + LCTW 80	1:220
Proteus rettgeri NCTC 7475	Nutrient broth	Nutrient broth + LCTW 80	1:250
Proteus vulgaris NCTC 4636	Nutrient broth	Nutrient broth + LCTW 80	1:200
Pseudomonas aeruginosa NCTC 1999	DA	Nutrient broth + LCTW 80	1:20
Salmonella gallinarum NCTC 5775	Nutrient broth	Nutrient broth + LCTW 80	1:210
Salmonella typhi NCTC 786	RW	Nutrient broth + LCTW 80	1:180
Shigella flexneri NCTC 8516	Nutrient broth	Nutrient broth + LCTW 80	1:200
Shigella shigae NCTC 4837	Nutrient broth	Nutrient broth + LCTW 80	1:140
Shigella sonnei NCTC 2941	Nutrient broth	Nutrient broth + LCTW 80	1:250

TABLE 4 The Bactericidal Activity of Dettol, diluted in distilled water, at 37°C against Standard Bacterial Cultures assessed by the killing dilution technique

Organism	Culture Broth	Sub culture Broth	Killing dilution of Dettol
Staphylococcus aureus NCTC 4163	FDA	Nutrient broth + LCTW 80	1:800
Pseudomonas aeruginosa NCTC 1999	DA	Nutrient broth + LCTW 80	1:60
Escherichia coli NCTC 86	Nutrient broth	Nutrient broth + LCTW 80	1:510
Streptococcus pyogenes NCTC 8191	Nutrient broth	Nutrient broth + LCTW 80	1:1,700
Salmonella typhi NCTC 786	R.W.,	Nutrient broth + LCTW 80	1:770

TABLE 5 The rapidity of the bactericidal activity of Dettol against Standard Bacterial Cultures

Organism	Time taken for 1:40 Dettol to kill test culture when diluted with:		
	soft water	5% serum	2.5% blood
Staphylococcus aureus NCTC 4163	< ½ minute	2 minutes	5 minutes
Streptococcus pyogenes NCTC 8191	< ½ minute	< ½ minute	< ½ minute
Streptococcus faecalis NCTC 8213	< ½ minute	< ½ minute	< ½ minute
Salmonella typhi NCTC 786	< ½ minute	< ½ minute	< ½ minute
Escherichia coli NCTC 86	< ½ minute	< ½ minute	< ½ minute
Proteus vulgaris NCTC 4635	< ½ minute	< ½ minute	< ½ minute
Pseudomonas aeruginosa NCTC 1959	< ½ minute	< ½ minute	< ½ minute
Shigella sonnei NCTC 2941	< ½ minute	< ½ minute	< ½ minute
Proteus rettgeri NCTC 7475	< ½ minute	< ½ minute	< ½ minute

TABLE 6 The Bactericidal activity of Dettol acting on dried micro-organisms

Test Organism	Concentration of Dettol	Average time taken to achieve a total kill	Range	Number of Tests
Staphylococcus aureus) NCTC 4163)	1:40	3½ minutes	(2½ - 5)	8
	1:100	2½ minutes	(½ - 10)	11
Pseudomonas aeruginosa) NCTC 1999)	1:40	½ minute	(both same)	2
	1:100	2 minutes	(½ - 2½)	3

TABLE 7 The Bactericidal activity of Dettol acting on dried organisms embedded in an organic film

Organism	Concentration of Dettol	Average time taken to achieve a total kill	Range	Number of Tests
Staphylococcus aureus NCTC 4163	1:40	21 minutes	10 - 40	8

recent hospital isolates of haemolytic Streptococci assessed by the killing dilution technique

Hospital	Source of culture	Killing Dilution of Dettol in distilled water
Bangour Broxburn	Pus from sinus Throat swab Throat swab	1:340 1:400 1:535
Glasgow Royal Maternity Hospital	Scarlet fever Scarlet fever Acute tonsilitis Acute tonsilitis Acute tonsilitis Puerperal sepsis Scarlet fever Acute tonsilitis	1:405 1:365 1:560 1:405 1:320 1:270 1:430 1:735
City Hospital Edinburgh	Wound swab Vaginal swab Puerperal sepsis Sputum TB Throat swab Throat swab	1:265 1:560 1:595 1:940 1:530 1:450
Northern General Hospital, Edinburgh	Skin infection	1:370
Royal Infirmary Glasgow	Throat	1:260
Leeds University Bacteriological Dept.	Bronchial aspirate Skin lesion	1:220 1:300
Wright Fleming Institute, St. Marys Hospital Med. School	Throat swab	1:300
Royal Devon & Exeter	Tonsil	1:330

