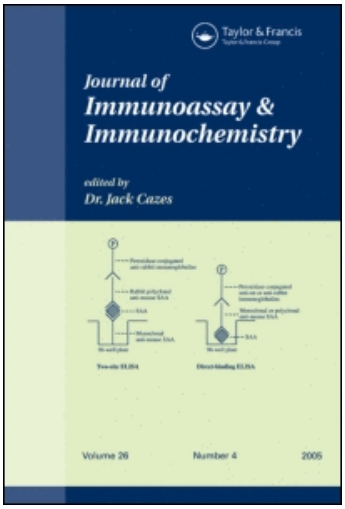

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Inflammatory Activity as an Indicator of Water Quality: The use of human whole blood cultures

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INFLAMMATORY ACTIVITY AS AN INDICATOR OF WATER QUALITY: THE USE OF HUMAN WHOLE BLOOD CULTURES

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ABSTRACT

A rapid whole blood culture (WBC) assay system was developed to monitor the inflammatory potential of water samples collected in the Western Cape, South Africa. Water contaminated with inflammatory substances induced the pro-inflammatory hormone interleukin 6 (IL-6). All water samples collected from the Eerste River, Stellenbosch, induced IL-6 secretion, and the quantity of IL-6 secreted is dependent on the concentration and origin of the sample. The lowest IL-6 inducing activity for river water was obtained for samples collected near the origin of the river. Samples at subsequent points downstream showed an increase in IL-6 inducing activity. Drinking water samples collected from selected towns in the Western Cape showed that there were major differences between the inflammatory potential of the water. Of the 15 samples assayed, 7

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had low inflammatory activity, 4 had an intermediate inflammatory activity and 4 had high inflammatory activity. The water sources that have a high inflammatory activity may pose a health risk to consumers.
(KEY WORDS: Inflammatory activity, water quality, IL-6, whole blood culture)

INTRODUCTION

When microbes or microbial contaminants are introduced into mammals, an inflammatory response, which is characterized by fever, diarrhoea and in severe cases by anaphylactic shock (1,2), results. One of the pro-inflammatory hormones, interleukin 6 (IL-6), is directly responsible for the synthesis of all the hepatic acute phase proteins, and this hormone is also responsible for fever in mammals (3). Although tap water is the major source of drinking water in urban areas in South Africa, river and canal water are still used by some people in lesser developed areas. In 1997 approximately 11% of urban South African households lacked adequate water supplies (4). Rural South Africa has a more severe problem and up to 29% of infant deaths is due to diarrhoea caused by, amongst others, poor sanitation and water supplies (5). Polluted recreational water supplies is also a major cause for concern and studies have shown a higher incidence of gastrointestinal, respiratory and skin problems amongst swimmers at specific beaches around Cape Town, South Africa (6). Due to the severity of these problems and specifically in light of the high incidence of inadequate drinking water associated infant deaths, there is a great need to develop user-friendly and rapid *in vitro* human assays for screening water quality.

Several different methods for measuring the microbial content of water are being used at present (7). Most of these systems employ an initial enrichment step (microbial culture), followed by a detection step to positively identify the specific microbe. An inherent problem with microbial enrichment procedures are that only viable organisms grow and multiply during the culture period, resulting in a distorted, and quite often underestimated, picture of what was present in the original water sample. These methods also require long incubation periods (8).

The IL-6 pro-inflammatory response to microbial contamination, which occurs *in vivo* as a response to microbial invasion, can also be simulated *in vitro* using cell culture assays (9,10). Previous studies carried out by the authors and other researchers have shown that inflammatory substances such as bacteria, bacterial products and fluids contaminated with microbial breakdown products, stimulate human blood cultures to secrete cytokines such as interleukin-1, interleukin-2, tumour necrosis factor (TNF) and IL-6 (11,12,13,14).

The aim of the present study was to do a preliminary investigation to determine whether the WBC assay can be used to detect and quantitate inflammatory agents in water from different sources.

