

THE AQUABRITE SYSTEM

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SUMMARY

The Aquabrite System is a non-chlorine, fresh water method of disinfection of swimming pools. The latest trials investigated bacterial mortality against time using an improved formulation. Mortality rates for chlorine against time were also examined. Organisms from two sources, laboratory cultures and raw sewage were used to assess die-off rates. The Aquabrite System was highly effective in promoting bacterial mortality from both sources. Chlorine displayed less rapid efficacy on laboratory cultured organisms and no demonstrable die-off was observed on raw sewage bacteria during the test period. This paper attempts to cover the following objectives:

Report recent efficacy trials investigating bacterial mortality against time, using The Aquabrite System, incorporating a new formulation.

Describe The Aquabrite System, its applications and unique advantages as a practical pool disinfectant.

Compile and summarise available background data on the development of The Aquabrite system.

BRIEF REPORT

The data obtained by this series of controlled tests indicated that when a low level of a specially blended persulphate oxidiser (Aquabrite) was used with copper and silver ions in water containing urea (organic nitrogen) *excellent results* were obtained.

Chlorine, under similar circumstances, did not perform as well, probably due to the formation of chloramines. In heavily used swimming pools, the water will certainly contain ammonia and urea so that these compounds will be present in the pool water. Unless large quantities of chlorine are being continuously added to achieve the desired free chlorine concentration then inadequate disinfection will result. Public pools often have such a facility but domestic pools do not. It is in such circumstances that The Aquabrite System can be of benefit.

The Aquabrite System displayed the additional benefit of longevity of antimicrobial activity. This was demonstrated by storing treated water for fourteen days before conducting mortality versus time tests using *Ps.aeruginosa sp* as the test organism.

1.0 THE CHLORINE TREATMENT SYSTEM

1.1 Introduction

Historically, chlorine has been favoured as a water disinfectant. It exhibits rapid bactericidal activity within certain constraints. The efficacy of chlorine is limited by pH, alkalinity (ability of the water body to resist changes in pH), organic content, nitrogen content and exposure to ultraviolet light (White, 1972).

These conditions do not often receive the attention required to maintain the efficacy of chlorine. The dosing of chlorine into water with an incorrect balance of the above-mentioned parameters may lead the operator into a false sense of security. These parameters are discussed below.

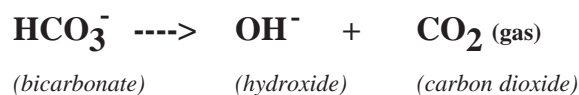
1.2 pH and Alkalinity

Chlorine is only effective as a disinfectant when in the form of hypochlorous acid (HOCl). This form of chlorine exists when the pH of the water body is less than 9.0. Above pH 9.0 the hypochlorite ion (OCl⁻) predominates. Hypochlorite ion, at concentrations normally found in swimming pools, is a very poor sanitiser (White, 1972). Refer to table 1.

pH	HOCl%	OCl% at 20 C
5.0	99.7	0.3
6.0	96.8	3.2
7.0	75.2	24.8
8.0	23.2	76.8
9.0	2.9	97.1

Table1: Content of HOCl decreases with increase in pH and content of OCl increases with increase in pH (White, 1972).

Spas may be particularly prone to unstable pH as the large volume of air injected through the water in a spa may cause carbon dioxide stripping of the water. Loss of carbon dioxide causes a rise in pH. At a given temperature and pressure, carbon dioxide is in fixed equilibrium with bicarbonate ion in the water.

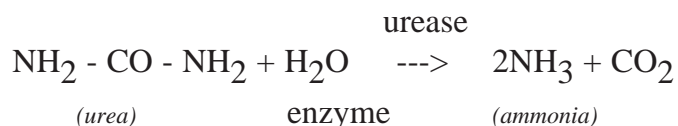


From the reaction above it is apparent that the removal of carbon dioxide forces the equilibrium to the right, causing the formation of the alkali hydroxyl ion which increases the pH.

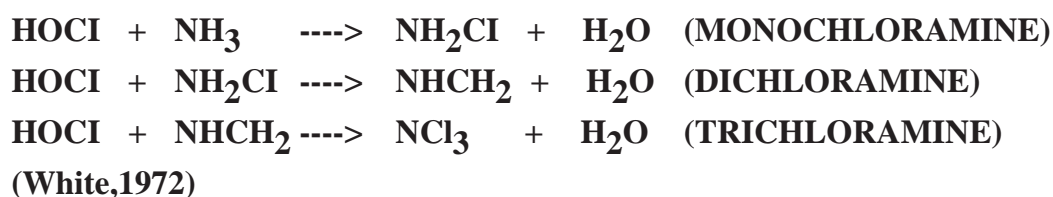
1.3 Organic and Nitrogen Content

The concentration of nitrogen (as ammonia and urea) and organic matter present in pool water is of critical importance. The active form of chlorine, hypochlorous acid (HOCl), is an extremely reactive chemical and is able to combine with these constituents to form a wide variety of

compounds. Some of these compounds are toxic and irritating. The hypochlorous acid that combines in this way is rendered less effective as a disinfectant. In order to maintain appropriate conditions the water body must remain essentially free of constituents which will combine with chlorine; however these are often difficult to control. Nitrogen may enter the water in the form of urea from urine and sweat, sunscreen lotion and quaternary ammonium algacides. Organic matter may enter the water through leaves, dust, skin cells, make up and faeces. Urea, a component of urine and sweat, is the main source of ammonia nitrogen. Urea is converted to ammonia by hydrolysis with water under the influence of the urease enzyme in the following way.



The product of the reaction of hypochlorous acid with ammonia is the formation of a group of compounds known as chloramines. These are unpleasant chemicals causing red eyes and mucosal irritation and have the characteristic "chlorine smell". The odour is misleading since this form of chlorine is not very effective as a disinfectant and long contact times may be required (White, 1972). Hypochlorous acid reacts to form a series of chloramines. They are corrosive gases and often cause damage to buildings which enclose swimming pools (see reactions below).



The above reactions are dependent upon pH, ammonia concentration and temperature. Chloramines can be destroyed by superchlorination. Superchlorination is the addition of very high concentrations of chlorine to the water. The pool cannot be used until the concentration of chlorine falls to within acceptable limits for swimming. Nitrogen trichloride (or trichloramine), an extremely objectionable gas, may be formed at this point (White, 1972).

Chlorine is able to form compounds with organic or carbon based matter and can perform addition, substitution and free radical reactions so that a wide range of chlorinated compounds is possible (Harold and Schuetz, 1966). Chloroform, which is toxic and a suspected carcinogen, is often found in chlorinated swimming pool waters, along with a series of chlorine-resistant organic nitrogen compounds (proteins) such as creatinine (White, 1972) .