TABLE 9 The bactericidal activity of Dettol against recent hospital isolates of Pseudomonas aeruginosa assessed by the killing dilution technique

Hospital	Source	Clinical Diagnosis	Killing dilution of Dettol in Distilled water (1 in -)
Charing Cross Hospital Med. School	Faeces	Diverticulitis	> 51
Memorial Hospital Shooters Hill	Ear	Otitis Media	42
General Hospital Birmingham	Urine	Bladder Infection	20
Cardiff Royal Infirmary	Urine	Chronic Cystitis	48
Royal Devon & Exeter Hospital	Stock culture	Originally isolated from Urine, Cystitis	> 64
"	Urine	-	55
Northern General Hospital Edinburgh	Ileostomy pus	-	70
Royal Infirmary Glasgow	Urine	-	120
Wright Fleming Institute St. Mary's Med. School	Urine	-	55

TABLE 9 Continued

Hospital	Source	Clinical Diagnosis	Killing dilution of Dettol in Distilled water (1 in-)
West Middlesex Hosp. Isleworth	Ear	Otitis Externa	48
Post Graduate Med. School London	Urine	Bladder infection after Prostatectomy	32
"	Breast Abcess		24
Leeds University Bacteriology Dept.	Ear discharge	-	80
"	Wound swab	-	30
City Hospital Edinburgh	Rectal Swab	Dysentry	25
"	Wound Swab	Tetanus	>51
"	Rectal Swab	Poliomyelitis	16
"	Faeces	Unspecified	26
"	Faeces	Dysentry	12
"	Faeces	Dysentry	24
"	Faeces	Dysentry	48
"	Rectal Swab	Dysentry	30

TABLE 9 Continued

Hospital	Source	Clinical Diagnosis	Killing dilution of Dettol in Distilled water (1 in -)
County Hospital York	Urine)	Unspecified	60
"	Urine)	Cystitis	46
"	Urine)		40
"	Burn	Burning	60
"	Burn	Burning	60
Glasgow Royal Maternity Hospital	Faecal isolate	-	20
"	Faecal isolate	-	30
"	Faecal isolate	-	30
"	Faecal isolate	-	30
"	Brain swab	-	15
"	Faecal isolate	-	15

TABLE 10 The bactericidal activity of Dettol against recent hospital isolates of Escherichia coli assessed by the killing dilution technique

Hospital	Source of Culture	Killing dilution of Dettol in Distilled water
Royal Glasgow Maternity Hospital	Urine	1:420
	Urine	1:550
	Urine	1:480
Hull Royal Infirmary	Wound Swab	1:440
Northern General Edinburgh	Ileostomy	1:200
City Hospital Edinburgh	Rectal Swab	1:220
Royal Infirmary Glasgow	Faeces	1:220
Royal Devon & Exeter	Urine	1:400
Wright-Fleming Institute St. Mary's Medical School	Urine	1:180

TABLE 11 The bactericidal activity of Dettol against recent hospital isolates of Staphylococcus aureus assessed by the killing dilution technique

Hospital	Source of Culture	Killing dilution of Dettol in distilled water. (1 in -)
Bangour Hosp. Broxburn	Septic finger	230
" " "	Sub-mandibular abscess	300
" " "	" "	200
" " "	Graft to thumb	> 300
" " "	Burns of abdomen	200
" " "	Burns of breast	> 300
" " "	Stitch line	200
" " "	Maxillary abscess	200
County Hospital, York	Abscess of arm	300
" " "	Conjunctiva	150
" " "	Mastoid cavity	200
Glasgow Royal Mat. Hosp.	Boil on fore-arm	150
" " "	Baby's eye discharge	130
" " "	Baby's mouth, monilial infection	130
Kent & Canterbury Hosp.	Subcutaneous abscess	230
" " " "	Carbuncle of neck	160
Postgraduate Med. School, London	Wound	150
	Boil	280
Cardiff Royal Infirmary	Chronic blepharitis	150
General Hospital Birmingham	Stitch abscess	200
Kidderminster & District Gen. Hospital	Recurring carbuncle	150
Luton & Dunstable Hosp.	Infected finger	200
Memorial Hosp. S.E.18	Case of furunculosis	250