METHODS

Collection of River Water Samples

Water samples were collected at various points along the Eerste River, Stellenbosch, Western Cape, South Africa (Figure 1). The samples were

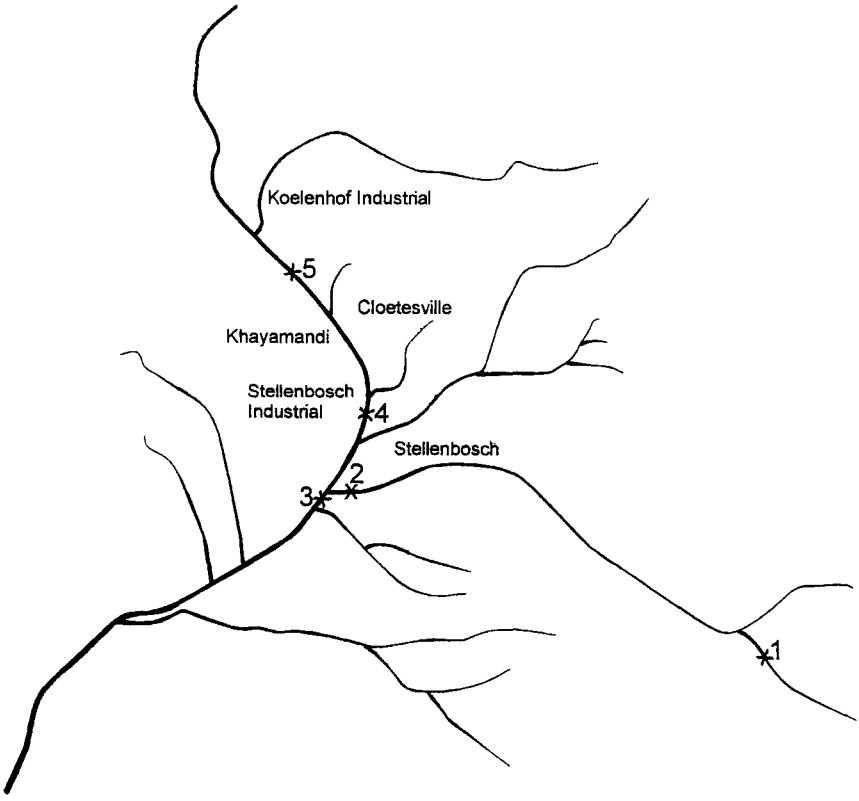

Figure 1. A map of the Stellenbosch area and the Eerste River, Western Cape, South Africa. Sampling sites (1-5) are indicated on the map.

collected in clean microfuge tubes (Elkay) and stored overnight at 4 °C. Samples for longer term storage were frozen at -65 °C.

Collection of Drinking Water Samples

Tap water samples were collected from several towns in the Western Cape, South Africa (Figure 2). All the samples assayed, except the drinking water samples from Buffelsjagsdam Canal and Caledon Waterfall, were tap water.