1.4 Ultra-Violet Light and Temperature

Heat and ultra-violet light must also be considered. At elevated temperatures, chlorine can be destroyed (Yahya et.al., 1989). High temperatures can exist within water heating systems. Ultraviolet light breaks down hypochlorous acid. Swimming pools are particularly prone to loss of sanitiser efficacy due to the action of sunlight. (White 1972).

Isocyanuric acid is a sparingly soluble cyclic aliphatic compound that acts as a U. V absorber in the water. It is often called “pool conditioner” or “stabiliser” because it is able to prolong the life of chlorine in pool water. It forms a series of chlorinated isocyanurates having various compositions.

These compounds form a chlorine reservoir. Hydrolysis of N-Cl bond causes the release of hypochlorous acid (White, 1972). Studies of outdoor chlorinated pools show that pools without these stabilisers lose about 90 percent of their total chlorine residual on a sunny day in two or three hours. Pools treated with 25 to 50 milligrams per litre of cyanuric acid, however, under the same conditions, lose only 10 to 15 percent of their total chlorine (White, 1972). When “pool conditioner” is used at concentrations up to 100 mg/L, the free chlorine residual must be increased from 0.4 mg/L to 1.5 mg/L. Its use above 100 mg/L is not recommended. There is some concern about the toxicity of the dismutated N-base and its accumulation in pool waters.

1.5 Application of Chlorine

A typical ten-metre pool contains about sixty thousand litres of water. The pool is often heated in order to extend the swimming season. A cupful of dry chlorine is routinely added every day. The cupful equates to about 150 g of calcium hypochlorite (55 kg per annum) which contains 65% available chlorine*. When chlorine gas dissolves in water only half of its chlorine content forms hypochlorous acid, the active sanitiser (HOCl). * The term “available chlorine” means available as chlorine gas.



Of the original 150g less than one third (~50g) can be effective as a disinfectant. The concentration of chlorine added to the pool water is therefore only 0.8 mg/L.

If the pool is in use then this concentration of chlorine is totally inadequate. Children, in particular, are prone to spontaneous urination (micturation), when they are immersed in warm water. Urine is the main source of nitrogen in pool water and contains about 2.5% of urea by weight. Urea is almost 50% nitrogen. One part of ammonia nitrogen uses up to 10 parts of chlorine. Therefore, less than half a litre of urine would be sufficient to destroy all of the free chlorine in the pool. Additional nitrogen or organic substances will continue to build up in the pool water; ideal nutrients for algae and bacteria, and then impose an even higher chlorine demand for the next day. The urine itself does not normally contribute any bacteria to the pool, just the nutrients to enable bacteria to grow.

The majority of domestic pool owners operate their pools on the basis of economy first so that the pump and filter are under utilised resulting in less than optimum water turn over. This is followed by a token dose of chlorine on a daily basis followed by a plethora of chemicals such as clarifiers (polyelectrolytes) algicides (quaternary ammonia compounds) pool conditioners (isocyanuric acid) and stain removers (organic acids) from the pool shop because “the pool went off” .

At this time the pool gets a “hit” of chlorine, usually about five litres of liquid pool chlorine to clean up the algae. The pH goes up because of the highly caustic nature of the hypochlorite solution and the pool becomes cloudy due to precipitation of calcium from the water. Mineral acid must be added to correct the situation but now the total alkalinity has fallen again, and so

it goes on! The main permanent active sanitiser is not free chlorine but monochloramine. Monochloramine *is a very poor bactericide*.

Melbourne's Waverley City Council conducted a survey of the concentrations of free residual chlorine (hypochlorous acid) in swimming pools. Although operators believed they were dosing chlorine in accordance with health standards, 76% of pools tested were below the recommended levels (Nat. Times, 1980). Some of these pools contained organisms in numbers which were in breach of public health criteria. In a similar survey, Queensland University found that 50% of pools sampled contained coliform bacteria (Nat. Times, 1980).

Such high failure rates imply that the pool operators did not understand the principles of pool chlorination and consequently were unable to maintain their pools in a sanitary condition using chlorine.

2.0 THE AQUABRITE SYSTEM

2. Introduction

The Aquabrite system is based upon the principle of “oligodynamics”. The term “oligodynamic” is used to differentiate between ordinary poisoning death by metallic salts on living organisms at high concentrations and antibacterial activity of metal ions at much greater dilutions. “Oligodynamics” was first used by a researcher, Von Nageli (1893) when he formed it from two Greek words “oligos” meaning small and “dynamis” meaning power (Lawrence and Block, 1966).

There have been numerous studies of oligodynamics dating from about 1890 to the present time. The general conclusion is that there is a form of silver ion which is antimicrobial, stable and nontoxic to man (Lawrence and Block, 1966). Silver may be added to water as a sparingly soluble salt, colloidal metallic dispersions or generated electrolytically. The water may also be passed through media coated with metals. **The Aquabrite System utilises electrolytic copper and silver.**

In practice, copper and silver metals are made into an alloy from which electrodes are cast. When the electrodes are placed in the pool filtration system and a small current of electricity is allowed to flow between the two electrodes, copper and silver ions are released into the water from the positive electrode (anode). The ions are swept into the pool by the rapid flow of water and become part of the pool water chemistry. These metal ions are completely safe to use and provide a residual disinfectant and algicidal activity throughout the pool to prevent bacterial slime on pool surfaces and inhibit the growth of algae.

2.2 Copper

The World Health Organisation has imposed a limit for soluble copper in drinking water of 2.0 mg/L. The limit is set at that concentration because a metallic taste may be imparted to some waters. Copper ions serve as an effective algicide and its concentration can be measured with a simple test kit and therefore controlled. The Aquabrite system operates within the range of 0.2 - 1.0 mg/L. “There is no scientific data available to support the toxicity of copper in drinking water” (Dresher, 1995).

2.3 Silver

The world Health Organisation limit for silver in drinking water has been set at 0.05 mg/L. Excessive ingestion of silver can cause a rare condition called argyria * (Romans, 1966, a darkening of the skin. Silver is considered to be the least toxic of the heavy metals. The Aquabrite system normally operates within the range 0.01-0.05 mg/L.

The concentration of silver ions is so low that it cannot be measured with a simple comparator test kit. Its concentration is dependent on the solubility product of its least soluble salt (Glasstone and Lewis, 1965). Silver sulphide is the least soluble of the silver salts but oxidised pool water cannot contain sulphides. The most likely form of silver in pool water is silver chloride.



*Reports of argyria caused by silver used in oligodynamic applications have not been found in the literature.

If the chloride ion concentration increases, the solubility of silver chloride decreases (Glasstone and Lewis, 1965). If the temperature of the water increases, the solubility increases. The solubility of silver chloride also increases if salts other than chloride (except sulphide and bromide) are present.

