CSIRO LAND and WATER

Microbial Pathogens in Wastewater

Literature Review for Urban Water Systems Multi-divisional Research Program

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Summary

The reuse of wastewater represents a vast potential to remove many of the pressures on the world's freshwater resources. It has, in fact been claimed that unless steps are taken now, we will be creating difficulties for both the environment and future generations of countries. While wastewater reclamation has the potential to improve water usage and preserve global fresh water resources, a number of issues remain to be resolved. Many of these issues involve assessment of environmental and health risk.

This review of the literature determined that:

- Infection by pathogenic microorganisms is the major risk factor associated with the recycling of wastewaters.
- There are a wide range of microbial pathogen types which can be present in wastewater, with the type and number present being highly dependent on the socioeconomic conditions of communities creating the wastewater.
- Risk assessment is still an area which requires intensive research. There are many factors which can influence the risk associated with a proposed wastewater reclamation project, many of which are, at present, difficult to accurately determine.
- The World Health Organization's guidelines for wastewater reuse for agricultural purposes appears to be directed more towards developing nations and thus, may not be as applicable to more developed nations which have higher standards of living and greater sanitation regimes.
- The sensitivity and accuracy in the detection of pathogenic and indicator microorganisms is improving through the development of new methods, but further research and ratification of these methods is still required.
- There are a range of options available for the treatment and reuse of wastewater. Many factors can influence choices of treatment processes. These include the type of microbial pathogens present, their resistance to treatment and environmental attenuation processes, the intended use for the recycled wastewater, and the potential for contact with workers and the general public.

The aim of this review is to examine the extent of current knowledge regarding the microbiology of wastewaters; current areas of research relating to the detection, identification, and enumeration of the types of microbial pathogens in wastewaters; the health risks associated with microbial pathogens in wastewaters; the treatment methods used

for the removal of microbial pathogens from wastewater; and to identify deficiencies in the knowledge of microbial pathogens in wastewaters.

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1 Introduction

It has been predicted that, due to massive world wide increases in the human population, water will become one of the scarcest resources in the 21st century. As human numbers increase, greater strains will be placed on available resources and pose an even greater threat to environmental sources. It has already been claimed that in Australia's recent past, scant attention was paid to the use of the continent's water resources resulting in the environmental problems faced by the country today (Day 1996). Day concluded that current Australian laws, regulations and management strategies still do not address these problems and unless steps are taken now, future generation and the environment will be at great risk.

A report by the Secretary-General for the United Nations Commission on Sustainable Development (1997) has concluded that there is no sustainability in the current uses of fresh water by either developing or developed nations. The report stated that worldwide water usage has been growing at more than three times the world's population increase. The report also concluded that water shortages, combined with increasing pollution of water, was causing widespread public health problems, limiting economic and agricultural development (thus jeopardising global food supplies), and harming a wide range of ecosystems.

Polluted and/or untreated waters have a large health risk by causing waterborne disease. Despite large advances in water and wastewater treatment, waterborne diseases still pose a major world-wide threat to public health. It has been reported that waterborne pathogens infect around 250 million people each year resulting in 10 to 20 million deaths (Anon 1996). Many of these infections occur in developing nations which have lower levels of sanitation, problems associated with low socioeconomic conditions, and less public health awareness than in more developed nations. However, it has been documented that the incidence of waterborne disease in the US has actually increased in the past 20 years, with more waterborne outbreaks being recorded between 1971 and 1985 than in any previous 15 year interval since 1920 (Craun 1988).

Water treatment and sanitation has been traced as far back in history as 3000 BC with water sanitation methods used by the Minoan civilisation (Figure 1). Modern knowledge of the need for sanitation and treatment of polluted waters, however, really began with the frequently cited case of John Snow in 1855, in which he proved that a

cholera outbreak in London was due to sewage contaminated water obtained from the Thames river. Since Snow's findings, numerous studies have advanced the knowledge of wastewater treatment, and from 1910, on the advantages of wastewater reuse (Figure 1).

Wastewater offers a source of water that could drastically reduce the utilisation of precious natural water resources. Sources of wastewater can include collected stormwater runoff, industrial wastewaters, domestic greywater and sewage. Cities worldwide generate large amounts of different types of wastewaters, all of which require treatment and disposal. Current disposal methods often generate concerns over treatment costs, their environmental impact, and the loss of a potentially valuable resource. Wastewater reclamation and reuse is increasingly being viewed as an economically and socially viable enterprise.

Public concern over polluted water is a major issue in most countries worldwide. There can be a danger that the public, often through the media, gains an exaggerated opinion on the risks associated with wastewater. Examples of the level of public concerns and the need for the relevant authorities to be well informed are given in Figure 2. These examples highlight the dangers of how poor publicity, and public misperceptions and/or fears can possibly severely handicap attempts to use recycled waters. When the public is properly informed, however, studies have shown that they often prefer options which involve the reuse of wastewater (ARCWS 1995). Options such as marine dumping tend to be poorly supported by the public and the protection of the environment is also considered to be fundamentally important. Cost considerations are often an important issue for the public when considerations are made regarding the use of treated groundwater (ARCWS 1995).

The health risks for the public from wastewater can come from microbial pathogens, toxic chemicals, and heavy metals. This review will only focus on the health risks derived from the presence of microbial pathogens in wastewater. The aim of this review is to examine the extent of current knowledge regarding the microbiology of wastewaters; current areas of research relating to the detection, identification, and enumeration of the types of microbial pathogens in wastewaters; the health risks associated with microbial pathogens in wastewaters; the treatment methods used for the removal of microbial pathogens from wastewater; and to identify deficiencies in the knowledge of microbial pathogens in wastewaters.

2 Wastewater Reuse

There are a number of methods employed and/or being researched which involve the recycling of wastewaters for a variety of uses. Known collectively as wastewater reclamation or reuse, recycling of wastewater has been practised for centuries but has been used extensively in a number of countries only in the latter part of the 20th century. Some of the places where wastewater reclamation is practiced or researched include Australia (Anderson 1996, Dillon et al. 1996, Law 1996), Egypt (Shereif et al. 1995), France (Bontoux & Courtois 1996), Greece (Tchobanoglous & Angelakis 1996), Israel (Kanarek & Michail 1996), Japan (Asano et al. 1996), Jordan (Gur & Al Salem 1992), Nambia (Haarhoff & Van der Merwe 1996), Saudi Arabia (Al-A'ama & Nakhla 1995), Tunisia (Bahri & Brissaud 1996), Turkey (Tanik et al. 1996), and the USA (Crook & Surampalli 1996, Jolis et al. 1996, Olivieri et al. 1996).

The majority of countries produce large amounts of wastewater from domestic and industry. With growing human populations, many countries are turning to wastewater reuse as a means of reducing the demand on potable water supplies. Examples of different country's water requirements and the amount of wastewater reused are given in Table 1. Wastewater reuse is projected to increase in many countries as the demand on water sources escalate due to increasing populations. For example, Saudi Arabia predicts by 2000 that almost 10% of its total water demand will be met through wastewater reuse (USEPA 1992). Similarly, it has been predicted that Greece could be reusing up to 60% of its total wastewater effluent by 1999 (from less than 10% in 199 (Tchobanoglous & Angelakis 1996). Thus, the recycling of wastewater will become an important issue for many countries as the turn of the century approaches.

2.1 Types of Wastewater

Types of wastewaters used for recycling include treated and untreated sewage effluent (Asano et al. 1996, Haarhoff & Van der Merwe 1996, Mujeriego et al. 1996, Shereif et al. 1995), storm water runoff (Asano et al. 1996, Dillon et al. 1994), domestic greywater (Anderson 1996), and industrial wastewater (Asano et al. 1996, Guillaume & Xanthoulis 1996).

2.2 Uses for Recycled Wastewater

Recycled wastewaters have been used in a variety of applications. Reported major uses include groundwater recharge, agricultural irrigation, aquaculture, reticulation of parks and golf courses, formation of wetlands wildlife habitats and recreational impoundments. Other reported uses include flushing water for toilets (particularly in large hotels and buildings), industrial uses, snow melting/removal, make-up water for evaporation cooling towers, and potable water.

Agricultural irrigation is the biggest uses of recycled wastewaters. 41% of recycled water in Japan and 60% of Californian recycled wastewaters are used for this purpose (Asano et al. 1996). 15% of Tunisia's reclaimed wastewater is used for the irrigation of crops (Bahri & Brissaud 1996). Issues relating to the use of treated wastewater effluent for agricultural irrigation can vary between countries. Such variations depend on regulations controlling wastewater reuse, the percentage of wastewater recycled, the level of treatment prior to reuse, and, to some extent, the types of crops irrigated. The differences often depend on the dependence of the particular country for water, the political and social structure of the country, and the general availability of water.

For example, the use of wastewater for irrigation is considered a traditional practice in France, but is now strictly controlled by the health authorities with current trends moving away from agricultural irrigation and toward the irrigation of golf courses and landscaped areas (Bontoux & Courtois 1996). In the U.S.A., California has been using recycled water since in the early 20th century and wastewater reuse is now practised in several other states. Current U.S. EPA guidelines require that recycled wastewater undergo at least secondary treatment, and almost always that chemical disinfection is used prior to reuse of the wastewater (Crook & Surampalli 1996, U.S. EPA 1992).

A number of countries treat their wastewater prior to reuse using waste stabilisation ponds (Gur & Al Salem 1992, El Hamouri et al 1996). An Israeli study has reported the use of wastewater in unrestricted irrigation following infiltration of the untreated wastewater into, and then recovery from an aquifer which was used to cleanse the wastewater (Kanarek & Michail 1996). A novel technique practised in Suez, Egypt, passes raw sewage through plankton ponds, then through fish production ponds. The resulting effluent is then used

for irrigation of cereal crops and trees (Shereif et al. 1995). The fish in the production ponds are also used for human consumption.

Several advantages have been reported regarding the use of treated wastewater for irrigation purposes. do Monte and e Sousa (1992) reported that the use of effluent from a facultative treatment pond for the irrigation of crop pastures (sorghum, maize and sunflower) negated the need for the use of artificial nitrogen fertiliser on these crops. They estimated that this created savings of between $228 and $533/ha Another study comparing the use of groundwater with treated wastewater as an irrigation source found that treated wastewater attenuated the effects of water salinity significantly better than groundwater (El Hamouri et al. 1996). Guillaume and Xanthoulis (1996) reported on the reuse of wastewater produced by a food processing plant in Belgium. The wastewater was used by local farmers for the irrigation of vegetable crops. The effluent also contained fertilising elements (nitrogen and phosphates). The wastewater could be used untreated and greatly reduced the costs of crop production by reducing the need for application of artificial fertilisers.