TABLE 11 Continued

Hospital	Source of Culture	Killing dilution of Dettol in distilled water. (1 in -)
Preston Royal Infirmary	Not stated	200
Royal Devon & Exeter Hosp.	Acute tonsillitis	125
" " " " "	Boil	140
Royal Infirmary, Sheffield	Carbuncle	130
Walton Hospital, Liverpool	Infected baby's eye	100
Southmead Hosp. Bristol	Eye swab	250+
Charing X Hospital	Labial abscess	250
Chase Farm Hospital	Axillary abscess	200
Northern General Hospital, Edinburgh	Abdominal sinus	180
City Hospital, Edinburgh	Nasal swab	160
Wright-Fleming Institute St. Mary's Hospital Med. School.	Boil	200
Leeds University Bacteriology Dept.	Osteomyelitis	200

TABLE 12 The bactericidal activity of Dettol, in both hard and distilled water, against recent hospital isolates of Proteus vulgaris assessed by the killing dilution technique

HOSPITAL	SOURCE	Killing dilutions in: Hard Water		Distilled Water	
		Freshly Isolated Culture	6 Months Culture	Freshly Isolated Culture	6 Months Culture
		DETTOL	DETTOL	DETTOL	DETTOL
Glasgow Royal Mat. Hospital	Vaginal swab	200	260	330	290
" " " "	Pyelitis	230	290	230	290
City Hospital, Edinburgh	Faeces-Gastro-enteritis	230	230	230	260
General Hospital, Birmingham	Bowel - resection sarcoma	350	380	350	330
Postgraduate Medical School, London	Septic post op. wound	250	200	250	300
Royal Infirmary, Sheffield	Nasal swab	250	200	290	290
" " "	Urine	250	200	250	290
" " "	Urine	210	290	290	330
Royal Lancaster Infirmary	Pus	290	300	290	290
Queen Charlotte's Mat. Hosp. London	No History	230	290	230	290
Newcastle Public Health Lab.	No History	260	290	260	380
" " " "	No History	300	290	300	330
City Hospital, Aberdeen	Urinary tract	260	290	260	330
" " "	Urinary tract	200	330	300	360
" " "	Urinary tract	260	290	300	290
Royal Devon & Exeter Hospital	Urine - Cystitis	260	290	300	380
Queen Elizabeth Hospital, Birmingham	Stool - no abnormality	300	380	340	380
Average Killing Dilution		260	300	290	330

TABLE 13 The bactericidal activity of Dettol, compared directly with Phenol, against recent hospital isolates of Shigella sonnei assessed by the killing dilution technique

Hospital Source	Killing Dilution in Distilled water (1 in -)	
	Dettol	Phenol
Charing Cross Hospital	400	80
General Hospital, Newcastle-on-Tyne	300	75
Royal Devon & Exeter Hospital	300	70
Memorial Hospital, Shooters Hill	300	75
Royal Lancaster Infirmary	350	80
Royal Sussex County Hospital, Brighton	250	80
Glasgow Royal Maternity Hospital	250	70
Public Health Department, Salford	275	65
Lister Strain 8219	350	85
Lister Strain 8220	250	80
Lister Strain 8221	400	80

TABLE 14 The bactericidal activity of Dettol when applied to skin which has been inoculated with specific test cultures

Test Organism	Time of contact of Dettol on infected skin (minutes)	* Percentage reduction in viable organism obtained with Dettol compared to a water control	
		1:20 Dettol	1:40 Dettol
St. aureus NCTC 4163	5	99%	95%
	10	100%	99%
Ps. aeruginosa NCTC 1999	5	99%	79%
	10	100%	92%
E. coli NCTC 86	5	100%	94%
	10	100%	99%
Strep. faecalis NCTC 8213	5	99%	95%
	10	100%	99%

* These results are the average percentage reduction from experiments carried out on 10 human volunteers.