As silver ions can be easily reduced by organic matter, it is necessary to keep them in an oxidised state by maintaining a permanent chemical oxidiser in the pool water .

2.4 Aquabrite Oxidiser

Aquabrite oxidiser is a special blend of persulphates. Although it is not bactericidal at concentrations used in swimming pools, when copper and silver ions are present a powerful disinfectant is produced in the water. (The disinfectant properties are broad spectrum and residual throughout the pool for up to fourteen days after treatment.)

Aquabrite has an oxidation potential high enough to maintain silver ions in a high oxidation state even to Ag^{3+} (Zsoldos and Kowalski, 1970). In a similar way, copper is oxidised to Cu^{3+} . This is important for the removal of ammonia from pool water by the following suggested mechanism:



Organic matter is oxidised but no unpleasant compounds are formed.

The standard dose of Aquabrite is 20mg/L (1Kg per 50,000 litres of pool water) each week in summer, when the pool is in constant use and once per month when not in use. Aquabrite poses no risk to health on the ingestion of pool water, as its final reaction products consist of simple sulphates and oxygen.(Zsoldos et.al., 1970).

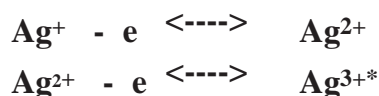
2.5 Maintaining Optimum Conditions

In practice, both copper and silver are lost from the system so that continuous replacement is necessary . Electrolytic generation of copper and silver ions is both practical and convenient and essentially automatic. Copper can be measured with a simple colourimetric test kit, and regulated accordingly. Aquabrite is added manually or as a strong (10%) solution and its concentration in the pool may be easily tested using Palin's DPD test method. A standard pool test kit utilising DPD No.1 tablets is required for this procedure.

As with any oxidiser, its duration in the pool depends on several factors:

- The organic loading on the pool such as leaves, number of persons using the pool, chemicals added such as citrates, oxalates, polyelectrolyte pool clarifiers, quaternary ammonium algicides, etc.
- Rainfall - when the excess water is run off to maintain the correct level of water in the pool, the active components of the system are lost.
- Backwashing of sand filters, or diatomaceous earth filters results in the loss of sanitiser from the pool.
- High chloride ion (salt) concentration shortens its duration.
- Use of reducing agents for pool cleaning such as metabisulphites, sulphonic acids etc.
- Generally the higher the pH of the pool water, the shorter the life of the oxidiser. (see 2.6 below)

A recent review of the literature (Thurman and Gerba, 1988) does not address the mechanism by which the Aquabrite System is able to rapidly disinfect. Researchers have dealt with copper and silver individually or in combination with chlorine (Yahya et.al., 1989) or iodine (Pyle et.al., 1992). Neither chlorine nor iodine is able to oxidise silver to its higher valency state as the necessary oxidation potential to do so must exceed 1.987 volts.



* It is not known conclusively that the trivalent ion exists in pool water, only that it is theoretically possible.

2.6 Advantages of the Aquabrite System .

- No corrosive gases or condensates are formed .
- Water remains taste and odour free
- Sunlight has no detrimental effects on the chemistry of the system .
- Efficiency increases directly with water temperature . Effective over a wide pH range (6.8 to 7.4- optimum range).
- Residual disinfectant action throughout the body of water. •Non-toxic chemicals and by products.
- Active components can be simply measured with colour test kits.
- Inexpensive to operate in most situations.
- Controls both algae and bacteria.
- Automatic operation with no daily chemical additions to the pool.
- Does not bleach towels and costumes.

- Pool conditioner (chlorine stabiliser) is not required
- Fresh water system which will not cause damage to porous pavers and sandstone or plants and grass around the pool- as opposed to salt water systems
- Backwash water may be used for irrigation of lawns and gardens as the water is free of chemicals (i. e. salt or chlorine laden backwash water may contaminate local water courses)
- Does not dry out skin and hair and is non-irritating to eyes .
- Compatible with chlorine should this be required as an alternative to “ Aquabrite”

Limitations

- Not compatible with pools containing salt water- the pool should be drained and refilled with fresh water
- Bromine or bromides are not compatible with this system
- Adjust pH and total alkalinity (T.A.) only with sodium bicarbonate (**Do not** use soda ash or caustic soda)
- Not compatible with Baquacil biocide

2.7 Applications of Aquabrite System

The first documented use of silver with persulphates to control bacteria in swimming pools was reported in 1970 and used successfully by Marotta Scientific Controls Inc. New Jersey, U.S.A. The persulphate was utilised to maintain the silver in an oxidised state. Silver ions were added to the pool in the form of silver nitrate (Zsoldos and Kowalski, 1970).

The Aquabrite system is capable of controlling algae and bacteria to within safe limits in both swimming pools and spas, including herbal spas. Laboratory tests under controlled conditions confirm its efficacy*.

The Aquabrite System has been applied by successfully to:

- Fountains at the Warringah Mall Shopping Centre, Brookvale
- A decorative water cascade at the AMP Centre, Circular Quay
- A 55 metre resort pool, Trinity Links, Cairns

Many thousands of domestic pool owners in Australia and overseas have adopted the system. The applications are numerous and the system virtually unlimited in scope.

A trial of the principle in a domestic swimming pool situation was conducted over a period of four months from November 1990 to March 1991 with successful results.

In July 1995 an Aquabrite system was fitted to a commercial herbal spa in a Korean hotel and bathhouse at Kings Cross, Sydney. Subsequent monitoring by both South Sydney Council and an independent N.A.T.A. registered laboratory demonstrated that the bacterial quality of the spa water complied with regulations contained within the Public Health Act, 1991. Two additional systems have since been installed in the same hotel.

- * *Laboratory efficacy studies were conducted from February to April 1991 at the NATA registered laboratories of Judell Platt Thomas and Associates. Results confirmed that the method was an effective disinfectant and that the treatment **remained active for a period of several weeks** after the water had been treated.*

The Aquabrite System may be considered as a viable option wherever algae and bacteria pose a problem in fresh water. It is especially valuable as an alternative to chlorination in cases where a medical condition such as dermatitis or an allergy to chlorine or chlorinated compounds would prevent the pleasures of swimming. The system is particularly useful for the treatment of pool water, which may be discharged into environmentally sensitive areas as the water is free of salt and chlorine compounds.

3.0 METHODOLOGY

3.1 Experimental Design

Two identical fibreglass tanks each with a capacity of 200 litres were set up. Each tank was fitted with an Onga MD10 (40W) pump, a Waterco Filter King-5 micron cartridge filter and an Email type 4503 (1500W) immersion heater. The plumbing for the tanks allowed for cross mixing so that the water in both tanks could be made chemically identical. During the period of the Aquabrite treatment however, cross mixing was prevented by operating a system of valves and each tank became a separate system with its own pump and filter.