Apart from irrigation of crops, pastures and orchards/vineyards, wastewater has been used or trialed for potential use in a number of other areas. Wastewater is used by industry in several countries as boiler feed water, cooling water and process water (Asano and Levine 1996, Crook 1996, Tanik 1996, Wijesinghe et al. 1996). The use of treated wastewater for toilet flushing is also becoming more common place particularly in newly constructed high rise buildings, hotels, and new domestic housing projects (Asano 1996, Crook 1996, Law 1996). Other major uses for recycled water include landscape irrigation (Bontoux & Courtois 1996, Mujeriogo et al. 1996), for environmental purposes such as flow augmentation or recreational impoundments, and groundwater recharge (Kanarek & Michail 1996, USEPA 1992).

3 Microbial Pathogens in Wastewater

3.1 Pathogenic Microorganisms

Microbial pathogens which can be potentially present in wastewater can be divided into three separate groups. These groups are the viruses, bacteria and the pathogenic protozoan/helminths. The majority of these pathogens are enteric in origin, that is, they are excreted in faecal matter, contaminate the environment and then gain access to new hosts through ingestion (i.e., the faecal-oral route). Examples of the different microbial pathogens are given in Table 2.

3.1.1 Viruses

Viruses are among the most important, and potentially most hazardous of the pathogens found in wastewater. Untreated wastewater can contain a range of viruses which are pathogenic to humans. Viral numbers have been detected in concentrations in excess of 10^3 - 10^4 viral particles/litre of wastewater. Viruses are generally more resistant to treatment processes, are more infectious, and require smaller doses to cause infection than most of the other pathogen types. Viruses are also generally more difficult to detect in environmental samples such as wastewater.

All of the prevalent pathogenic viruses found in wastewater which are discussed in the literature enter the environment through faecal contamination from infected hosts. In wastewater, the most commonly detected pathogenic viruses are the enteroviruses. This group consists of small, single-stranded RNA viruses and includes the poliovirus types 1 and 2, multiple strains of echovirus, enterovirus and coxsackievirus (International Nomenclature of Diseases 1983). The hepatitis A virus which is also often isolated from faecally contaminated wastewater has been tentatively classified in the enterovirus group (International Nomenclature of Diseases 1983). The enteroviruses are known to cause a wide range of diseases in humans including poliomyelitis, upper respiratory infections, acute gastroenteritis, aseptic meningitis, pericarditis, myocarditis and viral exanthema, conjunctivitis, and hepatitis (International Nomenclature of Diseases 1983).

Other viruses which have been detected in wastewaters include adenoviruses, rotaviruses, reoviruses, astroviruses, and caliciviruses such as Norwalk virus and other small round structured viruses. Like the enteroviruses, these viruses cause a range of infections including acute gastroenteritis, respiratory tract infections, diarrhoea, pneumonia, and conjunctivitis (Cruz et al. 1990, International Nomenclature of Diseases 1983, Marx et al. 1995). The rotaviruses are the most infectious of all the enteric viruses (Gerba et al. 1996) and thus can be considered to be a high health risk group if present in wastewaters.

While most members of the general population are susceptible to infection from enteric viruses, small children, the elderly and the immuno-compromised are the most at risk and have the highest

infection rate from these viruses. These population groups are also particularly at risk of developing the more rare forms of disease caused by these viruses.

3.1.2. Bacteria

Bacteria are the most common of the microbial pathogens found in wastewater. There are a wide range of bacterial pathogens and opportunistic pathogens which can be detected in wastewaters. Many of the bacterial pathogens are enteric in origin, however, bacterial pathogens which cause non-enteric illnesses (e.g., Legionella spp., Mycobacterium spp., and Leptospira) have also been detected in wastewaters (Fliermans 1996, Neuman et al. 1997, Wilson & Fujioka 1995).

Gastrointestinal infections are among the most common diseases caused by bacterial pathogens in wastewater. These include diarrhoea, the most well known examples are cholera caused by Vibrio cholera and salmonellosis caused by a number of Salmonella species; and dysentery, caused by variousShigella species as well as some Salmonella species. Dysentery-like infections have also recently been found to be caused by some strains of enteropathogenic E. coli (Grant et al. 1996). Typhoid, a disease cause by Salmonella typhiand other closely related Salmonella spp., has been traced food stuffs irrigated with wastewater (Bryan 1977).

Campylobacter, Helicobacter and Arcobacter are attributed with being the major causes of human acute enteritis (Wesley 1996). Koenraad (1997) concluded, that thermophilic Campylobacter contamination of recreational waters may be a greater infection risk than previously thought. Helicobacter pylori has also been implicated in causing stomach ulcers and has been linked to cancer (Wesley 1996).

Non-enteric bacterial diseases which can be transmitted by pathogens present in wastewater include legionellosis (Legionnaire's disease) a potentially fatal pneumonia caused by Legionella species; leptospirosis, a zoonotic infection causing a febrile illness caused by Leptospira interrogans; and melioidosis, a pneumonia-like disease cause by Pseudomonas pseudomallei.

The contamination of food by water containing known toxin producing organisms such as Staphylococcus aureus, Salmonella spp., E. coli, or Clostridium perfringens can cause outbreaks of food poisoning (often severe and wide spread). Mycobacterium ulcerans, the cause of

subcutaneous ulcerous lesions on body extremities has been implicated through epidemiological evidence to be present in wastewater and causing infections through contact with the wastewater (Johnson et al. 1996). M. Ulcerans has never been isolated from environmental samples, however, so this evidence is circumstantial only.

As well as the established pathogens, a number of opportunistic pathogens (microorganisms which cause infections and disease under optimal conditions, commonly in the very young, elderly and immuno-compromised) can be found in untreated and treated wastewaters. These opportunistic pathogens include Pseudomonas, Streptococcus, Flavobacterium and Aeromonas species (Ashbolt et al. 1995).

These opportunistic pathogens can be commonly isolated from a wide range of environmental water samples including wastewaters. They are often members of natural microbial populations and, at times, can be major members of these populations. Many opportunistic pathogens, being members of the natural microbial population, have the ability to rapidly increase in number when given sufficient nutrients. As wastewaters often have high nutrient loads, high numbers of these opportunistic pathogens can be present, increasing the risk of infections occurring from them.

3.1.3. Protozoa

Pathogenic protozoa are detected more prevalently in wastewater than in other environmental sources. There are a number protozoan pathogens which have been isolated from wastewater sources. The most common detected are Entamoeba histolytica, Giardia intestinalis (formerly known as Giardia lamblia), Cryptosporidium parvum.

E. histolytica, G. intestinalis, and C. parvum are all common enteric pathogens and have been frequently detected in wastewater which has been contaminated with faecal material. Infection from all three of these protozoan pathogens commonly occurs after consumption of food or water contaminated with the cysts or oocysts. Giardia and Cryptosporidium are ubiquitous in fresh and estuarine waters and have been detected in numerous countries around the globe (Ferguson et al. 1996, Haas & Rose 1996, Ho et al. 1995, Kfir et al. 1995, Ongerth et al. 1995, Wallis et al. 1996). E. histolytica can be detected in all parts of the world, although it is more prevalent in tropical regions (Feachem et al. 1983).

G. intestinalis has been implicated in the majority of U.S. waterborne disease outbreaks from water supplies that rely on surface waters (Craun 1988). Likewise, C. parvum has been found to be the cause of a number of major outbreaks involving drinking water. The most notable of these outbreaks was in Milwaukee, Wisconsin, where it was estimated that at least 403,000 people (approximately 25% of the city's population) became infected over a 2 month interval (MacKenzie et al. 1993). Estimations of the world prevalence of amoebal dysentery from E. histolytica infection range from 10 - 30% of such infections world wide (Feachem et al. 1983). These three parasites are of major concern to operators involved in the recycling of water, particularly water which has been in contact with human and animal faecal matter. E. histolytica commonly causes amoebiasis, (usually a gastroenteritis but can also be exhibited as a dysenteric disease (amoebic dysentery)) but has also been noted to infect the liver, lungs, pericardium, skin and brain. Both C. parvum and G. intestinalis cause an acute diarrhoea. The cysts of all three parasites have increased resistant to desiccation, increased temperature, changes in pH, and chlorination.

The main reservoir for C. parvum is man but it has also been shown to be able to infect several domestic and wild animals and birds (O'Donoghue 1995). Like C. Parvum, although the main reservoir for G. intestinalis is man, it has been shown to be able to infect a number of other warm blooded animals (Feachem et al. 1983). Likewise, the main reservoir for E. histolytica is man, however, primates, dogs and cats have been shown to be able to harbour the organism and pass it on to humans (Feachem et al. 1983). The implication regarding the ability of these protozoa to have multiple hosts is that the contamination of water and wastewaters by animals such as ducks, cattle and other domestic and wild animals could influence the health risk level of wastewater. This is particularly the case where wastewater, treated or untreated is stored in reservoirs or dams prior to reuse (Feachem et al. 1983, Graczyk et al. 1996).

3.1.4. Helminths

Helminths (nematodes and tape worms) are common intestinal parasites which, like the enteric protozoan pathogens, are usually transmitted by the faecal-oral route. Some of these parasites require an intermediate host for development prior to becoming infectious for humans. Helminth parasites commonly detected in wastewaters include the round worm (Ascaris lumbricoides), the hook worm

(Ancylostoma duodenale or Necator americanus), the whip worm (Trichuris trichiura) and the causative agent of strongyloidiasis Strongloides stercoralis.

It has been estimated that approximately 25 % of the world's human population is infected with the round worm nematode Ascaris lumbricoides (Elliset al. 1993). The prevalence of Ascaris infection is influenced by population density, education standards, sanitation levels, degree of agricultural development, education standards, and cultural and dietary habits (Khuroo 1996). Ascaris lumbricoides is endemic in regions of Asia, India, South America and Africa. Infection does occur in developed regions including Europe, North America, Japan and Australia although the infection rates are much lower (Khuroo 1996).

A number of other helminths are endemic in certain regions of the world depending on environmental and socio-economic conditions. For example, Strongloides stercoralis, is a soil transmitted parasitic nematode, is endemic in northern Australia, mainly infecting members of the Aboriginal population in these regions (Fisher et al. 1993, Prociv & Luke 1993). Strongloides infections are rare in the more southern regions of the continent. Thus, this parasite would need to be taken into consideration if the reuse of wastewater was considered in such a region.

The World Health Organization lists intestinal nematodes as the greatest health risk involving agricultural/aquacultural uses of untreated excreta and wastewater (WHO 1989). Infection levels are particularly endemic where human faecal matter is used as a fertiliser for growing vegetables (Khuroo 1996). Udonsi et al. (1996) have showr that children under the age of 19 years have the greatest prevalence of nematode infection with those over 30 years of age having the lowest infection levels. The results of this study indicate that helminth infection is a particular problem for infants and that infection, in particular chronic infection, begins at a young age. Chronic helminth infections have been shown to affect the physical and mental development of children due to malnutrition resulting from chronic infection (Khuroo 1996). Helminth eggs require moist shady soil for embyronation of the eggs over a period of five to ten days before they are able to cause infection. Following embyronation, however, the eggs can remain infectious in the contaminated soil for up to ten years (Khuroo 1996) This means that any soils which have been in contact

with recycled waters contaminated with faecal material could be considered as potential long-term sources of these parasites (Ellis et al. 1993, WHO 1989).