TABLE 15 The Trichomonacidal activity of Dettol

Key

+ denotes active, healthy trichomonads present

± denotes sluggish living and dead, trichomonads present

o denotes only dead trichomonads present

Strain of Tr. Vaginalis		Final Concn of Dettol	Time Exposure to Dettol			Room Temp.
Designation	Age		2 mins.	10 mins.	1 hr.	
B	7 months	1/200	o	o	o	23°C.
		1/400	o	o	o	
		1/600	o	o	o	
		1/800	±	±	±	
		/1000	+	+	+	
		Aq. dest. control	+	+	+	
		Inoc. control	+	+	+	
C	5 months	1/200	o	o	o	23°C.
		1/400	o	o	o	
		1/600	+	+	+	
		1/800	+	+	+	
		1/1000	+	+	+	
		Aq. dest. control	+	+	+	
		Inoc. control	+	+	+	
G	1½ months	1/200	o	o	o	26°C.
		1/400	o	o	o	
		1/600	±	±	±	

TABLE 15 The Trichomonacidal activity of Dettol

Key

+ denotes active, healthy trichomonads present

± denotes sluggish living and dead, trichomonads present

o denotes only dead trichomonads present

Strain of Tr. Vaginalis		Final Concn of Dettol	Time Exposure to Dettol			Room Temp.
Designation	Age		2 mins.	10 mins.	1 hr.	
B	7 months	1/200	o	o	o	25°C.
		1/400	o	o	o	
		1/600	o	o	o	
		1/800	±	±	±	
		/1000	+	+	+	
		Aq. dest. control	+	+	+	
		Inoc. control	+	+	+	
C	5 months	1/200	o	o	o	23°C.
		1/400	o	o	o	
		1/600	+	+	+	
		1/800	+	+	+	
		1/1000	+	+	+	
		Aq. dest. control	+	+	+	
		Inoc. control	+	+	+	
G	1½ months	1/200	o	o	o	26°C.
		1/400	o	o	o	
		1/600	±	±	±	

TABLE 15 The Trichomonacidal activity of Dettol

Key

+ denotes active, healthy trichomonads present

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Strain of Tr. Vaginalis		Final Concn of Dettol	Time Exposure to Dettol			Room Temp.
Designation	Age		2 mins.	10 mins.	1 hr.	
B	7 months	1/200	o	o	o	23°C.
		1/400	o	o	o	
		1/600	o	o	o	
		1/800	±	±	±	
		/1000	+	+	+	
		Aq. dest. control	+	+	+	
		Inoc. control	+	+	+	
C	5 months	1/200	o	o	o	23°C.
		1/400	o	o	o	
		1/600	+	+	+	
		1/800	+	+	+	
		1/1000	+	+	+	
		Aq. dest. control	+	+	+	
		Inoc. control	+	+	+	
G	1½ months	1/200	o	o	o	26°C.
		1/400	o	o	o	
		1/600	±	±	±	

The bacteriostatic and bactericidal activity of Dettol against a range of recently isolated mesophilic strains including members of the normal flora and cutaneous pathogens of the skin. Submitted and performed by Reckitt and Colman, Pharmaceutical Division.

A previous study was done earlier in a similar way. It is also included in this summary. A new selection of isolates was tested by determining the Minimal Inhibitory Concentration in doubling dilutions and standard MIC technique. The dilutions showing no growth were reinoculated with a drop of culture from the highest concentration in which growth occurred. The procedure was repeated every 3 days until no positives occurred. After 3 negative inoculations, the Final MIC (table) was read. Although some one-tube changes occurred, no resistance development occurred.

Bacterial killing concentration was also determined. Because of the lack of solubility of PCMX in water, the bactericidal concentrations were not done with PCMX alone. The bactericidal and bacteriostatic concentration of the formulated product, Dettol, did not differ significantly, indicating that the activity of PCMX is essentially bactericidal. Phenol was also tested for comparison and use in determining a phenol coefficient. The increase in activity obtained when phenol is chemically substituted or halogen molecules are added is easily seen in these values.

The inclusion of both the lipophilic and non-lipophilic and anaerobic diptheroids is interesting because of their universal occurrence on the skin.

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Laboratory Report No. BL 75/4

Date April, 1975.

Title The bacteriostatic and bactericical activity of Dettol against a range of recently isolated mesophilic strains including members of the normal flora and cutaneous pathogens of the skin.

Authors T.J. Munton, B.Pharm., Ph.D., M.P.S.

J. Prince, B.Sc.

Summary

Dettol has been shown to be both bactericidal and bacteriostatic against a range of commensal and pathogenic skin bacteria. Phenol co-efficients are quoted for all bactericidal data. The concentrations of Dettol which achieve bacteriostasis are of the same order as those giving the relevant bactericical performance. The formulation of parachlorometaxylenol into Dettol has been shown not to adversely affect the activity of the chemical, on the contrary, the evidence points to an enhancement of activity.