Sydney tap water is low in dissolved mineral salts so it was necessary to add minerals in order to increase the electrical conductivity and stabilise the pH of the water by the resultant buffering effect. The following mineral salts were added to the tank water. The tanks were prepared on 6th November 1995.

<u>Mineral Salts</u>	<u>g/400L</u>	<u>mg/L</u>
Sodium Bicarbonate	34	85
Calcium Chloride (Dihydrate)	37	92
Sodium Sulphate (Anhydrous)	17	42
Sodium Bisulphate (Dry Acid)	to adjust the pH to 7.2	
Urea	2	5

Urea was added to simulate real swimming pool water and provide some nutrients for bacteria. The original (400 L) tap water was dechlorinated by using a solution of 10% sodium thiosulphate solution drop wise until no colour was produced in the samples tested with a Palin's test kit. (DPD No.1 tablet followed by a DPD No.3 tablet .) A chemical analysis of the test water and control water appear in appendix 4.

3.2 Choice of Challenge Organisms

Challenge organisms were chosen to coincide with the NSW Health Department's "Guidelines for Disinfecting Public Swimming Pools and Spa Pools", NSW Health Department, 1991 vis: *E.Coli*, *Ps.Aeruginosa*, *Standard Plate Count Organisms*.

3.3 Analytical Methods

3.3.1 Chemical

Analyses of both the control and test water was conducted by Analchem-Bioassay according to accepted A.P .H.A. Standard Methods. Low copper and silver concentrations were analysed by C.R.A. (see appendix 1)

3.3.2 Microbiological or Biocidal Efficacy Test Methods

See Appendix 2

3.4 Quality Assurance and Quality Control

- pH was measured by a two buffer calibrated meter using a combined glass electrode.
- Temperature was measured by a calibrated total immersion mercury in glass thermometer 0 - 50°C in 0.1°C divisions
- Copper ion in the original preparation of the test water was measured using a Taylor colourimetric test kit.
- Chlorine was measured by using the Palin's DPD method.

*Laboratory quality assurance and control procedures in accordance with NATA requirements included the following:

(i) Media check for:

- Sterility
- pH
- Positive control recovery
- Typical colony morphology

(ii) Calibration of Equipment/Instructions on the following:

- Incubators
- Water baths
- pH meters
- Thermometers
- Autoclaves

4.0 DISCUSSION OF RESULTS

Electrolytically generated copper and silver ions have been used for many years as a method of purifying swimming pool water. Although this method is capable of controlling algae and bacteria, it has been regarded by most authorities to be too slow in its response against micro-organisms. However, when a suitable oxidiser is present with the copper and silver ions, the response is improved by several orders of magnitude and thus the method becomes a viable alternative to chlorination. The purpose of this investigation was to compare the efficacy of chlorine with The Aquabrite System against *E. coli*, *Ps. aeruginosa* and raw sewage bacteria in synthetic swimming pool water to which organic nitrogen (urea) had been added, as proposed by Yahya et.al., (1989).

Results of the tests indicated that copper and silver ions (900 ug/L and 50 ug/L, respectively) when used in conjunction with 10 mg/L Aquabrite reduced numbers of *E. coli* by greater than 3.1 log₁₀ in approximately 0.5 minutes. The chlorinated system (1.2 mg/L chlorine) inactivated *E.coli* numbers 1.1 log₁₀ in the same time interval. See figure 1. (test results are presented in appendix 3).

Results of the Aquabrite system versus chlorine on E.Coli

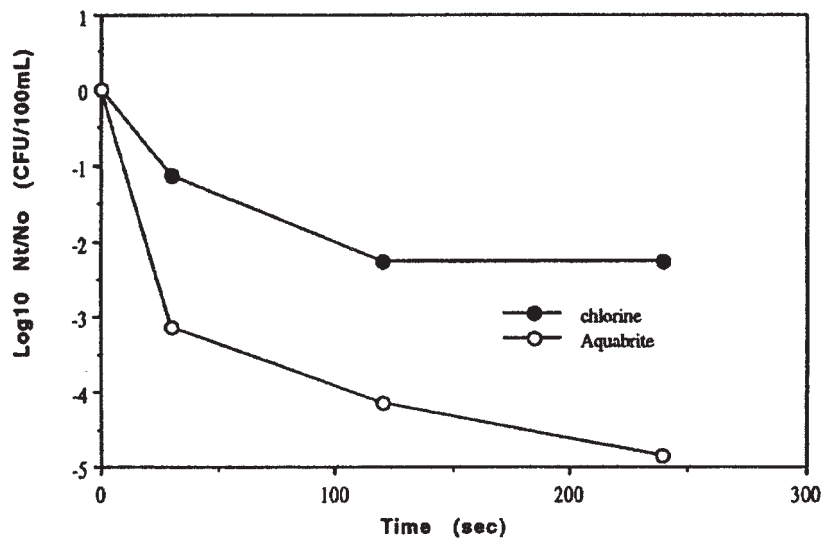


Figure 1. Mortality of E.coli after exposure to chlorine or Aquabrite System.

Note The above results are expressed in table form below

Time elapsed	Bacteria left alive with chlorine*	Bacteria left alive with Aquabrite system
2 minutes	54,000/100ml	< 100/100ml
4 minutes	64,000/100ml	< 10/100ml
8 minutes	63,000/100ml	< 2/100ml
16 minutes	10,000/100ml	< 2/100ml

Note *1.2 ppm chlorine
 < = less than

Results were obtained for *Ps. aeruginosa* which indicated that the organism resisted the effects of chlorination (1.2 mg/L chlorine) after an initial reduction of 2.2 log₁₀ in approximately 0.5 minutes, as no further reduction was observed even on prolonged contact. See figure 2 .(test results are in appendix 3).