3.2 Microbial Indicators

The detection, isolation and identification of the many different types of microbial pathogens known to contaminate groundwater would be a difficult, time consuming and hugely expensive undertaking if attempted on a regular basis. To avoid the necessity of undertaking such huge ventures, indicator microorganisms are used to determine the relative risk of the possible presence of pathogenic microorganisms in a sample. To function effectively as indicators for the presence of these pathogens, indicator microorganisms should be present in equivalent or higher numbers and be as, or more resistant to environmental factors and treatment processes than the pathogenic microorganisms.

As most of the microbial pathogens present in waters and wastewaters are faecal in origin, the detection of faecal contamination of water has been the main aim of water testing authorities. Historically, the faecal coliforms, in particular E. coli, have been used as indicators of faecal contamination of water sources (APHA 1989). E. coli is used as its growth characteristics and behaviour in the environment are relatively well known. Faecal coliforms which have been excreted by warm blooded animals can be grown on selective media at 44.5 ^{0}C. This ability to be cultured at elevated temperatures has lead them to be know as the thermotolerant coliforms (TTC) and they have become the mainstay indicator for the water industry.

Thermotolerant coliforms have the disadvantage in that they are more sensitive to environmental changes and treatment processes than a number of more resistant bacterial pathogens and almost all of the viruses, protozoan cysts and helminth eggs. The lower resistance of faecal coliforms in the environment when compared with viruses, protozoan cysts and helminth eggs is demonstrated in Table 3. The other major drawback regarding the use of the TTC as indicators of faecal pollution is the fact that coliform bacteria reside in the gut of many different warm blooded animals. Thus, the detection of TTC in a water source does not necessarily confirm the contamination of a water source with human faecal material or the presence of human pathogens. Their presence could, instead, be due to contamination from animal sources, which may not pose such a public health hazard,

particularly in respect to the presence of viruses (most viruses are species specific in their host range).

The inappropriateness of the faecal coliforms (or TTC) as indicators of human faecal contamination of water sources and of the effectiveness of treatment processes has lead to the search for more appropriate indicator microorganisms. A number of bacteria and bacteriophage have been studied for their suitability as indicators. Ferguson et al (1996) examined the behaviour and/or incidence of a number of potential indicator organisms in an estuarine environment and compared their concentrations with the occurrence of pathogens (Enteric viruses, *Aeromonas* spp., *Salmonella* spp., Giardia, and Cryptosporidium). The potential indicators examined included faecal coliforms, faecal streptococci, C. perfringens spores and F-RNA bacteriophage. C. perfringens spores were determined to be the most useful indicator of faecal pollution and the only reliable indicator for the presence of Giardia cysts. C. perfringens spores were also used by Hill et al. (1996) as a reliable indicator of the persistence of faecal material in the sediments at deep sea disposal sites.

Other bacteria which have been examined as potential indicators for microbial pathogens in water are the enterococci, bifidobacteria, and *Bacteroides* (Baker & Bovard 1996). The enterococci (or faecal streptococci) have been considered to possibly be useful as secondary indicators of faecal contamination of water sources (APHA 1989, Leclerc et al 1996). The enterococci are generally a little more resistant than the faecal coliforms to treatment processes and environmental factors. Studies comparing different potential indicators, however, have indicated that enterococci are not as accurate as other potential indicators (Ferguson et al. 1996, Jagals et al. 1995).

Faecal coliforms cannot be used as indicators of human faecal pollution due to their prevalence in the intestinal tracts of many different warm blooded animals. This makes the tracing of sources of faecally contaminated water virtually impossible when using faecal coliforms as the indicator. Thus, the potential for other more specific indicators has been examined.

It was demonstrated by Jagals et al. (1995) that the presence of sorbitol-fermenting bifidobacteria could be used as an indicator of water contamination with human faecal material. Bifidobacteria have the problem, however, of requiring strict anaerobic conditions for the culturing and identification on solid media. This can be a limiting

factor when large sample numbers are involved. Selective media do exist for bifidobacteria (Hartemink et al. 1996), however, problems associated with culturing and isolation still exist. Bifidobacteria were also shown to be more sensitive to environmental conditions than faecal coliforms, which makes it less suitable for use as a long term indicator of faecal pollution.

Bacteroides is another bacterium which has been closely examined for potential use as an indicator. Like bifidobacteria, Bacteroides are common in the intestines of humans and animals. Bacteroides is also an obligate anaerobe which has made its' potential use as an indicator of faecal pollution on a large scale inappropriate.

While Bacteroides is an obligate anaerobe like the bifidobacteria, the recent development of DNA probes for polymerase chain reaction (PCR) detection alleviates the requirement for culturing and improves the potential for using Bacteroides strains as indicators of faecal pollution (Kreader 1995). Likewise. PCR probes have been developed for identifying bifidobacteria isolated from food (Kaufmann et al. 1997). These probes and techniques could be used to improve the suitability of the bifidobacteria and Bacteriodes as indicators of human faecal pollution of waters.

One of the major problems associated with the use of bacteria as indicators for the presence of microbial pathogens in water is the greater resistance of protozoan cysts and viruses to environmental factors and treatment processes. Viruses in particular are difficult to detect in many water sources due to low numbers, and the difficulty and expense of culturing. To overcome these problems, bacterial viruses (bacteriophage) have been examined for use as indicators of faecal pollution and the effectiveness of treatment processes to remove enteric viruses. The most common bacteriophage studied are male-specific (F-RNA) bacteriophage (in particular MS2 and PRD-1) which infect gram negative bacteria containing the F^+ sex plasmid; somatic coliphages (bacteriophage which infect coliforms); and Bacteroides fragilis specific bacteriophage. Somatic coliphage and F-RNA bacteriophage have been shown to survive but not replicate for long periods in tropical pristine rivers (Hernández-Delgado & Toranzos 1995), indicating that they could be useful as indicators in environmental waters.

In a study of water from mains supply with different sources Amon and Kott (1995) found that F-RNA bacteriophage had a behaviour similar to that of coliforms, and B. fragilis bacteriophage had a similar incidence

and behaviour to that of E. coli. Grabow et al. (1995) compared the number of somatic coliphage, F-RNA bacteriophage and B. fragilis bacteriophage in faecal samples obtained from human and different animal sources. Their findings have been summarized in Table 4. The results indicated that the somatic coliphage and F-RNA bacteriophage were common in the faeces of humans and most of the animals sampled, while the B. fragilis bacteriophage was only detected in human faecal samples. They concluded that the B. fragilis bacteriophage could be useful as an indicator of human faecal contamination of water sources.

Jagals et al. (1995) also compared the detection efficiencies of somatic coliphage, F-RNA bacteriophage and B. fragilis bacteriophage in their assessment of the potential of these bacteriophage as indicators. They were unable to detect B. fragilis bacteriophage or enteric viruses from any of the samples of surface water run-off tested. They were able to detect large numbers of somatic coliphage in all of the samples tested, including those samples which were upstream of human habitation. F-RNA bacteriophage were only detected downstream of human habitation, indicating that these phage would be more likely to indicate human faecal pollution than the somatic coliphage.

The usefulness of F-RNA bacteriophage as indicators of the possible presence of enteroviruses in sewage wastes and the marine environment was questioned by Lewis (1995), due to detection irregularities, source uncertainty and low numbers in some samples (compared to the presence of enteric viruses). Similar results were obtained in a study by Carducci et al (1995), who found little correlation between coliphage and enterovirus numbers in sewage or aerosol samples.

Frederick and Lloyd (1995) suggested that Serratia marcescens bacteriophage could be useful as a model for predicting the removal of enteric viruses in waste treatment ponds. They based their recommendations on the observed survival rates of S. marcescens bacteriophage in such ponds and on previously published survival trends of poliovirus under similar conditions. It has been shown by other studies, such as those given below, however, that poliovirus has a very low survival rate compared to other enteric viruses. This makes extrapolations of survival rate correlations tenuous when using such comparisons. Also, due to the heterogenous nature of enteric viruses, different environmental conditions can produce highly variable survival patterns, thus making generic assumptions relating to similarities of survival rates imprecise at best.

One of the main interests in the use of bacteriophage as indicators is as an indication of the effect treatment processes have on the survival of pathogenic viruses. Meng and Gerba (1996) compared the survival of the F-RNA bacteriophages MS2 and PRD-1 with two adenovirus strains and poliovirus when they were exposed to UV irradiation. They found that the two bacteriophage were less resistant than the adenovirus strains but considerably more resistant than the poliovirus. The suitability of MS2 bacteriophage as an indicator of virus inactivation was also questioned by Joliset al. (1996) who noted that the sensitivity of the MS2 bacteriophage to UV light was not constant in a wastewater treatment plant which used UV light as a disinfection process.

Blanc and Nasser (1996) determined that bacteriophage were not good indicators for predicting the survival of enteric viruses in soil. In another study Havelaaret al. (1993) found that F-RNA bacteriophage correlated highly with enteric viruses in all wastewater types tested except for raw and biologically treated sewage. They did find, however, that enteroviruses could be isolated from some water samples in which F-RNA bacteriophage could not be detected. Jofre et al. (1995) examined the efficiency of three different water treatmen systems to remove bacteriophage from the water. They found that B. fragilis bacteriophage were more resistant to treatment processes (decimal reductions of 2.2-2.9) than other bacteriophage (decimal reductions of 2.3-5.2 for F-specific bacteriophage and >2.6-5.6 for somatic coliphage), and enteroviruses (decimal reductions of >2.9->3.4). They suggested that the B. fragilis bacteriophage should be studied further for its efficacy as an indicator of virus survival in water treatment plants. This suggestion was based on observed rotaviruses and hepatitis A virus resistant patterns, and the greater resistance to treatment of B. fragilis bacteriophage's when compared to the enteroviruses.

While a number of potential replacements for faecal coliforms have been studied for their possible use, none have been found to completely suitable. All of the potential indicators studied to date have one or more characteristics which prevents their implementation as replacements of the faecal coliforms. Thus, despite their drawbacks, faecal coliforms still remain the major organisms used to indicate faecal pollution and the effectiveness of treatment processes. However, the improvements in the detection of microorganisms which have been occurring in the last 10 years may mean that the use of indicators will no longer be required.

3.3 Detection of Microbial Pathogens in Water and Wastewater

It is imperative to be able to determine the presence or absence of any microbial pathogens in wastewater used in reclamation projects. Also, as different microbial pathogens have different infectious doses (number of infectious units required to cause an infection), the determination of the numbers of the different microbial pathogens in a wastewater sample is imperative. Also, the efficient enumeration of microbial pathogens in a wastewater sample pre- and post-treatment can allow an effective risk assessment to be made prior to the recycling of the wastewater. The ideal detection method would be rapid, sensitive, highly accurate, easy to perform, and able to run in high numbers.

There are a number of established methods for the detection of most microbial pathogens, however, most of these methods have major limitations. The majority of these limitations are associated with the time taken to isolate and/or identify the pathogen; with the determination of the numbers of that pathogen in a sample; and with the accuracy of detection. A large amount of research has been undertaken to develop methods which improve the detection of various microorganisms. Some limitations still exist with many of these newer methods and most still require extensive study to determine their efficacy under a wide range of conditions. Some of the established methods and newer methods are discussed below.