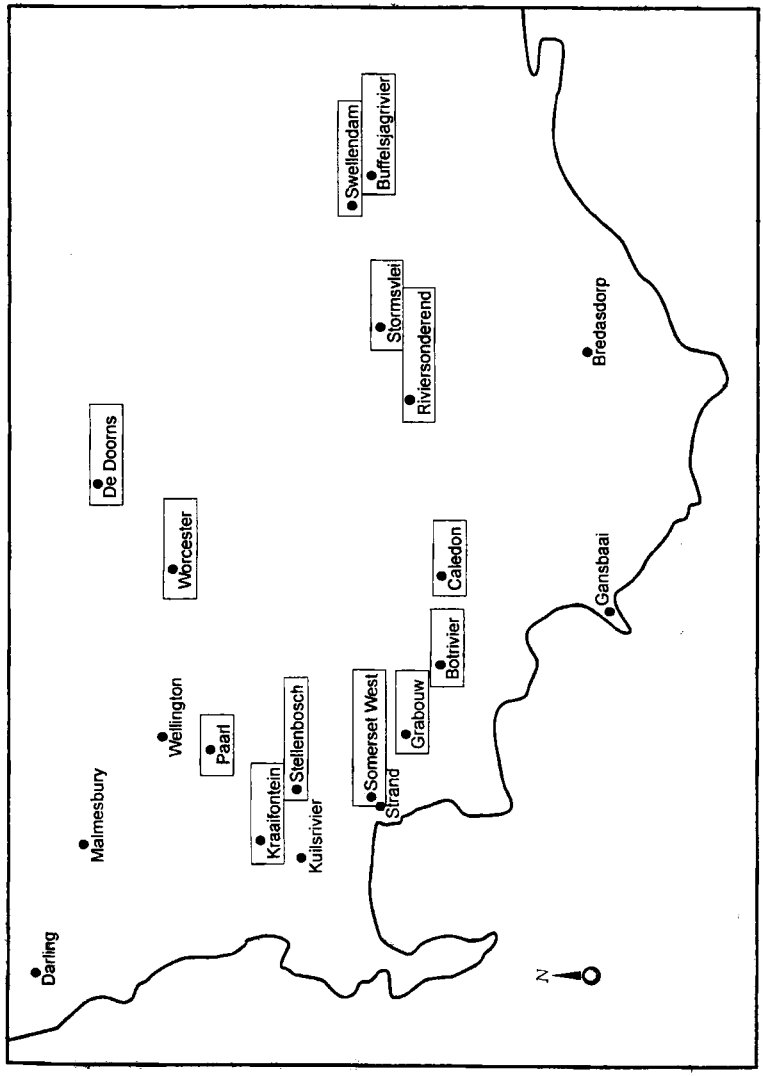

Figure 2. A map of the Western Cape, South Africa indicating the towns where drinking water samples was collected.

Samples were stored overnight at 4 °C or frozen at -65 °C for longer term storage.

Collection and culture of Whole Blood

Blood from healthy male donors was collected in heparinized tubes. The heparinized blood was diluted 1/5 with RPMI medium. Cultures were set up in 96 well plates (Nunc). Samples were diluted in WFI (SABAX, South Africa). WFI was also included in all assays as a negative control. Samples or controls were added to the wells (10 µl/well). Diluted heparinized blood (110 µl/well) was added to the controls or samples. The blood was then cultured for 18 hours at 37 °C. At the end of the culture period the culture supernatants were assayed for IL-6 using an in-house ELISA previously described 9. A recombinant IL-6 (Roche Molecular Biochemicals, South Africa) standard curve was included on each ELISA plate. This ELISA is linear for IL-6 concentrations between 12,5 and 400 pg/ml. A standard curve was generated for each ELISA plate using the Excell computer package and the IL-6 concentrations induced by the samples or controls were read off this curve.

Statistical analysis

All results are presented as the mean value of triplicate determinations. Linear regression analysis was used to determine the relationship between whole blood culture synthesised IL-6 and sample dilution.

RESULTS

The effect of sample dilution on IL-6 secretion by WBC

Dilutions of water samples collected from near the origin of the Eerste River (Jonkershoek) were tested for inflammatory activity. Inflammatory activity could be detected at dilutions equal and less than 1/96 (Figure 3). Regression analysis showed that the inflammatory activity is directly dependent on the dilution of the sample (Pearson correlation coefficient R = 0.98). All subsequent assays were done at a final sample dilution of 1/96.

The effect of the origin of the water sample on its inflammatory activity

All water samples collected from the Eerste River induced IL-6 secretion by WBC (Table 1). The amount of IL-6 secreted depends on the origin of the water sample. Triplicate assays showed that the variation between results obtained for the same sample was less than 5% for all samples tested. Water samples collected near the origin of the river induced lower levels of IL-6 than water samples collected at subsequent points along the river. Samples collected below the confluence with the Plankenbrug River showed a major increase in inflammatory activity when compared to samples upstream. Assays on water samples collected from the Plankenbrug River showed that this river is highly contaminated with inflammatory agents (586 pg/ml).

The Inflammatory Activity of Drinking Water Samples

Drinking water samples collected from towns in the Western Cape, South

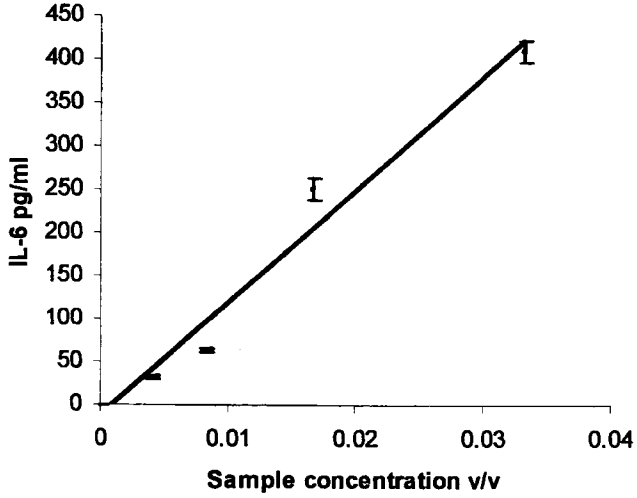

Figure 3. The effect of sample concentration on IL-6 secretion. Samples concentration is expressed as a function of the total incubation volume.