Results of the Aquabrite system versus chlorine on *P. Aeruginosa*

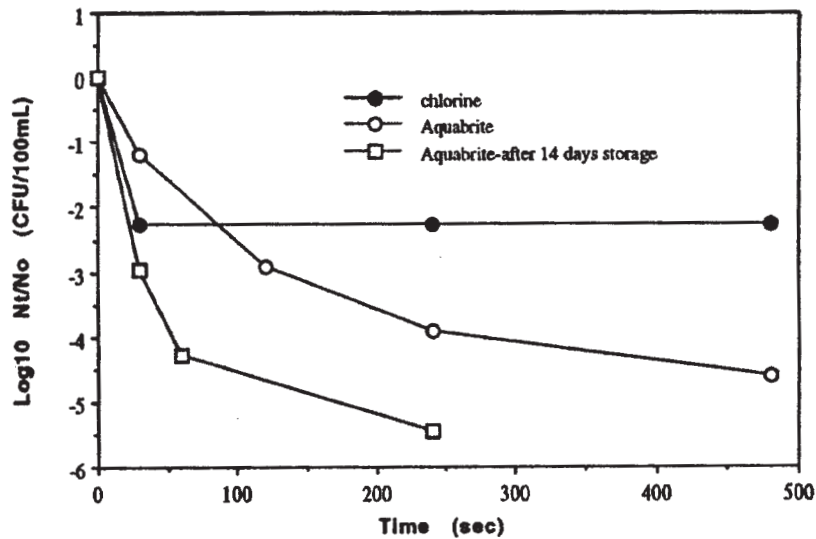


Figure 2. Mortality of *Ps. aeruginosa* sp. after exposure to chlorine or Aquabrite System.
Note The above results are expressed in table form below

Time elapsed	Bacteria left alive with chlorine*	Bacteria left alive with Aquabrite system
2 minutes	79,000/100ml	< 100/100ml
4 minutes	65,000/100ml	< 10/100ml
8 minutes	54,000/100ml	< 2/100ml
16 minutes	10,000/100ml	< 2/100ml

Note *1.2 ppm chlorine
 < = less than

The Aquabrite System reduced bacterial numbers by 1.2 log₁₀ after approximately 0.5 minutes and continued to reduce bacterial numbers by 3.9 log₁₀ at 4 minutes contact time. The same water which was stored for 14 days, and which had no further chemical treatment, was re-tested and demonstrated an improved anti-bacterial performance by reducing bacteria numbers by 3.0 log₁₀ in approximately 0.5 minutes and 5.4 log₁₀ in 4 minutes contact time.

In another trial, raw sewage bacteria were obtained from Warriewood Sewage Treatment Plant and after filtration and dilution, used to inoculate the test and control tanks.

Results indicated that the Aquabrite System was able to reduce bacterial numbers by 2.6 log₁₀ in approximately 0.5 minutes, but the results for chlorine (1.2 mg/L) followed a similar response to that which was observed for *Ps. aeruginosa*. After an initial reduction of 1.8 log₁₀ for 0.5

minutes contact almost no further reduction took place. Aquabrite, however, continued to reduce bacterial numbers to 4.4 log₁₀ after 8 minutes contact time; see figure 3 (test results are presented in appendix 3).

Results of the Aquabrite system versus chlorine on raw sewage

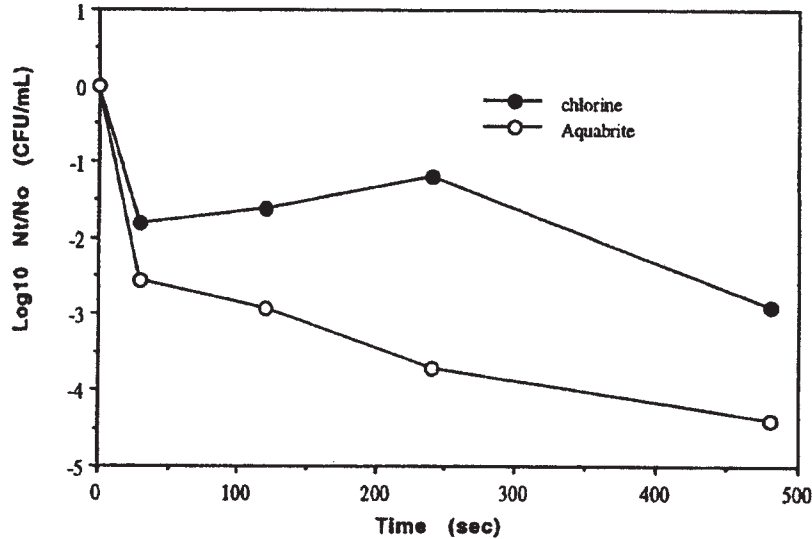


Figure 3. Mortality of raw sewage after exposure to chlorine or Aquabrite System.

Note The above results are expressed in table form below

Time elapsed	Bacteria left alive with chlorine	Bacteria left alive with Aquabrite system
2 minutes	2,400/ml	< 120/ml
4 minutes	1,500/ml*	< 19/ml
8 minutes	650/ml	< 4/ml

Note 1.2 ppm chlorine

*Estimate

Tests were performed in the following laboratory to determine the efficacy of the Aquabrite System versus legionella bacteria. Excellent results were obtained and no re-growth was observed up to four hours after the addition of the culture. This is important when aerosols may be formed for example in spas, water features and fountains.

Results of the Aquabrite system on Legionella

Time elapsed	Bacteria left alive with with Aquabrite system	Control
0 minutes	250/100ml	52,000/100ml
30 minutes	< 10/100ml	60,000/100ml
60 minutes	< 10/100ml	42,000/100ml
120 minutes	< 10/100ml	38,000/100ml
240 minutes	< 10/100ml	37,000/100ml

< = less than

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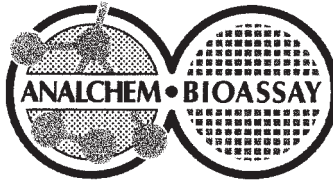
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Appendix 1 Chemical Analyses of Water

Appendix 2 Biocidal Efficacy Test Methods




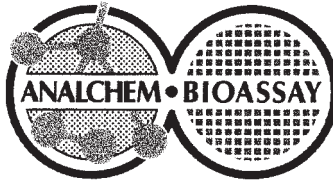
ANALCHEM BIOASSAY PTY LTD A.C.N. 000 678 160
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Tel: (02) 9818 1033 Fax: (02) 9810 8771
Incorporating JUDELL, PLATT THOMAS & ASSOCIATES

CERTIFICATE OF ANALYSIS

DATE: 6 Dec 95
CLIENT: Aquamatics Pty Ltd **Attn:** Les Chedzoy
REFERENCE No: 95/5437
IDENTIFICATION: Control, Ionised & Aquabrite
SAMPLES: Waters (2)
DETERMINATION: pH, conduct, Na, Ca, Cu, Ag, Cl, sulphate, alk
METHOD: APHA, AAS
RESULTS: Samples tested as received

	CONTROL	IONISED & AQUABRITE
pH	7.55	7.21
Conductivity (~S/cm)	470	507
Sodium (mg/L)	53	56
Calcium (mg/L)	32	32
Copper (mg/L)	Less than 0.05	0.90
Silver (mg/L)	Less than 0.01	0.05
Sulphate (mg/l)	66	65
Chloride (mg/L)	65	68
Bicarbonate (mg/L)	73	70
Kjeldahl Nitrogen (mg/L)	5.9	6.1
Free Ammonia (mgN/L)	0.7	0.4


David Carter BSc MRACI
Authorising Analyst



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BIOCIDAL EFFICACY TEST METHOD FOR AQUAMATICS PTY LTD

A. Assessment of biocidal efficacy of “Aquabrite” process system

Test date: 8 Nov 95

Simulated swimming pool water, treated using the “Aquabrite” process system (Test water) and untreated (Control water) will be provided by Aquamatics.