3.3.1 Established detection methods

Most established detection methods either rely on the culturing of pathogens using an artificial medium or cell culture, or, when they cannot be cultured, through direct detection usually involving the use of microscopy.

Viruses are only able to replicate within host cells, and thus, can not replicate in the environment. The common method for the detection of viruses in water sources is by concentrating the viral particles from large volumes of the water sample, and then culturing the concentrated viruses in plates of animal tissue culture. This has the disadvantage of being highly inaccurate, very time consuming, expensive and requiring highly trained personnel. There is the added problem that some viruses such as the Norwalk virus can not be cultured in vitro and can only be cultured by infecting volunteers.

The detection of most viruses in cell culture relies on the detection of a cytopathogenic effect. The viral particles kill infected tissue culture cells causing zones of clearing (plaques) in the monolayer of tissue culture cells. Quantitation of the number of infective viral particles in a sample relies on the use of the Most-Probable-Number (MPN) method where serial dilutions of a sample are used to inoculate a cell line. The highest dilution in which plaques are detected is used to calculate the number of viral particles in the original sample. To obtain a result which has some statistical validity, this requires the use of a large number of bottles of cultured cells to obtain an effective dilution series and replicate number.

Different cell lines are usually used to grow different viruses. The cell line chosen is usually based on viral growth rates, cytopathogenic effect of a particular virus, infectivity efficiency etc. in the differen cell lines. This has resulted in a number of different cell lines being recommended for the detection of the different enteric viruses in environmental samples. Thus, the routine assessment of wastewater for the presence of viruses is extremely unwieldy and expensive. Recently, however, Pintó et al. (1995) demonstrated that rotaviruses, poliovirus, coxsackievirus, enterovirus 70, astrovirus and adenoviruses could be detected using one particular cell line (CaCo-2), thus making routine analysis for these viruses much simpler and straight forward.

Another problem with conventional cell culture techniques is that detection of the cytopathogenic effect can often be difficult, or doe not occur at all, even though the cell culture has become infected. This situation requires the use of other methods such as enzyme-linked-immuno-assay (ELISA) to detect the infection of the cell culture (Nasser et al. 1995, Sellwood & Wyn-Jones 1995). Also cell tissue cultures are extremely sensitive to many substances (e.g., humic compounds, divalent cations) present in many water sources, particularly wastewaters. These compounds can inhibit the growth of, or kill cell cultures, irrespective of the presence or absence of infective viral particles.

Protozoan and helminth parasites exist in wastewater and soils as cysts, oocyst or eggs and tend to be in low numbers when compared to bacterial numbers. Established detection methods for these parasites are laborious, expensive and inaccurate. Difficulties exist in detecting these pathogens as they cannot be cultured on artificial media. Also, the cysts/eggs are usually present in low numbers in wastewater samples necessitating the concentration of large volumes in order to obtain accurate results. The resistance of the cysts/oocysts/eggs to

environmental influences and treatment processes and their low infective dose levels necessitates their efficient detection.

The common method used for the detection of Giardia cysts and Cryptosporidium oocysts involves the concentration of several litres of water, usually by filtration. The concentrated solids are then resuspended off the filter and the cysts/oocysts separated from non-cellular matter using gradient centrifugation. The collected cysts/oocysts are then detected under a microscope, often after they have been stained with a fluorescently-labelled antibody (Kfir et al. 1995, Haas & Rose 1996, Wallis et al. 1996).

Detection using such methods is time consuming, expensive and requires highly trained personnel to accurately detect and identify the cysts. It has also been demonstrated that there is significant loss of cysts during the centrifugation-clarification stage (LeChevallier et al. 1995, Nieminski et al. 1995, Whitmore & Carrington 1992). The inability to determine the viability of detected cyst/oocysts is also a major drawback of these established methods (Abbaszadegan et al. 1997a). Some expertise is also required in detecting and identifying Cryptosporidium oocysts as they can be variable in their staining characteristics depending on their age, viability and stage of development (O'Donoghue 1995).

Helminth and tapeworm ova are currently only detected in wastewaters by the concentration of large volumes of water, usually using centrifugation/sedimentation followed by floatation and microscopic examination for the ova (Gaspard & Schwartzbrod 1995, WHO 1989). Similar to the detection of Giardia cysts and Cryptosporidium oocysts, this is time consuming, and requires considerable experience to be able to detect and identify the different helminth and tapeworm eggs.

Unlike the protozoa and helminths, most bacterial pathogens can be isolated and maintained on solid media (the best known exception is Mycobacterium leprae, the cause of leprosy). Approved methods and media for the isolation of the common bacterial pathogens and indicator bacteria from water and wastewater are well established. These methods can be found in detail in the Australian Standards on water microbiology (1995) and the American Manual for the Examination of Water and Wastewater (APHA 1989).

Despite the fact that most bacterial pathogens can be easily of cultured, there are a number of problems associated with attempts to detect the presence and numbers of these pathogens in wastewater

samples. Difficulties can exist in the identification of bacterial pathogens on isolation media, often requiring the distinction between the pathogenic microorganisms and contaminating saprophytic microorganisms which may also be present in the sample. The contaminating saprophyte may be closely related to the pathogenic strain sought, making distinction between the two strains difficult.

Colony morphology is rarely a significantly distinct enough feature to be able to conclusively distinguish between colonies of a pathogenic bacterial strain and contaminating non-target species. Presumptive isolates of the suspected pathogenic bacterium usually need to be subcultured and have their identity confirmed. This confirmation can be time consuming and expensive, especially if part of an epidemiological study. The whole process from isolation to identification is time consuming (with time frames from 4 to 14 days or more from processing of the environmental sample to a definitive result) and expensive, both in labour time and materials.

Attempts have been made to remove the contaminating saprophytic/non-pathogenic bacterial strains through the use of selective isolation methods and/or selective media (Gacriel & Lamb 1995, Gaudet et al1996, Handfield et al. 1996, Kersters et al. 1996, Nieme & Ahtiainen 1995). The aim is to greatly reduce the number of contaminating saprophytic/non-pathogenic microorganisms while maintaining the numbers of the target strain. However, the use of selective isolation procedures and/or media usually also reduces the number of the target organism recovered. This can be a particular problem when the pathogenic bacterial cells targeted are low in number, or stressed due to environmental pressures and/or treatment processes.

Another dilemma frequently encountered is that many bacterial strains in the environment enter a state where they are viable but nonculturable. Viable-but-nonculturable (VNBC) stands for a physiological state entered into by the cells of many non-spore forming bacterial species. It is surmised that the bacterial cells undergo this transformation when they become injured, or as a survival tactic in unfavourable environments. The cells reduce in size and cellular content (e.g. RNA and enzyme activity) (Porter et al. 1995). The VNBC state can be equated to a dormancy state. While these cells have been shown to be metabolically active, they can not be cultured on artificial media (Porter et al. 1995). VNBC can cause difficulties in trying to detect pathogenic bacteria on/in artificial media, if they exist in such a non-culturable state. Thus, VNBC can give a false negative result, or

an underestimation of the number of a pathogenic microbial cells in a sample. VNBC cells of pathogenic strains remain a heath risk as they are still capable of causing infection or, if environmental conditions improve, reverting to a full metabolic state and potentially increasing in number.

Detection of bacterial pathogens can be particularly difficult if the pathogenic strains form only a small proportion of the total bacterial population. In such situations the pathogenic strains can be lost during attempts to remove the non-pathogenic bacteria (e.g. through dilution) The detection of *Salmonella* in wastewater, for example, usually requires the selective and/or non-selective enrichment of the sample to increase the number of *Salmonella* cells present prior to pretreatment and plating on a selective medium. This results in the detection of Salmonella being a qualitative presence/absence test only, with no ability to determine the Salmonella numbers present in the original wastewater sample.

3.3.2 Polymerase Chain Reaction

With the inherent limitations associated with the established methods used for the detection of the various microbial pathogens in wastewaters, researchers have looked for other more sensitive, accurate and quicker detection methods. One of the most common of the new methods examined involves the use of the polymerase chain reaction (PCR). PCR can be used as the standard method or modified to semi-nested PCR or nested PCR methods (Gajardo et al. 1995, Le Guyader et al 1995, Mayer & Palmer 1996, Straub et al. 1995a). Semi-nested and nested PCR improve detection efficiencies through the further amplification of amplified DNA either by using one or both of the original primers (semi-nested) or a completely different set of more selective primers (nested). Both of these modified methods have been demonstrated to significantly increase the detection efficiency of the PCR method (Gajardo et al. 1995, Straub et al. 1995a).

Detection limits for PCR methods have also been increased through the use of membrane hybridisation detection of PCR products with specific DNA probes (Hay et al. 1996, Laberge et al. 1996, Schwab et al. 1995) or by using enzyme-linked immunoassay (ELISA) (Ritzler & Altwegg 1996). As it is highly probable that wastewater samples contain more than one microbial pathogen, multiplex PCR can be used to detect more than one target in a single PCR reaction (Pepper et al. 1997, Picone et al. 1997, Rochele et al. 1997, Way et al. 1993). Multiplex PCR involves the use of a number DNA primers, each of which are designed to detect

specific microbial strains, in a PCR single reaction (Picone et al. 1997).

PCR has been trialed for the detection of a number of different pathogens in environmental samples. A number of enteric viruses have been detected in water, wastewater and soil environments using PCR. Detected viruses include Norwalk virus (Atmar et al. 1995, Wolfaardt et al. 1995, Schwab et al. 1995), enteroviruses (including hepatitis A virus) (Atmar et al. 1995, Gilgen et al. 1995, Green & Lewis 1995, Le Guyader et al. 1995, Pintó et al. 1995, Reynolds et al. 1995, Schwab et al. 1995), rotavirus (Gajardo et al. 1995), and astrovirus (Marx et al 1995, Pintó et al. 1996). Comparison of PCR with the conventional cell culture methods indicated that PCR was at least comparable in sensitivity to cell culture (Wyn-Jones et al. 1995) if not superior (Schwab et al. 1996)

The use of PCR has also been examined for its ability to improve detection efficiencies, reduce processing time and to determine viability of the *Giardia* cysts and *Cryptosporidium* oocysts (Abbaszadegan et al 1997b, Laberge et al. 1996, Mayer et al. 1996, Rochelle et al. 1997, Stinear et al. 1996). These studies have reported rapid specific detection of the cysts/oocysts. PCR has also been employed to determine the viability of *Giardia* cysts and Cryptosporidium oocysts through the detection of a heat shock protein mRNA (Abbaszadegan et al. 1997a, Abbaszadegan et al. 1997b). As mRNA has an extremely short lifespan in both live and dead cells, the amplification of mRNA from within the cyst/oocyst can be used to confirm the viability of the cyst/oocyst.

