Report on the efficacy of Anolyte in hospitals

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FORMULATION

Anolyte is a microbicidal solution containing a mixture of radicals (Cl2, ClO2, HCIO, CIO-,OH-, 0-, H02-, H2O2, O3, CIO-, S2O82, C2062-) which have strong oxidising properties,

The solution tested has to have a pH In the range of 5 -7 and a ORP of >1000 mV to be microbiologically effective and non-corrosive.

The manufactured Anolyte has a use life of 24 hours at ambient temperature and it is recommended to discard immediately after use.

1 To test the bactericidal activity of Anolyte under clean and dirt conditions using a modified European suspension test.
2. To test the sporicidal activity of Anolyte under clean and dirty conditions.
3. To assess the 'in vivo' activity of Anolyte in an automated endoscope washer disinfector,

In vitro microbiological tests were performed on a freshly prepared solution and following storage for 24 hours post preparation to establish the stability of the product,

For comparative purposes, test were also carried out with a widely used 2% activated alkaline glutaraldehyde (Asep, Galen Ltd, Craigavon, Northern Ireland), 2% glutaraldehyde is, at present, recommended as the disinfectant of choice for flexible fibreoptic endoscopes and other heat sensitive instruments.

TEST METHODS

Recovery/Neutraliser Broth

Anolyte	Nutrient broth (Oxoid No, 2) containing 1% sodium thiosulphate and 0.75% lecithin/tween 80 mixture (i.e, 30g Tween and 5g lethicin)
2% glutaraldehyde	Double strength nutrient broth (Oxoid No, 2) containing 10% horse serum

These recovery media were established as suitable in recovering small numbers of test organisms in the presence of the disinfectants under test. They were not inhibitory but neutralised disinfectant residues carried over to the recovery system.

BACTERICIDAL ACTIVITY

Pseudomonas aeruginosa
A non-sporing, gram negative bacilli, moderately NCTC 6749 resistant disinfectants and widely used for disinfectant testing

Enterococcus faecalis
A non-sporing gram positive cocci moderately resistant NCTC 775 disinfectant testing and qualifying thermal disinfection

A. Suspension test: clean and dirty conditions

1 ml of an overnight nutrient broth culture of *Pseudonomas aeruginosa or Enterococcus faecalis was* added to 9ml of freshly prepared disinfectant. The mixture was gently swirled to mix and, at specific time intervals of 30 secs, 1, 5, 10 and 20 mins, 1ml was removed and added to 9ml of recovery/ neutraliser broth. This was mixed thoroughly and 10 fold diluted in ¼strength Ringers solution. The recovery broth and dilutions wore plated onto tryptone soya agar plates and incubated for 18 hours at 37ºC to give damaged bacteria the opportunity to grow before plating out on to tryptone soya agar.

The test was repeated in the presence of 1 and 10% horse serum (dirty conditions) and also on solutions generated and stored (aged) for 24 hours.

SPORICIDAL ACTIVITY

Test Bacillus subtilles var niger NCTC 10073 Organism (A chemically tolerant spore forming bacteria widely used to validate Ethylene oxide and other chemical sterilisation processes)

A. Suspension test: clean-and dirty conditions

1 ml of a suspension of Bacillus subtilles var niger containing >107 spores/ml, heat shocked (80ºC for 1 min) to eliminate non-sporing organisms, was added to *9ml* of freshly prepared disinfectant. The mixture was gently swirled to mix and, at specific time intervals of 30 sec., 1, 5, 10, 20, 30, 60 and 120 mins, 1 ml was removed and added to 9ml of recovery broth. This was mixed thoroughly and 10 fold diluted in ¼ stretch Ringers solution. The recovery broth and dilutions were plated onto typtone soya agar plates and incubated for 18 hours at 37ºC and examined for surviving test organisms.
The number of colony forming units (cfu's) of surviving test organisms were enumerated and the results tramposod to Log 10 counts, The recovery broths were incubated for a further 7 days at 37ºC to give damaged spores the opportunity to grow before plating out onto typtone soya agar.

The test was repeated in the presence of 1 and 10% horse serum (dirty conditions) and on solutions stored (aged) for 24 and 48 hours,

B. Surface test

Bacillus subtilus var niger spores are deposited onto Aluminium foil and prepared to DoH specification TSS/81330-012 obtained from Steriscal Ltd, Rodditch, Worcs, were immersed in 10ml of disinfectant. At specific time intervals, i.e, 1, 5, 10, 20, 30, 60 and 120 mins, the spore, strips were aseptically removed and placed in 10ml of recovery broth. Five sport strips were immersed in separate universal containers for each time interval. *One of the five strips was* agitated with glass beads in the recovery broth diluted and counted as described for the suspension lest. The remaining dilutions and other 4 scraps were incubated at 37ºC and examined for growth up to 14 days. The spores were recovered in the same manner from 3 untreated strips to determine the pre-disinfection challenge.