1. Prepare suspensions of challenge organisms in sterile water. Store at 4°C until required. The challenge organism may be laboratory cultured organisms (*Escherichia coli* or *Pseudomonas aeruginosa*) or filtered, diluted sewage collected from Warriewood Sewage treatment plant (supplied by Aquamatics).
2. Fill 2 tanks with Test and Control water, adding 10L to each tank. Record temperature.
3. Add to both tanks simultaneously a suspension of the challenge organism to be examined. Immediately start laboratory timer.
4. Mix the two tanks in parallel thoroughly with paddles.
5. After 15 seconds remove the Time 0 sub-samples from each tank using a gamma sterilised container. Test immediately. Continue mixing of the tanks.
6. Enumerate organisms using the following procedures:

<i>E. coli</i>	STM MW05
<i>P. aeruginosa</i>	STM MW12
Total plate count (using membrane filtration procedure)	STM MW02
7. At each required time interval remove sub-samples simultaneously from the test and control tanks and test immediately.
8. After required incubation periods remove plates and count colonies. Confirmations are not required. Quote results as the count of test organism (per 100mL) or total plate count (per mL), as appropriate.

Aquamatics Pty Ltd
Biocidal Efficacy Test Method (continued)

B. ASSESSMENT OF BIOCIDAL ACTIVITY OF CHLORINE
Test date: 8 Nov 95

Simulated untreated swimming pool water inoculated with test organisms, as described in Part A above, (ie. Control tanks from Part A) will be used for this trial.

1. Assess numbers of organisms by tanking a sub-sample of test water before addition of chlorine and enumerate using the appropriate method (as described in Part A).
2. Add commercial liquid pool chlorine to give a free chlorine level of approximately 1.2 p pm. Use the free chlorine determination by the water laboratory, rather than the manufacturer's label claim to calculate the required volume to be added. Set laboratory timer.
3. Mix the two tanks in parallel thoroughly with a paddle.
4. After 15 seconds remove the Time 0 sub-samples from the tank using a gamma sterilised container. Test immediately. Continue mixing of the tanks.
5. Determine the total plate count of the water using a membrane filtration procedure and the test method STM MWO2.
6. At each required time interval remove sub-samples from the tank and test immediately.
7. After required incubation periods remove plates and count colonies. Determine total plate count for each time interval, for each tank.

C. ASSESSMENT OF BIOCIDAL EFFICACY OF "AQUABRITE" PROCESS SYSTEM
Test date: 22 Nov 95

Repeat the procedure as described in Part A above using water supplied by Aquamatics which has been stored for 2 weeks since initial testing without further processing.

NOTE (Parts A, B and C): Results are to be quoted for each sub sampling time after an initial period of 15 seconds mixing. In reading the reported results it should be noted that the membrane filtration procedure may take from 15 seconds to 2 minutes to complete, depending on the number of dilutions to be tested. As a result of this further die-off of bacteria may occur in the sub-sample itself.

Aquamatics Pty Ltd
Biocidal Efficacy Test Method (continued)

Reference methods for Analchem Bioassay test procedures

STM MW05

Reference: APHA-AWWA-WEF (1992) “Standard Methods for the Examination of Water and Waste Water” faecal coliform procedure.

STM MW12

Reference: Australian standard AS 4276.13 (1995) *Pseudomonas aeruginosa*- membrane filtration method.

STM MWO2

Reference: Australian standard AS 4276.3.1 (1995), 37°C/48 hour count, (membrane filtration procedure used.)



ANNE GERRY, MAppSc, MASM
Chief Microbiologist

Appendix 3 Biocidal Efficacy Results



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CERTIFICATE OF ANALYSIS

DATE: 22 Nov 95

CLIENT: Aquamatics Pty Ltd

Attn: Les Chedzoy

JOB No: 95/5421A

ORDER No:

SAMPLES: Water supplied by Aquamatics Pty Ltd

EXAMINATION: Trial to assess biocidal efficacy of "Aquabrite" process, system, challenge using Escherichia coli

METHOD: Biocidal efficacy test method for Aquamatics , Enumeration of E. coli using STM MW05


RESULTS

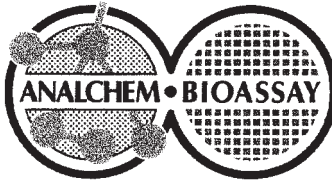
Control water: Simulated swimming pool water provided by Aquamatics
Test water: Simulated swimming pool water subjected to "Aquabrite" process system

Temperature of test and control tanks: 33°C
Test and control tanks inoculated with challenge organisms simultaneously . Inoculated water was mixed for 15 seconds before removal of initial samples for testing.

	<u>E. coli</u> (cfu /100mL)	
TIME (minutes)	TEST	CONTROL
0	<100	1.4x10 ⁵
2	<10	Not tested
4	<10	Not tested
8	<2	Not tested
16	<2	1.1x10 ⁵

< - less than,


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Chief Microbiologist



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CERTIFICATE OF ANALYSIS

DATE: 22 Nov 95

CLIENT: Aquamatics Pty Ltd

Attn: Les Chedzoy

JOB No: 95/5421B

SAMPLES: Water supplied by Aquamatics Pty Ltd

EXAMINATION: Trial to assess biocidal efficacy of "Aquabrite" process, challenge using Pseudomonas aeruginosa

METHOD: Biocidal efficacy test method for Aquamatics , Enumeration of P. aeruginosa by STM MW12

RESULTS


Control water: Simulated swimming pool water provided by Aquamatics
Test water: Simulated swimming pool water subjected to "Aquabrite" process system

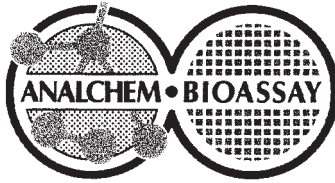
Temperature of test and control tanks: 32.5°C
Test and control tanks inoculated with challenge organisms simultaneously. Inoculated water was mixed for 15 seconds before removal of initial sample for testing.