No information could be found on the use of PCR for the detection of the eggs of human pathogenic helminths in wastewater. However, PCR has been used to study the biology of helminths (Geary 1996) and to detec ovine helminths (*Echinococcus multiocularis*) in fox faecal matter (Mathis et al. 1996). Semenova et al. (1996) used the randomly amplified polymorphic DNA (RAPD-PCR) to study the genetic differences between the helminth groups, showing that there is a significant genetic differentiation between species. The results of these studies suggest that PCR detection methods could be highly applicable to the detection, differentiation, and viability determination of helminths in wastewater and soil samples.

The PCR method has been tested for the rapid detection of bacterial pathogens in a number of recent studies. Bacterial species for which PCR has been tested as a detection method include *Bacteroides* (Kreader 1995), *Campylobacter* (Jackson et al. 1996), *E. coli* (Tamanai-

Shacoori et al. 1996, Tsai et al. 1993), Helicobacter pylori (Nilsson et al. 1996), Legionella (Frahm, et al. 1995, Fricker & Fricker 1995, Hay et al. 1995, Palmer et al. 1995, Roll & Fujioka 1995), Leptospira (Letocart et al. 1996), Listeria (Jensen et al. 1993) Salmonella (Cohen 1996, Jensen et al. 1993, Lin & Tsen 1996, Way et al. 1993) and Staphylococcus (Jensen et al. 1993).

PCR has been used to identify bacterial strains which have been isolated as colonies on solid media. Examples include the identification/speciation of Arcobacter (Harmon & Wesley 1996), different Leptospira species (Letocart et al. 1996), and various Mycobacterium species (Neumann et al. 1997). PCR has also been found to be effective for the differentiation of Vibrio cholera strains (Rivera et al. 1995), and the determination of virulence among Salmonella strains (Swamy et al. 1996). Hay et al. (1995) used PCR amplification to demonstrate that Legionella cells encysted within cells or cysts of the amoebae Acanthamoebae castellanii were viable despite being nonculturable on artificial media.

The determination of a distinct area of the 16S rRNA gene for Mycobacterium ulceranshas lead to the possibility that PCR could be used for the rapid detection of this organism (Portaels et al. 1996). This would be useful as this organisms has never been isolated from environmental sources, therefore its mode of transmission has never been clearly established.

One of the advantages of PCR is its ability to detect small amounts of target DNA in a sample. Wyn-Jones et al. (1995) found that PCR had a detection limit of 5 plaque forming units (pfu)/sample for enteroviruses in river and marine waters. Other researchers determined detection limits of 2 pfu/sample for enteroviruses in water (Schwab et al. 1996), 3 pfu/sample for astroviruses in water (Pintó et al. 1996), 20 pfu/mL for rotaviruses from sewage samples (Gajardo 1995), and a theoretical detection limit of 1 pfu/L for enteric viruses from wastewater (Green & Lewis 1995). With optimised reaction conditions, Sunun et al (1995) were able to achieve a maximum sensitivity of Qβ 0.3 coliphage pfu/reaction.

The PCR detection of bacteria has generally only been used as a qualitative presence/absence test, with little reported on quantitative measurements. However, Roll and Fujioka (1995) reported that the commercially available Enviroamp PCR kit for the detection of

Legionella cells in environmental samples is semi-quantitative, being able to determine if there are greater or less than 10^3 Legionella cells/mL in a sample.

Although PCR has demonstrated that (i) there can be a great reduction in the time required to detect pathogenic microorganisms in wastewater samples, (ii) that viable-but-non-culturable status does not affect detection, and (iii) that it is at least comparable if no superior in detection sensitivity to traditional methods, it does, however, have a number of problems. Due to the sensitivity of the method, common PCR detection methods are not capable of distinguishing between viable and non-viable pathogenic microorganisms (or their resting stages). This is principally because DNA is relatively stable in the environment, particularly when encased in the membrane of a dead cell. The sensitivity of PCR means that there is a strong possibility that this DNA will be amplified despite being in a non-viable cell. DNA has also been shown to exist in a naked state (i.e., outside of cell membranes) in water (Maruyama et al. 1993, Paul et al. 1991, Romanowski et al. 1991, Tsai et al. 1993).

Even RNA, which is environmentally less stable than DNA, can give false positive PCR results. Lévêque et al. (1995) examined the effect UV irradiation had on the detection efficiency of hepatitis A virus in seawater. They found that after just 15 minutes irradiation, no infectious virus could be detected by cell culture, but hepatitis A virus RNA could be detected in the solution using RT-PCR and probing with a labelled DNA probe, even after 60 minutes of irradiation. Tsai et al. (1995) studied the persistence of naked viral RNA in filtered and unfiltered sea water at 4 ^{o}C and room temperature (approximately 23 ^{o}C). They determined that the naked RNA could not be detected by RT-PCR after 2 days incubation in the unfiltered seawater held at either temperature. While this demonstrated that RNA does not persist in the seawater environment for any appreciable length of time there is still a significant chance of detecting viral RNA which is not capsid bound.

The PCR method is also capable of giving false positives, again due to the inherent sensitivity of the method, usually from contamination by extraneous nucleic acids, often through contact with contaminated laboratory equipment. Thus, great care must be taken in the processing of the samples and the running of the PCR method in order to achieve a valid, reliable result.

The polymerase enzyme, which is central to the PCR method, is also

highly susceptible to a number of contaminants commonly found in wastewaters (e.g., humic compounds, high divalent cation concentrations, and salts). At times, particularly with wastewaters, considerable effort must be made to remove these inhibiting compounds prior to testing (Straub et al. 1995b).

There are a wide range of methods which can be used to remove the inhibitory compounds, including the use of polyvinyl polypyrolidine, sephadex columns, ion exchange resins, and caesium chloride gradient ultracentrifugation. Recently, Nilsson et al. (1996) reported on a method which used immunomagnetic bead-labelled oligonucleotide probes for the selective extraction of Helicobacter pylori DNA from faecal samples. Schwab et al. (1996) and Graff et al. (1993) both used an antibody-capture method for the selective isolation of enteroviruses and hepatitis A virus from water and wastewater samples prior to extraction of the RNA. Thus, methods are currently being developed which will alleviate many of the factors causing inhibition and false positives and negatives

While small numbers of target nucleic acids can be detected in a sample using PCR, the detection limit can be affected by the extraction or concentration efficiencies for different pathogenic microorganisms from a wastewater sample. The need to concentrate large volumes of water, particularly for viruses, protozoan cysts and helminth eggs, risks significant loss of the target organism during the concentration process. Quoted recovery efficiencies for viral particles have varied from 32% of enteroviruses from sewage sludge and amended soil (Straub et al. 1995b), to 77% for poliovirus seeded into artificial sea water (Reynolds et al. 1995). The concentration of protozoan cysts from water samples have similar problems. In a survey of published methods, LeChevallier et al. (1995) reported that most methods had recorded recovery efficiencies of less than 50% for Giardia cysts and Cryptosporidium oocysts from water samples. Trials and comparison of these methods by LeChevallier et al. determined that Cryptosporidium oocyst losses could be as high as 30% for each centrifugation step. These reported losses during the concentration and/or recovery procedures can greatly decrease the detection sensitivity of the PCR method.

Thus, while the use of PCR shows great promise for the detection of microbial pathogens in wastewaters, a number of issues need to be resolved before it can be considered to be a standard for the wastewater industry.

3.3.3 Other isolation/detection methods

Apart from PCR, researchers have studied several other methods for their ability to detect and enumerate microbial pathogens in wastewater samples.

Viruses have been detected in wastewater samples using enzyme-linked-immuno-assays (ELISA). Nasser et al. (1995) indicated that while plaque assay gave a slightly greater sensitivity for the number of poliovirus in groundwater and wastewater, a positive result using the ELISA method indicated that active viral particles were present. Sellwood and Wyn-Jones (1995) combined cell culture and ELISA for a simple, sensitive, low cost method for the detection of rotaviruses from water.

Hurst et al (1988) demonstrated that the use of virus specific labelled-DNA probes in an in situ hybridisation method was approximately 40% more sensitive for the detection of adenovirus infected cell cultures than cytopathogenic assays or immunofluorescence detection in the same cell cultures. Following concentration steps, Genthe et al. (1995) tested gene probes for the detection of adenoviruses in South African waters. Using the probes they were able to detect adenoviruses in up to 59% of raw and treated water samples tested. Similar detection levels were found for both chlorinated and non-chlorinated samples indicating that, like with PCR, hybridisations with gene probes cannot distinguish between active and inactive virus particles. An oligonucleotide hybridization assay was used by Beekwilder et al. (1996) to identify and enumerate F-RNA bacteriophage present in surface waters. Such a method could be considered to be useful in the isolation of specific bacteriophage being used as indicators for the presence and/or survival of enteric viruses in water systems such as wastewater.

Kakubayashi et al. (1996) developed a method combining two cellular stains to determine the viability of isolated Giardia cysts. The method involves staining the cysts with the nuclear stain 4',6-diamidino-2-phenylindole (DAPI), which specifically stains DNA, and propidium iodide, which can only stain cysts which have disrupted walls. The DAPI stain is used for cyst detection and the propidium iodide is used to determine the viability of the cyst. Cysts which take up the propidium iodide stain are classed as non-viable, while those which do not stain with the propidium iodide are considered viable. The basis of this method has also been used combined with flow cytometry for the detection, enumeration and determination of Cryptosporidium oocysts

(Veal 1996). Flow cytometry has been used to discriminate, enumerate and identify a number of different species and types of microorganisms in environmental samples using fluoropores as well as labelled antibodies and oligonucleotides (Davey and Kell 1996).

Fluorescent nuclear stains combined with stains which determine cell membrane integrity, along with flow cytometry have been used to assay the physiological status of *E. coli* seeded into sterile lake water (Porter et al. 1995). This study was able to demonstrate that the viability of viable-but-non-culturable cells could be determined and that total viable cell numbers could be rapidly and efficiently determined. The use of computer-assisted laser scanning microcopy linked to video image analysis was demonstrated to achieve a detection limit of 5.2×10^2 *Cryptosporidium* oocysts/g of soil, sediment or faecal material (Anguish & Ghiorse 1997). While these methods have improved detection sensitivity, the accuracy of enumeration and identification, along with removing some amount of operator error, they still require expensive equipment and highly trained staff.

A method has been developed for the assessment of the viability of nematode eggs (Gaspard et al. 1996). This method involves the isolation of the eggs from sludge or wastewater followed by culturing the eggs in aerated deionized water for up top 16 days (incubation time is dependant on helminth species) at 30^{o}C. The cultured eggs are then sonicated to disrupt the wall of the egg and the degree of development of the helminth within the egg determined. Those eggs which have reached the laval stage are considered to be viable. Like the general isolation and detection method for helminths, this method is time consuming and requires a high degree of technical expertise to obtain an accurate, reproducible result.