The degree to which organisms were capable of developing resistance to Dettol was determined using a bacteriostatic procedure. The final minimum inhibitory concentrations were shown to be marginally greater than the initial figure, particularly with Gram-negative organisms.

1. Introduction

A previous document (1) showed Dettol to be active against a wide spectrum of standard bacterial and fungal cultures and also recent hospital isolates. This present document extends this work to include a new series of recent skin isolates and determines both the bacteriostatic and bactericidal activity of Dettol in direct comparison to phenol and parachlorometaxylenol. In addition the development of resistance to Dettol and parachlorometaxylenol is also determined.

2. Experimental procedures

2.1. Products

Dettol, standard production formula used throughout

Phenol, analar grade (BDH, Poole, England).

Para-chlorometaxylenol, raw material grade used in the manufacture of Dettol.

All dilutions of the products were made in distilled water. Parachlorometaxylenol is soluble at 1:3,000 and solutions were prepared by slightly warming the mixture.

2.2. Micro-organisms and method of culture

Micro-organisms were isolated from the skin by a utilization of sterile swabs which were then plated onto non-selective nutrient agar. Identification of the resultant colonies was achieved by standard procedures (2, 3)using selective media and fermentation techniques.

Cutaneous pathogenic organisms were obtained from hospital skin swabs and, in general, commensal organisms were obtained from skin swabs of laboratory personnel.

All bacterial cultures were grown in Nutrient Broth No. 2 (Oxoid Ltd) for three subsequent generations at 37°C for 18 hours except the diphtheroid species which were grown in thioglycollate medium (Oxoid) for 48 hours at 37°C.

2.3. Evaluation of bactericidal activity

The bactericidal activity of the three products was evaluated by the A.O.A.C. phenol co-efficient test. The method varied only insofar that other organisms in addition to those normally recommended have also been tested.

A series of five dilutions of the product under test, each of five mls, were made in distilled water in sterile containers and placed in a water bath at 20°C. 0.5ml of the test culture was added to each dilution. The mixture was agitated and loopfuls removed after 5 and 10 minutes contact time and transferred into a suitable sub-culture medium containing 0.3% Lecithin and 3% Tween 80 as neutralizer.

Subcultures were incubated at 37°C for 48 hours and the presence or absence of growth noted.

2.4. Evaluation of bacteriostatic activity

Evaluation of bacteriostatic activity was achieved using the Minimum Inhibitory Concentration (MIC) technique.

Five ml of the test product solution was pipetted into 5ml double strength nutrient broth and mixed. Double strength thioglycollate medium was used for the diphtheroid species. Five ml of this mixture was then pipetted into 5ml of single strength broth. This doubling dilution procedure was continued to obtain an adequate range of final product concentrations.

Each tube was inoculated with one drop (0.02ml) of the test culture broth from a pasteur pipette.

All tubes were incubated at 37°C for three days and the tube containing the lowest concentration of product which showed no growth was taken as the MIC.

2.5. Development of resistance

Development of resistance was evaluated using a modified version of the minimum inhibitory concentration procedure detailed above. Following the initial determination of the MIC value one drop (0.02ml) from the tube containing the highest concentration of product which permitted growth was subcultured into each of the preceding negative tubes. This procedure was repeated every three days until no further positives were obtained. Following three negative inoculations the new MIC was read and compared to the initial MIC in order to evaluate the development of resistance.

3. Results

3.1. Bactericidal activity

The results are shown in Table 1. Due to the fairly low solubility of the pure chemical, parachlorometaxylenol, this compound was not assessed for bactericidal activity as the un-formulated product.

Dettol was found to be bactericidal against all strains of organisms used. In general both Gram-negative and Gram-positive bacteria were equally sensitive to the activity of Dettol. The strains of Pseudomona sp. tested were found to be the most resistant organisms.

3.2. Bacteriostatic activity

The results are shown in Table 2. The minimum inhibitory concentration of Dettol is of the same order as the bactericidal activity. This shows Dettol to be a highly bactericidal product with, on dilution, little remaining bacteriostatic ability. In general the level of PCMX required for activity was greater in terms of the active ingredient, than Dettol itself. Therefore, the formulation of PCMX in Dettol does not in any way detract from the antibacterial activity of PCMX, on the contrary, there is some evidence to show an enhancement of activity.