P. aeruginosa/100mL)

TIME (minutes)	TEST	CONTROL
0	5.0×10^3	7.9×10^4
2	<100	Not tested
4	<10	Not tested
8	<2	Not tested
16	<2	6.0×10^4

< - less than


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CERTIFICATE OF ANALYSIS

DATE: 28 Nov 95

CLIENT: Aquamatics Pty Ltd

Attn: Les Chedzoy

JOB No: 95/5700

SAMPLES: 2 waters for biocide testing, supplied by Aquamatics Pty Ltd

EXAMINATION: Trial to assess biocidal efficacy of water treated using the "Aquabrite" process system which had been stored for 14 days.

METHOD: Biocidal Efficacy Test Method for Aquamatics.
Enumeration of *P. aeruginosa* using STM MW12.

RESULTS

Control water: Simulated swimming pool water supplied by Aquamatics, prepared 14 days prior to testing.

Test water: Simulated swimming pool water subjected to "Aquabrite" process system

Temperature of test tank: 30°C Temperature of control tank: 29°C

Test and control tanks not inoculated and tested concurrently due to time schedule for testing required.

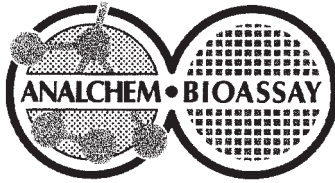
Inoculated water was mixed for 15 seconds before removal of the initial samples for testing .

P. aeruginosa (cfu/100mL)

TIME (seconds/minutes)	TEST	CONTROL
0	5.6×10^5	7.5×10^4
30 sec	$>6.0 \times 10^2$	Not tested
1 min	30	Not tested
2 min	40	Not tested
4 min	<2	Not tested
8 min	<2	Not tested
16 min	<2	2.2×10^3

< - less than, > - greater than


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Chief Microbiologist



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CERTIFICATE OF ANALYSIS

DATE: 22 Nov 95

CLIENT: Aquamatics Pty Ltd

Attn: Les Chedzoy

JOB No: 95 / 5421C

SAMPLES: Water supplied by Aquamatics Pty Ltd

EXAMINATION: Trial to assess biocidal efficacy of "Aquabrite" process system - sewage inoculation study, using a total plate count method.

METHOD: Biocidal Efficacy Test for Aquamatics, Total Plate Count using STM MW02


RESULTS

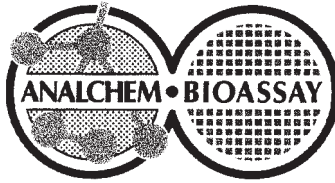
Control water: Simulated swimming pool water provided by Aquamatics
Test water: Simulated swimming pool water subjected to "Aquabrite" process system

Temperature of test and control tanks: 31°C
Test and control tanks inoculated with diluted sewage simultaneously. Inoculated water was mixed for 15 seconds before removal of initial sample for testing .

		Total Plate Count/mL
TIME (minutes)	TEST	CONTROL
0	2.7×10^2	$>10^5$
2	1.2×10^2	Not tested
4	19	Not tested
8	4	Not tested
16	4	$>10^5$

> - greater than


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Chief Microbiologist



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CERTIFICATE OF ANALYSIS

DATE: 22 Nov 95

CLIENT: Aquamatics Pty Ltd

Attn: Les Chedzoy

JOB No: 95/5421D

ORDER No:

SAMPLES: Water supplied by Aquamatics Pty Ltd

EXAMINATION: Trial to assess efficacy of chlorination of water against inoculated E. coli, P. aeruginosa and bacteria in diluted sewage.

METHOD: Biocidal efficacy test method for Aquamatics , Enumeration using Total Plate Count method STM MW02 + membrane filtration procedure

RESULTS:

Initial Counts immediately prior to chlorination

<u>E. coli</u> tank:	Total Plate count/100mL: $>10^7$
<u>P. aeruainosa</u> tank:	Total Plate count/100mL: $>10^7$
Diluted sewage tank:	Total Plate count/mL: $>10^5$

Chlorine added to each test tank: Approximately 0.1mL of 8% liquid pool chlorine to each 10L tank (as per Aquamatics dosing instructions). Water was mixed for 15 seconds after addition of chlorine before removal of initial sample for testing .

	Time after chlorine addition (minutes)	Total Plate Count/100mL
E. coli seeded tank	0	7.3×10^5
	2	5.4×10^4
	4	$\sim 6.4 \times 10^4$
	8	$\sim 6.3 \times 10^4$
	16	$>1.0 \times 10^4$


Page 1 of 2

Aquamatics Pty Ltd 95/5421D

	Time (minutes) after chlorination	Total Plate Count/100mL
<u>P. aeruginosa</u>	0	5.5×10^4
seeded tank	2	7.9×10^4
	4	$\sim 6.5 \times 10^4$
	8	$\sim 5.4 \times 10^4$
	16	$> 10^4$
		Total Plate Count/mL
Diluted sewage tank	0	1.5×10^3
	2	2.4×10^3
	4	$\sim 6.3 \times 10^3$
	8	$\sim 6.5 \times 10^2$
	16	$> 10^2$

~ - approximately, > greater than,

NOTE: Results for E. coli and P. aeruginosa trials reported per 100mL.
Results for diluted sewage trial reported per mL.


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Chief Microbiologist

Appendix 4 Legionella results



ANALCHEM BIOASSAY PTY LTD A.C.N. 000 678 160
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CERTIFICATE OF ANALYSIS

DATE: 13 Oct 97

CLIENT: Aquamatics Pty Ltd

Attn: Les Chedzoy

JOB No: 97/4769

ORDER No:

SAMPLES: 2 Waters rec'd 30/9/97

EXAMINATION: Legionella challenge test

METHOD: As per Aquamatics Legionella challenge test protocol
(Procedure No 3, deionised water sample not included)

RESULTS:

Samples tested as received
Testing performed 8 Oct 97
Temperature of challenge test: 35°C
Initial count cfu/mL: 5.0×10^4

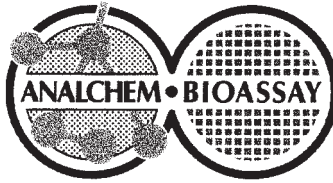
CONTROL WATER

	<u>Legionella</u> cfu/mL
0 minutes	5.2×10^4
30 minutes	6.0×10^4
60 minutes	4.2×10^4
2 hours	3.8×10^4
4 hours	3.7×10^4

TEST WATER IONISED & AQUABRITE

	<u>Legionella</u> cfu/mL
0 minutes	2.5×10^2
30 minutes	<10
60 minutes	<10
2 hours	<10
4 hours	<10

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CERTIFICATE OF ANALYSIS

DATE: 15 Sept 97 **DATE:** 4 Sept 95
CLIENT: Aquamatics Pty Ltd **Attn:** Les Chedzoy
REFERENCE No: 97/4317B
SAMPLES: Waters (2)
IDENTIFICATION: See below O/N 2195
DATE RECEIVED: 4 September 1997
DETERMINATION: See below
METHOD: APHA4500H+B, 2510B, 2320B, C07A, 2340B Palin Method, CSIRO RptS1
RESULTS: Samples tested as received