Immunoassays have also been used for diagnosis of helminth infection and the identification of helminth species (Romarís et al. 1996). ELISA tests have been developed by Kehayov et al. (1991)for the detection *Trichinella spiralis* infections (trichinosis), and by Chandrashekar et al. (1993) for the detection of *Ochocerca volvulus* infections (Onchocerciasis). To be effective in the detection of helminths in wastewater an ELISA test would need to be able to detect antigens present on the surface of the eggs of the different helminths. While no literature could be found on the use of ELISA for the detection of helminth eggs in wastewaters, the above reported uses of ELISA tests indicate that there is at least a possibility of developing such a test.

Other researchers have worked on the use of specific enzymes for the rapid detection of specific groups of bacteria in food and environmental samples. The total coliform group is known to contain the enzyme β-D-galactosidase, which cleaves galactose. The faecal coliforms, a sub-set of the total coliform group which are found in the intestinal tracts of warm blooded animals, also possess the enzyme β-D-glucuronidase which cleaves glucuronide. This enzyme is not found in the remaining members of the total coliform group. The presence of these enzymes in the coliform groups have been used to develop rapid identification methods for total and faecal coliforms. These methods are based on the linking of β-D-galactoside and β-D-glucuronide with a marker compound such as 4-methylumbelliferone. The essence of these methods is that the galactosidase or glucuronidase enzymes cleave the parent compound releasing the marker compound. In the example of 4-methylumbelliferyl-β-D-galactoside and 4-methylumbelliferyl-β-D-glucuronide, this cleavage of these compounds releases 4-methylumbelliferone which fluoresces under long wavelength UV light. The use of these compounds in conjunction with the Most Probable Number (MPN) technique can give a quantitative result in as little as 12 hours (Park et al. 1995).

4-methylumbelliferyl-β-D-galactoside has also been used for the detection of enterococci which also contain the β-D-galactosidase enzyme (Niemi & Ahtiainen 1995). Other reported variations on this method have used o-nitrophenyl-β-D-galactopryranoside (which gives a yellow colour when cleaved) for the detection of faecal coliforms in sewage (Apte et al. 1995), chlorophenol red-β-D-galacto-pryranoside in combination with 4-methylumbelliferyl-β-D-glucuronide for the enumeration of total coliforms and E. coli in water and wastewater (Bitton et al 1995), and 5-bromo-6-chloro-3-indoyl-β-D-glucuronide, which produces visible blue colonies when cleaved by β-glucuronidase, for the detection of E. coli in a range of water samples (Ciebin et al. 1995). Comparisons with standard media showed that there was good correlation between these new enzymatic-based detection methods and standard isolation techniques on solid agar for coliforms, faecal coliforms and enterococci (Bitton et al. 1995, Ciebin et al. 1995, Niemi & Ahtiainen 1995). The enzyme based methods have the advantage, however, of providing a result within 18 hours, compared to the standard 24 - 36 hours required by the standard techniques.

Other methods studied for the analysis/identification of bacterial pathogens isolated from water sources include the use of fatty acid methyl ester (FAMEs) for the analysis of the diversity of Aeromonas

species in drinking water (Huys et al. 1995); analysis of antibiotic resistance patterns in faecal streptococci isolated from natural waters (Wiggins 1996); the detection of Mycobacterium spp. in water using gas chromatography/mass spectrometry (Slosárek et al. 1996); and the analysis of enterogenic repetitive intergenic consensus sequences of pathogenic and non-pathogenic Vibrio cholera (Rivera et al. 1995).

Most of the new or novel methods (including PCR) discussed above, while being quick, accurate and/or highly sensitive remain to be completely validated for their efficacy in detecting microbial pathogens, in particular viruses and protozoa/helminths, in wastewater. More research will need to be done, and is currently being undertaken by a number of different researchers.

3.3.4 Commercial detection and identification kits

Several of the methods detailed above have been used for the production of commercial kits for the detection of various microorganisms in clinical, food and environmental samples. These kits have the appeal that they are usually easy to use, and have excellent internal quality control standards. In addition, they also remove the necessity to spend time producing media or solutions; greatly reduce processing time; and are usually able to give very rapid results. These time saving factors are often make them very economical for small government and commercial labs. Several of these kits are detailed below and the brochures of some of these kits are given in Attachment 1.

The presence of the β-galactosidase and β-glucuronidase in coliforms and enterococci have been used in the development of several commercial kits for the rapid detection of these organisms in sewage, wastewaters and water samples. Examples of these kits include ColiPAD® (IDEXX), ColiLert® (Environetics), Colisure™ (Millipore Corporation), ColiComplete™ and ColiTrak Plus™ (Biocontrol). These kits rely primarily on the use of a chromogenic or fluorogenic substrates to give a presence/absence result. This result is usually combined with a MPN dilution method to give quantitative number of coliforms in a sample.

Other identification/detection kits commercially available include an immunodiffusion method for the detection of Salmonella bacteria in enrichment cultures (1-2 Test® (Biocontrol)); test strips for the rapid

identification of isolated bacterial strains (e.g., API identification strips (Biomererieux) and BBL Crystal (Becton Dickinson)); and a PCR kit specifically designed to detect Legionella species in environmental water samples (EnviroAmp (Perkin Elmer)).

Some problems, however, still exist with a number of these kits. Van Poucke and Nelis (1997) reported that enzymatic presence-absence tests used for the detection of total coliform bacteria could be limited by false positive results from non-coliform bacteria possessing the β-galactosidase enzyme. The same study showed that higher-than-expected results for the detection of faecal coliforms were obtained due to the presence of β-glucuronidase in non-E. coli bacteria. Hanai et al. (1997) compared the standard U.S Food and Drug Administration (USFDA) method with six commercially available kits designed to detect Salmonella in food and water. They found that only one of the kits was comparable to the USFDA method for the detection of Salmonella serovars seeded into food samples.

4 Microbiological Issues Related to Wastewater Reuse

It has been generally acknowledged that the greatest hazard associated with the recycling of wastewaters is the potential presence of microbial pathogens. Guidelines have been created by a number of countries which regulate microbial levels, application methods, and treatment processes for wastewater reuse. These guidelines are all designed to minimise the health risk associated with microbial pathogens in recycled wastewater. There are a number of factors which are important when determining the health risks in wastewater reuse. These are discussed below.

4.1 Microbiological Health Risks Associated With Wastewater Reuse

The determination of health risks associated with wastewater reuse is a difficult, subjective, and often emotive issue. There are a number of factors which need to be considered to properly assess health risks prior to the establishment of a wastewater reuse scheme. Basher and Shahalam (1989) indicated that issues which need to be considered prior to the reuse of wastewater include the proximity of human habitation to the wastewater reuse site and possible forms of human contact; possibility of human ingestion of aerosols and the direct exposure of the wastewater to workers' skin; the socioeconomic status

of populations likely to be exposed to the wastewater; and the duration and frequency of human contact with the wastewater.

As the greatest health risk associated with wastewater is the presence of microbial pathogens, there are many factors to be considered regarding the health risk associated with the different pathogens groups. Prost (1987) determined that there are three levels of risk relating to microbial pathogens in wastewaters: (1) Theoretical risk: which is the type(s) of microbial pathogens present in the wastewater; (2) Experimental risk : which is the known survival attributes of the pathogen and the known dose required to cause infection; and (3) Actual risk : which is the risk as determined from epidemiological evidence.

Other risk factors identified are related to the individual pathogen. These included the quantity of a pathogen excreted by the host; the period of latency required before the excreted pathogen becomes infectious; the length of time the pathogen can survive in the environment; the ability to multiply in the environment; the dose (or a number of pathogen) required to infect a susceptible host; and the hosts response (usually relating to an immune response due to previous exposure) (Prost 1987). These risk factors were used to classify the different pathogens into different risk categories. Category I consisted of pathogens which have a low infectious dose and are immediately infectious. All viruses and intestinal protozoa made up this category. Category II contained the pathogens which require a higher infectious dose than category 1 pathogens. They tend to be able to survive for long periods of time or increase in number in the environment due to their ability to multiply therein. This category was comprised exclusively of bacteria. Category III contained those parasites which have long latency period. The parasites in this category comprised of helminths such as Ascaris lumbricoides or Necator americanus. Parasites with complex life cycles, for example, having the requirement of an intermediate host, made up the remaining three categories (IV, V, & VI) (Prost 1987).

Taking all of these risk factors into account, the infection risk from a pathogen still relies on the host susceptibility. Susceptibility is dependent on physical, immunological, and sociological status. For example, viruses have a low infectious dose, an ability to survive treatment methods and a reasonable survival time in the environment. Their high prevalence in communities in developing countries, however, means that the population tends to develop immunity to these pathogens at a young age. Examples include the hepatitis A and polio

viruses (Shuval 1991). Thus, the presence of such pathogens in wastewater presents a low risk to these communities. Conversely, pathogens which do not induce an immune response, for example helminths, pose a greater risk to these communities due to the low sanitary conditions present, the chance for on going chronic infection, and for reinfection to occur.

The opposite risks could be considered to occur in developed nations with greater standards of sanitation and public health. There is less contact with pathogens such as poliovirus or hepatitis A virus in early childhood, thus, less community immunity to such diseases. The risk factors for such diseases are therefore, much more pertinent in developed nations than developing nations. Also, pathogens such as helminths are less of a wastewater hazard to communities in developed nations due to higher sanitation standards.

The WHO microbiological guidelines for wastewater reuse (see Table 5) identified helminths as the major health risk in wastewaters, partly due to the resistance of the eggs to environmental factors, and partly because the ingestion of less than 10 eggs has been shown to have a high probability of causing infection. Of particular risk to helminth eggs are farm works in contact with wastewaters used for irrigation purposes and people who consume raw or poorly cooked contaminated food (WHO 1989).

The WHO guidelines were based on the following pathogen risk factors (1) long persistence in the environment; (2) low minimal infective dose; (3) short or no immunity; (4) minimal concurrent transmission through other routes such as food, water and poor personal or domestic hygiene; and (5) long latent period and/or soil development stage required (Shuval 1991). These factors lead the WHO committee which developed the guidelines to identify helminths as the major hazard to wastewater reuse. Protozoa and bacteria were deemed to be an intermediate risk. Viruses, despite being highly infective and having reasonably long survival periods in the environment, were identified as low risk. This was due to concurrent routes of infection in the home due to poor hygiene, thus giving high immunity levels from a young age.

The WHO guidelines were formed on the assumption that wastewater would be reused in developing nations with no, or low cost treatment. Thus, more developed nations would need to consider forming guidelines and criteria which would be more relevant to their conditions. Haas et al. (1993) examined the risk of a person contracting a viral infection from water using exposure estimates,

dose response, and the morbidity and mortality probabilities. They determined that there could be a life time risk as high as 1 in 20 of death from exposure to a waterborne virus. This is clearly a very high risk, indicating that viruses still need to be carefully considered when contemplating recycling wastewater.

A number of epidemiological studies have been undertaken to determine the incidence of infection due to microbial pathogens in wastewater. Most studies have concluded that there is little or no greater risk to workers and the community due wastewater reuse when compared to the incidence of disease in the general community (Clark 1987, Kindzierski & Gabos 1996).