3.3. Development of resistance

A comparison of the initial and final MIC values listed in Table 2 indicates the degree of the development of resistance to the specific

test organism with both Dettol and parachlorometaxylenol.

The results show that development of resistance does not occur with the Gram-positive organisms tested. With the Gram-negative organisms the MIC was lowered to give growth in the immediately preceding tubes within the series dilution. However, resistance did not develop to more than four times the initial MIC value with any culture tested.

TJM/PAK

Table 1 The Bactericidal Activity of Dettol and Phenol against a range of skin isolates

Organism	Source	Killing Dilution		Phenol Coefficient
		Dettol	Phenol	
Staphylococcus Group I (1)	HRI	1:100	1:65	1.5
Staphylococcus Group I (2)	HRI	1:100	1:70	1.4
Staphylococcus Group II (3)	HRI	1:300	1:65	4.6
Staphylococcus Group IV	HRI	1:660	1:65	10.2
Staphylococcus Group V	HRI	1:550	1:80	6.9
Micrococcus spp. (aerobic)	R&C	1:75	1:55	1.4
Streptococcus faecalis Type C	HRI	1:550	1:85	6.5
Streptococcus faecalis Type D (1)	HRI	1:300	1:65	4.6
Streptococcus faecalis Type D (2)	HRI	1:440	1:65	6.8
Streptococcus faecalis Type D (3)	HRI	1:370	1:65	5.7
Streptococcus pyogenes Type A	HRI	1:400	1:70	5.7
Neisseria catarrhalis	R&C	1:100	1:70	1.4
Acinetobacter anitratum	HRI	1:370	1:85	4.4
Escherichia coli AA 9395	RHG	1:420	1:100	4.2
Escherichia coli AA 9721	RHG	1:550	1:90	6.1
Escherichia coli AA 9803	RHG	1:480	1:110	4.3
Escherichia coli 10245	HRI	1:440	1:100	4.4
Escherichia coli	HRI	1:440	1:80	5.5
Citrobacter sp.	HRI	1:300	1:90	3.3

Table 1 (continued)

Organism	Source	Killing Dilution		Phenol Coefficient
		Dettol	Phenol	
Kleosiella aerogenes CA 22552	RHG	1:550	1:100	5.5
Klebsiella aerogenes CA 23535	RHG	1:440	1:110	4.0
Klebsiella aerogenes	WH	1:420	1:100	4.2
Klebsiella aerogenes	HRI	1:370	1:90	4.1
Proteus mirabilis BA 23919	RHG	1:550	1:100	5.5
Proteus mirabilis BA 25702	RHG	1:370	1:100	3.7
Proteus mirabilis BA 25945	RHG	1:420	1:100	4.2
Proteus mirabilis (1)	R&C	1:440	1:100	4.4
Proteus mirabilis (2)	R&C	1:440	1:110	4.0
Proteus mirabilis	HRI	1:550	1:90	6.1
Providence (A)	HRI	1:550	1:90	6.1
Providence (B)	HRI	1:440	1:100	4.4
Pseudomonas aeruginosa 12211	HRI	1:44	1:90	0.47
Pseudomonas aeruginosa	RHG	1:48	1:90	0.53
Pseudomonas aeruginosa 65376	HRI	1:35	1:90	0.39
Pseudomonas aeruginosa Thornham	HRI	1:32	1:85	0.35
Enterobacter spp.	R&C	1:400	1:110	3.6
Serratia marcascens	R&C	1:600	1:120	5.0
Mycobacterium smegmatis	R&C	1:300	<1:80	>3.8

Table 1 (continued)

Organism	Source	Killing Dilution		Phenol Co-efficient
		Dettol	Phenol	
Diphtheroid (lipolytic)	R&C	1:150	1:25	1.7
Diphtheroid (non-lipophilic)	R&C	1:300	1:80	3.8
Diphtheroid (anaerobic)	R&C	1:220	1:90	2.4

KEY

HRI	Hull Royal Infirmary
R&C	Reckitt and Colman
RHG	Royal Hospital for Sick Children, Glasgow
WH	Warstead Hospital, London

TABLE 2

The Bacteriostatic Activity of Parachlorometaxylenol (PCMX)
in Dettol Vehicle and Distilled Water
Against a Range of Skin Isolates