IN VIVO TEST': ASSESSMENT OF BACTERICIDAL ACTIVITY OF MIRCOCIDE PA IN THE KEYMED AUTODISINFECTOR

Cleaning and disinfection regime evaluated

The widely used KeyMed Autodisinfector Mark 3 was used in these tests. This cleans, disinfects and rinses all channels and external surfaces of flexible fibreoptic endoscopes.
Neutral detergent was placed in the detergent reservoir (changed after 5 cycles), Anolyte (freshly prepared and changed after each cycle) in the, disinfectant reservoir and freshly drawn tap water in the rinse reservoir (changed after each cycle). Anolyte was used with a 4 min contact time. This is the contact time currently recommended by the British Society of Gastroenterology (1988) for gastrointestinal endoscopes. For comparative purposes a further series of tests wore performed using neutral detergent and 2% activated alkaline glutaraldehyde, The glutaraldehyde was used repeatedly for 20 cycles and then discarded.
Method

The biopsy, suction, air and water channels of an 0lympus gastroscope GIF Q10 were contaminated with an overnight broth culture Pseudomonas aeruginosa NCTC 6749 (UK disinfectant test strain) enriched with 10% horse serum, and left to drain/dry for 10 min at room temperature before sampling (pre-disinfection count) or processing and sampling (post-disinfection count). The scope was i.e. re-contaminated for each test cycle. In previous studies with neutral detergent and 2% glutaraldehyde, the Autodisinfector has been shown to be highly effective in cleaning and disinfecting fibrescopes, After processing the endoscope, channels were sampled by flushing with 1ml of sterile water which was Collected in a sterile container at the distal tip. The method of sampling is described in detail elsewhere (Bradley & Babb (1987) Evaluation of the Autodisinfector, Acta Endospica 17:17-22), Samples were 10 fold diluted and the recovery fluid and the dilutions plated onto tryptone soya agar. These were incubated at 37ºC for 18 hours and the number. of colony forming units of the test organism enumerated. Counts were transposed to the Log 10 system and the Log reduction (RF) calculated for each *cycle, i.e.*

Log10 pre-disinfection count - Log 10 post-disinfection count = Log 10 reduction (Rf)

Table 1a BACTERICIDAL ACTIVITY:

Suspension tests Pseudomonas aeruginosa NCTC 6749

Solution	pre-count	30 secs	1 min	2 mins	5 mins	10 mins	20 mins
Anolyte clean conditions	7.84	0	0	0	0	0	0

fresh 24 hours	7.93	1.96	1.15	0	0	0	0
Anolyte dirty (1% serum) fresh 24 hours	7.84 7.93	2.17 5.93	0 3.72	0 0	0 0	0 0	0 0
Anolyte dirty (10% serum) fresh	7.93	6.2	6.2	0	0	0	0
2% glutaraldehyde clean 1%serum 10%serum	7.96 7.96 7.92	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

Log 10 remaining after

Table 1b BACTERICIDAL ACTIVITY:

suspension tests Enterococcus faecalis NCTC 775

Solution	pre-count	30 secs	1 min	2 mins	5 mins	10 mins	20 mins
Anolyte clean conditions fresh 24 hours	7.74 7.52	0 0	0 0	0 0	0 0	0 0	0 0
Anolyte dirty (1% serum) fresh 24 hours	7.74 7.52	0 0	0 0	0 0	0 0	0 0	0 0
Anolyte dirty (10% serum) fresh	7.52	6.34	5.38	3.1	0	0	0
2% glutaraldehyde clean 1%serum 10%serum	7.73 7.73 7.69	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

Log 10 remaining after

Table 2a SPORICIDAL ACTIVITY

Suspension tests

Solution	pre-count	30 secs	1 min	2 mins	5 mins	10 mins	20 mins	30 mins	1 hr	2 hrs
Anolyte clean conditions fresh	7.54	0	0	0	0	0	0	0	0	0
Anolyte dirty (1% serum) fresh	7.20	5.18	2	2	0	0	0	0	0	0
Anolyte dirty (10% serum) fresh	7.5	7.3	7.08	6.97	6.93	6.76	5.88	4. 34	0	0
2% glutaraldehyde clean 1%serum 10%serum	7.89 7.78 7.82	7.51 7.58 7.62	7.54 7.49 7.56	7.40 7.46 7.49	7.42 7.51 7.53	7.35 7.28 7.38	6.99 7.26 7.06	5.81 6.08 5.94	4.72 4.63 4.83	0 0 0

Log 10 remaining after

Table 2b Surface test: clean conditions only

Solution	pre-count	5 mins	10 mins	20 mins	30 mins	1 hr	2 hrs
Anolyte	5.64	0	0	0	0	0	0