Published epidemiological studies have focused on workers in the wastewater treatment industry, in particular sewer workers (Clark 1987,Kindzierski & Gabos 1996, West & Locke 1990). Increased antibody titres to the bacterial disease leptospirosis has been observed among sewer workers in Canada (Kindzierski & Gabos 1996). Clark (1987) cited studies which demonstrated that inexperienced workers at wastewater treatment plants had increased antibody titres for the Norwalk virus, and echoviruses. Other studies cited in the same review showed that wastewater treatment workers in Alaska had increased incidences of hepatitis A infections, and sewage workers in Copenhagen had increased antibody titres to hepatitis A virus. Other studies quoted in the same review on residents surrounding wastewater treatment plants did not demonstrate any exposure effects. There was, however, a perceived increased risk to communities living nearby wastewater treatment plants from Legionella.

Further studies cited in the review by Clark (1987) indicated that an increased risk may occur for wastewater workers from intestinal protozoan pathogens such as Giardia intestinalis and Entamoeba histolytica but there was little risk for the general public. Many of these studies determined that incidences of infection or raised antibody titres among wastewater workers decreased with improved hygiene, increased protection measures, and increased training and education of the workers.

Most of the studies reviewed by Clark (1987) involved studies with workers who were in direct contact or close association with wastewater. There has been little evidence associated with wastewater reuse for increased rates of infections among the general public, regardless of the level of treatment or type of use. Further epidemiological research will be needed assessing the risks,

particularly in developed countries with regard to viral infections. Infection caused by viruses in wastewater is difficult to determine due to the common occurrence of some enteric viruses in communities, the possible presentation different symptoms by different infected hosts, and the possibility of non-specific infections. A risk assessment on the potential for gastrointestinal disease from waterborne rotavirus by Gerba et al. (1996) indicated that there was a significant risk of infection from recreational waters contaminated with rotavirus particles. Such a risk assessment could easily be extrapolated to the assessment of risks associated with the presence of rotavirus in wastewater.

Most reported cases of illness due to contact with contaminated wastewater or recreational waters have occurred where either the water has not be treated or where treatment processes have been either inadequate or have broken down (MacKenzie et al. 1994, Shuval 1991). Bryan (1977) lists 65 cases of disease outbreaks associated with foods contaminated by sewage or wastewater. The majority of these cited cases were typhoid fever, viral hepatitis, helminth infections (mostly fascioliasis), salmonellosis, and cholera. An increase in the incidence of hepatitis A and dysentery in China has been linked to insufficient progress in the treatment of wastewater and excreta (Prost 1989).

Other factors which can have an influence on the risks associated with wastewater reuse include wastewater type, the method of application, the potential for human contact, and treatment levels.

Risks associated with the type or source of wastewater are primarily influenced by the degree of contact with faecal material, and the nutrient load. Clearly, increased chances of faecal contamination increases the risk of microbial pathogens being present in the wastewater. Therefore, a wastewater from an industrial plant would have a lower risk from microbial pathogens than domestic greywater, which in turn would pose less of a risk than sewage effluent.

Guillaume and Xanthoulis (1996) described the use of effluent from a food processing plant for irrigation in the local market gardens. As there was no mixing of the industrial effluent with sewage effluent, the presence of microbial pathogens was not an issue. Rose et al. (1991) studied the microbial quality in domestic grey water from different households and the persistence of selected pathogens in grey water samples. Their findings showed that households with young children had increased faecal coliform numbers than households

without young children. They also demonstrated that Salmonella typhimurium, Shigella dysenteriae and poliovirus seeded into samples of grey water persisted for several days.

Sewage effluent, particularly from high population density areas such as urban developments, carry the full spectrum of faecally excreted human pathogens in high concentrations, and thus, has a correspondingly high risk for workers and the general public (Feachem et al. 1983, Shuval 1991) (see Table 6 for an example of the pathogen load in sewage for a hypothetical tropical town in a developing country).

The presence of utilisable nutrients in wastewaters can increase the risks associated with bacterial pathogens due to their ability to multiply in such environments. Rose et al. (1991) demonstrated that coliform bacteria and standard plate count bacteria increased in number in domestic grey water held at 25°C by one order of magnitude within two days. In a study of Salmonella spp. and Vibrio cholera seeded into reconditioned wastewater, Rajkowski et al. (1996) found that both pathogens were able to grow at temperatures between 10 °C and 27 °C. At some temperatures, both strains increased in number by a factor of almost 3 $\log_{10}$.

The results of these studies indicate that low numbers of bacterial pathogens in a wastewater could be a health risk if the wastewater is held and/or not treated. In such an event there is the potential for the pathogens to increase to numbers so that infection could occur. This risk is even greater for opportunistic pathogens such as Aeromonas hydrophila and Pseudomonas aeruginosa These bacterial species are commonly found in water environments and are more efficient at replicating in these environments than many pathogenic bacteria such as Salmonella.

The other major risk factor involving pathogens in wastewater is the potential for contact with workers and the public. Contact can come about through the manner in which wastewater is reused, for example application methods, and what the wastewater is used for, e.g., the type of crop irrigated.

Application methods are particularly important in cases of irrigation. The most common forms of agricultural irrigation are spray irrigation, drip and other localised forms of irrigation, and surface irrigation. The general consensus is that spray irrigation creates the greatest health

risk, and therefore requires the highest treatment standards. Shuval et al. (1989) studied the presence of enteric bacteria and viruses in aerosols created during spray irrigation using wastewater. They found that enteric bacteria and viruses could be detected in aerosols up to 730 m downwind from the irrigation sprinklers. In contrast, localised and drip irrigation methods are considered to be the least hazardous and often only require enough treatment to prevent clogging of the drip nozzles (Marcos de Monte et al. 1996, US EPA 1992).

Fattal et al. (1987) studied the incidence of viral infections of two groupings of kibbutzim, one group using spray irrigation of treated wastewater and the other group using treated wastewater for fish ponds. They found that there was no significant difference between the two groups except for a rise in echovirus type 4 infections (mostly in the 0-5 years age group) in the kibbutzim which used spray irrigation. This rise in infections, however, was attributed to a major national echovirus type 4 epidemic which occurred shortly before collection of blood samples for analysis. It was concluded that there was insufficient evidence of the transmission of viral diseases by spray irrigation. Likewise, a study of the effect of wastewater spray irrigation on rotavirus infections in an exposed population found that there was no significant increase in rotavirus infections due to the use of spray irrigation (Ward et al. 1989). This study did not, however, attempt to isolate rotavirus in the aerosols emitted from the irrigation, or the potential distance travelled by microorganisms in aerosol droplets, or for increases in the incidences of other viral or bacterial diseases.

Where wastewater is used for crop irrigation, crop type is an important consideration. Most guidelines use a scale of treatment required which depends on the crop type. Where there is little chance of human contact with the wastewater, e.g., plantation irrigation, industrial and seed crops etc, little or no treatment is considered to be necessary. However, where crops will be eaten raw, the wastewater needs to be heavily treated. This is particularly important with root vegetables and other crops such as cucumbers and melons which are in contact with the soil.

Application methods are important with crops. Spray irrigation tends to wet the surface of the plants, thus coating the surface of the plants with pathogens. El Hamouri et al. (1996) demonstrated that dense crops such as alfalfa increased the survival of pathogens on the surface of the plants due to the reduction of desiccation and sunlight effects. They also showed that the use of drip irrigation greatly reduced the

potential risk for crops which do not have contact with the soil (e.g., tomatoes and fruit trees).

The last risk factor relates to where the recycled wastewater is used and it's potential to come in contact with the public. For example, tree plantations irrigated with wastewater are not usually situated near towns or cities. Such uses, therefore, pose little or no threat to the general community, regardless of the application or treatment method. Conversely, the public has a much greater chance of coming in contact with recycled wastewater when it is used to irrigate golf courses, community parks and sporting ovals. In these cases, there is a much greater risk to the general public. In these cases, therefore, much higher levels of treatment and disinfection are required, along with the use of application methods which create low risks to the public.

4.2 Pathogen Dispersion and Movement In Surface And Subsurface Soils.

As some of the most popular treatment methods or reuses of wastewaters involve application onto/in surface and/or subsurface soils, a knowledge of the movement of different microbial pathogens in these environments is vital. There are several reviews available which cover the movement of microorganisms and viral particles in surface water, groundwater, soil and subsurface soils (Gerba & Bitton 1984, Lawrence & Hendry 1996, Pavelic et al. 1996, Yates & Yates 1988). Numerous other studies have been conducted on the movement of bacteria and viruses introduced into soil or an aquifer. The movement and survival of microorganisms in soil and the subsurface is a highly complex issue which depends on the pathogen type, soil type and conditions, water characteristics, temperature, light availability, the composition and viability of the indigenous microbial population, and the geographical conditions (e.g. Tropical, temperate, or desert). These are given in Table 7.

Soils and subsurface sediments have a large influence on the transport of microorganisms. One of the major influences of soils are as filters, which is dependant on pore sizes and grain size. Field studies on the movement of microorganisms through soil and in the subsurface have shown that many microorganisms can move rapidly through the soil matrix due to preferential flow caused by the presence of macropores (Sinton 1986, Abu-Ashour et al. 1994).

The other major influence is as an adsorbable material. The degree of adsorption is dependent on the soil composition (i.e. clay content, % of

iron hydroxides present etc), the presence of organic matter, cation concentration, and pH. Organic matter present in the soil matrix tends to compete with bacterial cells and viral particles for adsorption sites and thus increases the transport of microorganisms through the soil matrix (Johnson & Logan 1996, Powelson et al. 1991). The presence of organic matter was shown to increase the transport of the bacteriophage MS-2 under unsaturated-flow conditions (Powelson et al. 1991). This was attributed to competition of the organic matter an the virus particles for sorption sites.

Soil composition and pH influence the adsorptive ability of the soil matrix. Adsorption has a strong influence on the movement of bacteria and viruses in soils and the subsurface. Pathogenic viruses have been shown to reach adsorption equilibrium within 2 hours while bacteria have reached adsorption equilibrium within 24 hours (Matthess et al. 1988). Batch experiments have also demonstrated that fine-grained colloidal material was ten times more effective in adsorbing viruses than sand particles (Matthess et al. 1988). This is an important issue regarding the effect of sediment on microbe removal, particularly for wetlands used for wastewater treatment.

Sorption interactions between microorganisms and particulates can occur in two different processes. Colloidal clays, which have positively charged edges, may sorb to microbial surfaces, often even to the extent of forming an envelope around the entire surface of the microbe. This is purely an electrostatic interaction (Roper & Marshall 1979). Sorption of microorganisms to the surface of larger particles, however, only occurs under high electrolyte conditions. Under low electrolytic conditions both the microbe and the particle carry a negative charge and repulsion occurs preventing sorption (Roper & Marshall 1979).

Sorption is also dependent on the pH of the environment (increasing sorption at acidic or neutral pH and little adsorption at pH values above 8). The rate of adsorption of viruses to surfaces at different pH values is also dependant on the virus type. The concentration of cations also has a large influence on the adsorption of bacteria and viruses to surfaces, as does the clay content of the soil (Matthess et al. 1988). Adsorption of microbes to soil surfaces tends to be reversible and movement of microorganisms through soil and the subsurface has been observed to rapidly increase following rainfall events (Gerba & Bitton 1984).