Organism	Source	Minimum Inhibitory Conc. (g PCMX per ml)			
		Dettol Vehicle		Distilled Water	
		(Initial MIC)	(Final MIC)	(Initial MIC)	(Final MIC)
Staphylococcus Group 1 (1)	HRI	1:16700	1:8300	1:6000	1:6000
Staphylococcus Group 1 (2)	HRI	1:8300	1:8300	1:6000	1:6000
Staphylococcus Group II (3)	HRI	1:16700	1:8300	1:6000	1:6000
Staphylococcus Group IV	HRI	1:8300	1:8300	1:6000	1:6000
Staphylococcus Group V	HRI	1:16700	1:8300	1:6000	1:6000
Micrococcus spp. (aerobic)	R&C	1:26700	1:13300	1:12000	1:12000
Streptococcus faecalis Type C	HRI	1:16700	1:16700	1:12000	1:12000
Streptococcus faecalis Type D (1)	HRI	1:8300	1:8300	<1:6000	<1:6000
Streptococcus faecalis Type D (2)	HRI	1:8300	1:8300	1:6000	1:6000
Streptococcus faecalis Type D (3)	HRI	1:8300	1:8300	<1:6000	<1:6000
Streptococcus pyogenes Type A	HRI	1:8300	1:8300	1:6000	1:6000
Neisseria catarrhalis	R&C	1:16700	1:16700	1:6000	1:6000
Acinetobacter anitratus	HRI	1:8300	1:8300	1:12000	1:12000
Escherichia coli AA 9395	RHC	1:13300	1:6700	1:24000	1:24000
Escherichia coli AA 9721	RHC	1:13000	1:3300	1:6000	1:6000
Escherichia coli AA 9803	RHC	1:13300	1:3300	1:6000	1:6000
Escherichia coli 10245	HRI	1:13300	1:3300	1:6000	1:6000
Escherichia coli	HRI	1:8300	1:4200	1:6000	<1:6000
Citrobacter sp.	HRI	1:6200	1:2100	1:6000	<1:6000
Klebsiella aerogenes CA 22852	RHC	1:13300	1:6700	1:6000	1:6000
Klebsiella aerogenes CA 23535	RHC	1:13300	1:6700	1:6000	1:6000
Klebsiella aerogenes	HR	1:13300	1:6700	1:6000	1:6000
Klebsiella aerogenes	HRI	1:8300	1:2100	1:6000	1:6000

TABLE 2

(continued)

Organism	Source	Minimum Inhibitory Conc. (g PCX per ml)			
		Dettol Vehicle		Distilled Water	
		(Initial MIC)	(Final MIC)	(Initial MIC)	(Final MIC)
Proteus mirabilis BA 23919	RHC	1:13300	1:3300	1:6000	<1:6000
Proteus mirabilis BA 25702	RHC	1:13300	1:6700	1:6000	1:6000
Proteus mirabilis BA 25945	RHC	1:6700	1:3300	1:6000	1:6000
Proteus mirabilis (1)	R&C	1:13300	1:3300	<1:6000	<1:6000
Proteus mirabilis (2)	R&C	1:13300	1:3300	1:6000	<1:6000
Proteus mirabilis	HRI	1:8300	1:4200	1:6000	<1:6000
Providence (A)	HRI	1:8300	1:4200	1:6000	1:6000
Providence (B)	HRI	1:4200	1:4200	<1:6000	<1:6000
Pseudomonas aeruginosa 12211	HRI	1:830	1:420	<1:6000	<1:6000
Pseudomonas aeruginosa	RHC	1:670	1:330	<1:6000	<1:6000
Pseudomonas aeruginosa 65376	HRI	1:670	1:170	<1:6000	<1:6000
Pseudomonas aeruginosa Thornham	HRI	1:670	1:170	<1:6000	<1:6000
Enterobacter spp.	R&C	1:13300	1:6700	1:6000	1:6000
Serratia marcencens	R&C	1:13300	1:6700	1:6000	<1:6000
Mycobacterium smegmatis	R&C	1:8300	1:4200	1:6000	<1:6000
Diphtheroid (lipolytic)	R&C	1:26700	1:26700	1:12000	1:6000
Diphtheroid (non-liophilic)	R&C	1:13000	1:13300	1:12000	1:12000
Diphtheroid (anaerobic)	R&C	1:13300	1:13300	1:6000	1:6000

Table 2 The Bacteriostatic activity of Dettol and parachlorometaxylenol (PCMX) against a range of skin isolates