2% glutaraldehyde	5.57	5.62 (5/5)	4.38 (5/5)	4.29 (5/5)	3.86 (5/5)	1.30 (5/5)	0 (0/5)

Table 3 Endoscope tests

Solution	Channel	Pre-disinfection count	Mean post disinfection count	Mean Log 10 RF	No. of cycles
Anolyte	Biopsy	7.69	0	7.69	20
	Suction	7.58	0	7.58	20
	Air	7.83	0	7.83	20
	Water	7.93	0	7.93	20
2% glutaraldehyde	Biopsy	7.88	0.49 (0-2.3)	7.39	25
	Suction	7.94	0.23 (0-3.08)	7.71	25
	Air	7.95	0.24 (0-2.48)	7.71	25
	Water	7.99	0.15 (0-2.28)	7.84	25

The pH of Anolyte solutions used for these tests was in range 5.4-6.5 with an ORP of >1000 mV.

RESULTS

The bactericidal activity of freshly generated Anolyte and 'aged' samples (24 *hrs)* is shown in tables 1a and 1b. In the absence of an organic load, Anolyte was effective in under 2 mins with Pseudomonas aeruginosa and in under 30 sec. with Edterococcus faecalis. Increasing the organic load (10% serum) has a marked effect on bactericidal activity and 10-20 mins is required to destroy the test bacteria. Aged samples were slightly less effective but we would support use up to 24 hrs.

The European suspension tests requires a 5 Log reduction in non-sporing organisms in 5 mins and the tests under clean and low organic load conditions easily met this requirement with freshly generated and 24 hour aged sample. The pseudomonoacidal activity of 2%. glutaraldehyde, was slightly better than Anolyte and this activity was maintained in conditions of high organic load.

Tables 2a and 2b indicate the sporicidal activity in suspension and surface tests. At least a 6 Log 10 reduction in test apples is required to substantiate 'sterilant' claims. This was achieved in under 5 mins with freshly generated under clean and low organic load conditions. Increasing the level of serum to 10% reduced sporicidal activity with 1 hr immersion was required to destroy 5 Log spores, 2% glutaraldehyde on the other hand, took 2 hrs to kill spores but the addition of organic matter had no effect on sporicidal activity.

During these tests, no observable damage was sustained to the endoscope or washer disinfector, however, 20 cycles is insufficient to form an opinion as this only represents one mornings use, We would expect endoscopes and processing equipment to withstand several years exposure to disinfectants and cleansing agents without damage. Further tests will be necessary to establish instrument and processing equipment compatibility.

A slight smell of chlorine was noted when generating and using Anolyte although this was insufficient to cause concern.

CONCLUSION

Anolyte, in the 5-7 pH range, and with a ORP of $\geq$ 1000 mV, is highly microbicidal and disinfection occurs within 2 mins. Increasing the organic load reduces the efficacy and items must therefore be thoroughly cleansed first.

The activity of Anolyte against non-sporing bacteria is similar to 2% glutaraldehyde. The sporicidal activity of Anolyte is, however, far superior and a 6 Log 10 reduction in spores is obtained in under 5 mins. and under 30 secs in the absence of an organic load, This could reduce current sterilisation processing times. Increasing the organic load by adding 10% serum reduces efficacy and the sporicidal effect is then similar but not inferior to 2% glutaraldehyde.

The results of 'in vivo' tests in a widely used automated endoscope washer/disinfector showed that Anolyte is slightly superior to 2% glutaraldehyde and no test bacteria survived the automated cleansing and disinfection process.

On the basis of these tests we found the bartericidal / sporicidal activity of Anolyte encouraging. Providing other tests indicate that it is user friendly and non-damaging to instruments and processing equipment it could prove a viable alternative to glutaraldehyde for processing endoscopes and other heat sensitive equipment.

Other tests using Myrobactedum tuberculosis, or a surrogate, e,g. Mycobactedum terrae, may be necessary to establish suitability as a high level disinfectant.

Glutaraldohyde is, at present the disinfectant of choice for heat sensitive endoscopes but it is irritant and sensitising to the skin, eyes and respiratory tract and a less irritant alternative is sought. The performance of Anolyte in these initial tests showed activity was good and similar to the other recently introduced glutaraldehyde alternatives such as chlorine dioxide and peracetic acid. These products are however, expensive and unlike Anolyte not generated on demand. Providing a system is Produced for reliably and inexpensively generated Anolyte, at the specified pH and redox, it could greatly enhance the automated processing of heal sensitive endoscopes. Other possible uses of possible uses, which were not assessed in this study, are the benefits of using the Catholyte for, cleansing and the generation of bacteria-free water for rinsing processed instruments