	CONTROL	IONISED
pH	7.8	7.4
Conductivity ($\mu\text{S}/\text{cm}$)	660	740
Alkalinity (as CaCO_3 mg/L)	110	90
Hardness (as CaCO_3 mg/L)	170	166
Chloride (mg/L)	106	104
DPD after 15 min (as Cl_2 mg/L)	Less than 0.02	1.5
Oxidised nitrogen (mgN/L)	0.270	0.220
Copper (mg/L)	-	0.93
Silver (mg/L)	-	0.04


David Carter BSc MRACI
Authorising Analyst



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 Telephone: +61 2 9756 8222 Fax: +61 2 9743 5311

31 June 2002

Dr Geoff Hudepith
 Aquamatics Pty Ltd
 PO Box 54
 SEAFORTH NSW 2082

Dear Dr Hudepith

Enclosed is a copy of the 1998 NATA directory in the fields of
 Biological/Chemical Testing which states Analchem Bioassay Pty Ltd were
 accredited in that year.

Yours sincerely

T G Orlova
 MANAGER, BIOLOGICAL TESTING

Encl

Laboratory Accreditation • Training • Information Services • Inspection Accreditation
 National Association of Testing Authorities, Australia is a company limited by guarantee



1596 Uncle Ben's of Australia

Research and Development Analytical Laboratory
 Kelly Street
 WODONGA VIC 3690
 Ph: (060) 55 5200 Tx: 56002 Fx: (060) 55 5347
 Ph: (060) 55 5200 Tx: 56002 Fx: (060) 55 5347
FACILITIES: Normally not available for public testing

8.11 Microbiological tests on foods

- .03 Dairy products
- .17 Animal feeds
- .18 Microbiological tests for factory hygiene purposes
- .01 Surfaces
- .12 Microbiological condition of industrial waters

8.70 Waters, including effluents

- For the following determinations
- Dairy products
- Sterility testing of modified UHT milk products
- Pet foods
- Plate count, yeasts and moulds
- Swabs taken for plant hygiene purposes
- Plate count
- Industrial waters
- Coliforms, plate count

1599 The Egg Industry Cooperative Limited

Microbiological Laboratory
 Corner Kirkham and Chandler Roads
 KEYS BOROUGH VIC 3173
 Ph: (03) 9798 7077 Fx: (03) 9798 6163
FACILITIES: Normally not available for public testing

8.11 Microbiological tests on foods

- .04 Meat and meat products
- .05 Poultry and poultry products
- .06 Eggs and egg products
- .12 Vegetables and vegetable products
- for the following determinations
- Coliforms, lactic acid bacteria, plate count, yeasts and moulds
- Escherichia coli, Salmonella, Staphylococcus (coagulase producing strains) Moisture, pH, salt

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Microbiological Laboratory
 36-40 Halloran Street
 LILYFIELD NSW 2040
 Ph: (02) 98181033 Fx: (02) 98108771
FACILITIES: Public testing service

8.05 Sterility tests on pharmaceuticals

- .01 filtrable solutions and soluble preparations (membrane filtration)
- .02 Surgical dressings and devices
- .03 Non-filtrable preparations including ointments

8.06 Microbiological tests on veterinary products

- .12 Microbial count
- .13 Preservative efficacy
- .24 Microbioassay of antibiotics

8.07 Microbiological tests on pharmaceuticals

- .12 Microbial counts
- .13 Preservative efficacy
- .24 Microbioassay of antibiotics

8.09 Effectiveness tests on biocides

- .02 Bactericides

8.10 Microbiological tests on cosmetics, perfumes and essential oils

- .12 Microbial counts on cosmetics
- .13 Preservative efficacy
- .22 Microbial counts on perfumes
- .32 Microbial counts on essential oils

BIOLOGICAL TESTING

8.11 Microbiological tests on foods

- .01 Cereal products
- .02 Nuts and nut products
- .03 Dairy products
- .04 Meat and meat products
- .05 Poultry and poultry products
- .06 Eggs and egg products
- .07 Fish, crustaceans and molluscs
- .08 Edible fats and oils
- .10 Heat-processed foods in hermetically sealed containers
- .11 Sugar products, honey and confectionery
- .12 Vegetables and vegetable products
- .13 Fruit and fruit products
- .14 Beverages
- .17 Animal feeds
- .20 Mixed foods
- .25 Additives to foods

8.18 Microbiological tests for factory hygiene purposes

- .01 Surfaces
- .02 Air
- .03 Water

8.19 Microbiological tests on other materials

- .01 Surgical dressings and related materials
- .02 Medical devices

8.70 Waters, including effluents

- .11 Microbiological condition of potable waters
- .12 Microbiological condition of industrial waters
- .13 Microbiological condition of sewage
- .14 Microbiological condition of trade wastes
- .15 Microbiological condition of recreational waters
- .22 Biochemical oxygen demand of industrial waters
- .23 Biochemical oxygen demand of sewage
- .24 Biochemical oxygen demand of trade wastes

for the following determinations
 Bactericides
 TGA disinfectant test foods
 Anaerobes producing H₂S; coliforms, mesophilic spore-forming aerobic and anaerobic bacteria, plate count, rope spores, thermophilic bacteria, thermophilic spores (flat sour type), yeasts and moulds
Bacillus cereus, *Candida albicans*, *Clostridium perfringens*, *Enterobacteriaceae* *Escherichia coli*, *Lactobacillus*, *Listeria*, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus* (coagulase producing strains)
Vibrio parahaemolyticus
 Waters
 Coliforms, plate count, streptococci (faecal)
 Escherichia coli, Legionella, Pseudomonas aeruginosa, Pseudomonas, Biochemical oxygen demand, suspended solids, total grease and beach grease in waters
 Cosmetics and pharmaceuticals
 Coliforms, plate count, streptococci (faecal), yeasts and moulds
 Clostridium, Pseudomonas aeruginosa, Salmonella, Staphylococcus (coagulase producing strains)
 Antibiotics (presence of, including assays), efficacy of ethylene oxide sterilisation cycles with biological indicators (spore strips), preservative efficacy, sterility tests

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Technology Centre Microbiological Laboratory
 69 Charles Street
 COBURG VIC 3058
 Ph: (03) 92862222 Tx: 34139 Fx: (03) 93504658
CONTACT: Mr W N Guild
FACILITIES: Normally not available for public testing

8.11 Microbiological tests on foods

- .10 Heat-processed foods in hermetically sealed containers
- For the following determinations
 Commercial sterility of food