Organism	Source	Minimum Inhibitory Conc. (% w/v)			
		Dettol		PCMX	
		(Initial MIC)	(Final MIC)	(Initial MIC)	(Final MIC)
Staphylococcus Group I (1)	HRI	1:800	1:400	1:6000	1:6000
Staphylococcus Group I (2)	HRI	1:400	1:400	1:6000	1:6000
Staphylococcus Group II (3)	HRI	1:800	1:400	1:6000	1:6000
Staphylococcus Group IV	HRI	1:400	1:400	1:6000	1:6000
Staphylococcus Group V	HRI	1:800	1:400	1:6000	1:6000
Micrococcus spp. (aerobic)	R&C	1:1280	1:640	1:12000	1:12000
Streptococcus faecalis Type C	HRI	1:800	1:800	1:12000	1:12000
Streptococcus faecalis Type D (1)	HRI	1:400	1:400	<1:6000	<1:6000
Streptococcus faecalis Type D (2)	HRI	1:400	1:400	1:6000	1:6000
Streptococcus faecalis Type D (3)	HRI	1:400	1:400	<1:6000	<1:6000
Streptococcus pyogenes Type A	HRI	1:400	1:400	1:6000	1:6000
Neisseria catarrhalis	R&C	1:800	1:800	1:6000	1:6000
Acinetobacter anitratum	HRI	1:400	1:400	1:12000	1:12000
Escherichia coli AA 9395	RHG	1:640	1:320	1:24000	1:24000
Escherichia coli AA 9721	RHG	1:640	1:160	1:6000	1:6000
Escherichia coli AA 9803	RHG	1:640	1:160	1:6000	1:6000

Table 2 (continued)

Organism	Source	Minimum Inhibitory Conc. (1 in)			
		Dettol		PCMX	
		(Initial MIC)	(Final MIC)	(Initial MIC)	(Final MIC)
Escherichia coli 10245	HRI	1:640	1:160	1:6000	1:6000
Escherichia coli	HRI	1:400	1:200	1:6000	<1:6000
Citrobacter sp.	HRI	1:300	1:100	1:6000	<1:6000
Klebsiella aerogenes CA 22852	RHG	1:640	1:320	1:6000	1:6000
Klebsiella aerogenes CA 23335	RHG	1:640	1:320	1:6000	1:6000
Klebsiella aerogenes	WH	1:640	1:320	1:6000	1:6000
Klebsiella aerogenes	HRI	1:400	1:100	1:6000	1:6000
Proteus mirabilis BA 23919	RHG	1:640	1:160	1:6000	<1:6000
Proteus mirabilis BA 25702	RHG	1:640	1:320	1:6000	1:6000
Proteus mirabilis BA 25945	RHG	1:320	1:160	1:6000	1:6000
Proteus mirabilis (1)	R&C	1:640	1:160	<1:6000	<1:6000
Proteus mirabilis (2)	R&C	1:640	1:160	1:6000	<1:6000
Proteus mirabilis	HRI	1:400	1:200	1:6000	<1:6000
Providence (A)	HRI	1:400	1:200	1:6000	1:6000
Providence (B)	HRI	1:200	1:200	<1:6000	<1:6000

Table 2 (continued)

Organism	Source	Minimum Inhibitory Conc. (1 in)			
		Dettol		PCMX	
		(Initial MIC)	(Final MIC)	(Initial MIC)	(Final MIC)
Pseudomonas aeruginosa 12211	HRI	1:40	1:20	< 1:6000	< 1:6000
Pseudomonas aeruginosa	RHG	1:32	1:16	< 1:6000	< 1:6000
Pseudomonas aeruginosa 65376	HRI	1:32	1:8	< 1:6000	< 1:6000
Pseudomonas aeruginosa Thornham	HRI	1:32	1:8	< 1:6000	< 1:6000
Enterobacter spp.	R&C	1:640	1:320	1:6000	1:6000
Serratia marcescens	R&C	1:640	1:320	1:6000	< 1:6000
Mycobacterium smegmatis	R&C	1:400	1:200	1:6000	< 1:6000
Diphtheroid (lipolytic)	R&C	1:1280	1:1280	1:12,000	1:6000
Diphtheroid (non-lipophilic)	R&C	1:640	1:640	1:12,000	1:12,000
Diphtheroid (anaerobic)	R&C	1:640	1:640	1:6000	1:6000