TABLE 1

Comparison between the inflammatory activity of the river samples collected as assayed using WBC.

The inflammatory activity of river water, collected from different sites, was assayed using WBC. IL-6 results are given as mean ± standard deviation for triplicate assays.

	Site description	**IL-6 pg/ml**
Site number	Control Water	-1 ± 0.7
1	Eerste River at Jonkershoek	31 ± 2.1
2	Eerste River Pre-Plankenbrug	73 ± 0.9
3	Eerste River Post-Plankenbrug	427 ± 34.3
4	Plankenbrug River	586 ± 15.7
5	Plankenbrug River	484 ± 19.5

TABLE 2

Comparison between the inflammatory activity of drinking water samples collected from towns in the Western Cape, South Africa.

The inflammatory activity of drinking water collected from different towns was assayed using WBC. IL-6 results are given as mean ± standard deviation for assays done on duplicate samples assayed on different days.

Origin of drinking water sample	IL-6 pg/ml
Paarl	70.0 ± 9.8
Worcester	144.6 ± 23.3
De Doorns	49.0 ± 0.0
Kraaifontein	259.5 ± 12.0
Somerset West	28.5 ± 3.5
Buffelsjagsdam Canal	413.5 ± 29.0
Buffelsjagrivier	349.5 ± 3.5
Swellendam	122.5 ± 6.3
Stormsvlei	89.5 ± 6.4
Riviersonderend	104.5 ± 4.9
Caledon Waterfall	364.0 ± 12.7
Caledon	91.0 ± 5.7
Botrivier	5.5 ± 2.1
Grabouw	157.5 ± 2.3
Stellenbosch	48.5 ± 4.9
Water for injection	0 ± 0

Africa showed major differences in inflammatory activity (Table 2). The lowest inflammatory activity obtained was for water collected from the town of Botriver. The samples collected from the two non-tap sources had a higher inflammatory activity than the tap water samples. Of the 15 samples assayed, 7 had low inflammatory activity (≤100 pg/ml IL-6), 4 had an intermediate inflammatory activity (>100; <200 pg/ml IL-6) and 4 had high inflammatory activity (>200 pg/ml IL-6).

DISCUSSION

Both live microbes and the breakdown products of microbes can cause adverse reactions characterized by fever, diarrhoea and in very severe cases anaphylactic shock in human (1,2). At present most of the screening assays for microbial contamination rely on the presence of live microbes in the water, although it is well known that some of the most toxic bacterial breakdown products can withstand most of the treatments that are used to kill live microbes (15,16). There are some assays such as the LAL that can detect microbial breakdown products, but this assay is very specific for the gram negative bacterial product endotoxin, and will not detect any other pyrogenic substance present in water (17).

The present study shows that the WBC assay is an effective system for monitoring inflammatory activity in water samples. All samples collected from the Eerste River showed inflammatory activity. The amount of inflammatory activity was dependent upon the origin of the sample. It was particularly noticeable that there was a major increase in inflammatory activity of the samples from the Eerste River collected post confluence with the Plankenbrug River, compared to samples collected prior to confluence with this river. Assays on water samples from the Plankenbrug River also displayed high inflammatory activity. These results indicate that water, contaminated with inflammatory compounds, from this river could be a major source of inflammatory contamination of the Eerste River in the Stellenbosch area.

The drinking water samples collected from the different towns showed major differences in inflammatory activity. Repeat assays on a duplicate set of samples gave similar results indicating that the data obtained is a true reflection of the inflammatory activity present in the water samples.

The WBC assay for inflammatory agents detects live microbes as well as microbial breakdown products, and simulates the conditions that would be encountered by these when they enter an animal, namely the induction of the hormone that would normally set off the acute phase response. Previous studies by the authors have shown that this assay has a very high sensitivity and detects a wide range of microbes (18). The assay is also more sensitive than *in vivo* mammalian assays for inflammatory agents (9). This assay, being quantitative and very sensitive can be used on most aqueous samples and is a useful assay to monitor water quality.

CONCLUSIONS

This study shows that the WBC assay can detect differences in the inflammatory activity of water samples. Water sources that have a high inflammatory activity may pose a health risk to users as the high inflammatory activity is indicative of a substance/s in the water that will induce the immune system of mammals to react to it. We strongly suggest a more detailed study on the inflammatory activity of water resources and specifically an investigation to determine the clinical and epidemiological significance of the high inflammatory activity as a potential risk factor for users of the water resource.

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