The type of microorganisms also has an effect on it's movement

through soils. Cell size can have an influence on the ability of the soil to filter out the microorganisms, or retard one microbial type while allowing another to move rapidly through the medium. This often depends on the presence of macro pores and other fracture structures (Gerba & Bitton 1984). Harvey et al. (1997) compared the buoyant densities of bacteria and protozoa in order to predict their relative transport in groundwater. They were able to demonstrate that cell size had a significant influence on the buoyancy of the cells, thus influencing their sedimentation rate, and therefore, their rate of transport in the groundwater.

Many bacterial cells have the potential for motility which can allow the cells to negotiate through a porous medium. This effect of motility can often be due to a chemotactic response as the bacterial cells swim toward a nutrient source (Abu-Ashour et al. 1994). Viruses, on the other hand, being inactive outside of a host, merely move through soil and the subsurface by physical processes such as brownian motion, sedimentation and groundwater flow. Jansons et al. (1989a) and Powelson et al (1993) have both demonstrated, however, that viruses present in wastewater used for aquifer recharge travelled significant distances in the subsurface from the recharge basins.

Adsorption rates can also be influenced by microbial type. Some bacteria are known to possess pili and other appendages which are used to assist in attachment to surfaces. Different virus species have different isoelectric points depending on the composition of their capsids. These isoelectric points can change depending on the pH of the soil/groundwater, thus affecting their adsorption rate (Bales et al 1991). Yates et al. (1997) found that two bacteriophage, MS2 and ∅X-174, when passed through a column containing sand, had significantly different retention profiles. MS2 was not sorbed by the sand in the column while ∅X-174 was significantly retained. They concluded that this difference in sorption profiles was probably due to their different isoelectric points.

The ability of microbes to survive in an environment is also influenced by their capacity for motility. The longer introduced microorganisms are retarded in the soil or aquifer, the greater the chance that their inactivation and elimination will occur.

4.3 Survival of Pathogenic Microorganisms in Water and Soil Environments.

The persistence or survival of pathogenic microorganisms, and their resistance to treatment processes is an important wastewater issue. Survival can be related to the potential microbial types present, wastewater applications, health risk analysis etc. Pathogenic microorganisms remain a health risk as long as they persist in environments such as wastewater. The longer they survive in an environment the greater the potential they have of becoming mobilised if the chemical, physical or hydraulic conditions are suitable. Increased persistence and survival also increases the chance of their dispersion due to application procedures, for example spray irrigation. Therefore, the longer pathogens persist in wastewater, the chance that they could come into contact with workers and the general public increases.

Factors influencing the survival of viruses and bacteria in soil and groundwater are listed in Table 8. Such factors include the environment into which they are added, treatment type and type of microorganism. The activity of the indigenous microbial population, the rate of adsorption and the moisture content. Microorganisms have also been shown to have a wide range of survival times in soils and on crop surfaces depending on the environmental conditions (Feachem et al. 1983). The survival times for selected pathogenic microorganisms in soil and on crop surfaces are given in Table 3. The survival times quoted in Table 3 should be taken as “the usual case” only. Much longer survival times in soils and waters have been noted for some microorganisms. Thus, under optimal conditions, some pathogenic microorganisms could survive in soil or water for much longer periods of time than what is considered to be the norm.

Sobsey et al (1995) compared the survival rates of hepatitis A virus, poliovirus, echovirus and the bacteriophage MS-2 in laboratory columns packed with course sand, loamy sand, clay loam or organic muck. They found that, overall, the poliovirus had the largest reduction rate and echovirus the least. However, there was some variation in these results between soil types, with echovirus numbers being reduced more in organic muck than poliovirus. They also determined that increased organic matter and clay content in the soil, as well as increases in the organic content of the pore water, increased the reduction of viral numbers eluted out of the columns. Enriquez et al. (1995) compared the survival of adenovirus, poliovirus and hepatitis A virus in different water types. Adenovirus was found to be slightly more resistant than

poliovirus in wastewater and significantly greater than both hepatitis A virus and poliovirus in sea water and tap water.

E. coli cells suspended in sterile marine sediment have been shown to maintain their viability and ability to be cultured (i.e. not enter a viable-but-non-culturable state) for up to 70 days. This indicated that the sediment could provide a favourable, non-starvation environment for these bacteria (Davies et al. 1995).

Physical environmental conditions have also been shown to influence microbial survival. Light at the soil surface has a measurable bactericidal and viricidal effect. Meng and Gerba (1996) examined the amount of ultraviolet light irradiation required to inactivate 99% of poliovirus, two strains of adenoviruses, and the bacteriophages MS-2 and PRD-1. It was found that the adenovirus strains were the most resistant followed by the bacteriophage with poliovirus being the least resistant. The resistance of hepatitis A virus in seawater to UV irradiation was tested by Lévêque et al. (1995). They were able to demonstrate that infectious virus particles were no longer detectable after 15 minutes of irradiation.

Temperature has an affect on the survival of enteric viruses (Blanc & Nasser 1996). Negligible die-off of the viruses (less than a 1 $\log_{10}$ decrease) was observed at 10 ^{o}C over 20 days. Much greater reduction in viral numbers was observed at 23 ^{o}C (as high as a 5 $\log_{10}$ decrease) over the same time period. Poliovirus and the bacteriophage MS-2 were found to have a much greater reduction in numbers than hepatitis A virus and the bacteriophage PRD-1.

Increased oxygen concentrations was found to reduce the number of infective enteroviruses (echovirus strains, coxsackievirus, and poliovirus) in sterile groundwater (Jansons et al. 1989b). In this experiment, poliovirus was found to be the most stable to increasing oxygen concentrations and the most sensitive being one of the echovirus strains (echovirus type 6).

Other researchers have determined that the presence of a native population of microorganisms can have a profound negative influence on the survival of introduced microorganisms. Walter et al. (1995) attempted to isolate cytopathogenic viruses from the water column and sediments of two Austrian rivers. They found that 54% of the water column samples were positive for viruses but viruses could only be recovered from only 3% of the sediment samples. They surmised that

efficient virus inactivation may be occurring in rivers which are not heavily polluted and carry a high oxygen content, probably due to a healthy, active indigenous microbial population in the river sediments.

The rate of reduction in recoverable poliovirus numbers was observed by Kim and Unno (1996) to increase in a biological wastewater treatment system as the number of bacteria increased. The number of infectious virus particles removed from solution by the bacterial cells was observed to initially occur rapidly but then stabilize and not decrease any further. In contrast, it was observed in the same study that a mixed culture of bacteria and metazoa was able to reduce poliovirus numbers to zero. The authors determined that the first rapid reduction of viral numbers was due to adsorption of the virus particles to the bacterial cells, while the second reduction was due to the predation of the bacterial cells by the metazoa. Active antiviral activity has also been demonstrated to be directly due to some bacteria. The production of an compound with antiviral activity by Rhodopseudomonas capsulatawas shown by Hirotani et al. (1990) to inactivate over 80% of coliphages in a wastewater sample within 24 hrs.

While bacteria and other microorganisms have been demonstrated to remove viral particles from the free water, Quignon et al. (1997) found that viruses have a tendency to accumulate in biofilms. This adsorption to biofilms may increase the ability of virus particles to survive disinfection processes such as chlorination, to be released back into the free water at a later date.

Davies et al. (1995) demonstrated that the inhibition of protozoan predators in sediments collected from freshwater and marine sources increased the survival of faecal coliforms and faecal streptococci. They also found that Clostridium perfringens spores were unaffected by the presence of protozoan predators, indicating that the ability to form spores can greatly increase the survival ability of a microorganism. Bogosian et al (1996) demonstrated that the survival of *E. coli* strain added to water or soil was greatly dependent on the sterility of the soil or water. The seeded *E. coli* cells declined in number much quicker in the non-sterile soils than in the sterile soils. They were able to demonstrate that this decline was due to inactivation of the *E. coli* cells rather than through the induction of a viable-but-nonculturable state.

The addition of nutrients into sediments and aquifers has been shown to increase the metabolic activity of the indigenous bacterial

population (Capuano et al. 1995, Metge et al. 1993). Thus the addition of partially treated wastewater into soil and the subsurface will most likely increase the metabolic activity of the native population. This may well have an increased reductive effect on introduced microorganisms in these environments.

The active inactivation of introduced microorganisms by members of the indigenous microbial population is a poorly understood process. Some of the reason for this can be attributed to the difficulties associated with detection and enumeration of different microbial types. The inability to directly study the interaction between different microorganisms at a cellular level has also been an inhibiting element. However, much can also be blamed on the "Black Box" approach which has dominated in parts of the wastewater industry, where it has been considered that all can be solved through engineering plants and disinfection. These inhibiting factors should abate due to recent developments in detection methods, and a developing understanding that a knowledge of microbial interactions is important in the efficient and economical treatment of wastewaters.

Other influences on the survival of microorganisms in soils, sediments and the subsurface include adsorption rates and moisture contents. Generally increased ability to adhere to surfaces reduces the die-off rates in soils and groundwater for both bacteria and viruses (Gerba & Bitton 1984, Matthess 1989). For bacteria, adhesion to surfaces provides the advantage that the ability to obtain nutrients flowing past increases. For both bacteria and viruses, attachment allows integration into biofilms which decreases predation effects and other influences such as treatment processes and changes in the surrounding environment.

The sorption of bacterial cells to clay has been demonstrated to be advantageous to their survival. Roper and Marshall (1978) investigated the effect of microbial predators and parasites on E. coli cells adsorbed to montmorillonitic clay. They found that the interaction between the bacterial parasite Bdellovibrio and the E. coli cells was reduced by the presence of the montmorillonitic clay. They were also able to show that colloidal clay had little effect on the predation of the E. coli cells by microbial predators, but that predation was significantly reduced by crude clay. The inference of this study was that clays protect microbial cells by creating a barrier between them and microbial predators and parasites. This would have implications regarding the influence of predation/parasitism on bacterial pathogens in sediments. Protection by clays could increase the survival of microbial in

sediments and thus, be a health hazard should they be released again due events such as heavy rain.

Bacteria can be quite susceptible to moisture content of soils and the subsurface. Increased virus reduction has also been observed as the surrounding moisture decreases (Gerba & Bitton 1984).

Reduction efficiencies of various treatment processes and methods need to be viewed in perspective when considering the risks of reusing wastewater which has been treated to varying degrees. While it has been shown that a 99.9% reduction in viable pathogenic viruses and bacteria can occur in less than 20 days in soil (Abu-Ashour et al. 1994), it needs to be considered, that with high loadings of microbial pathogens onto soils or sediments, even a 99.9% death/inactivation rate means that some bacterial cells and/or viral particles will survive, thus creating a potential health risk.

The multitude of conditions described above influencing the movement and survival of introduced microorganisms in soil and groundwater point to the fact that each site is potentially different. There are a number of mathematical equations which have been used in an attempt to predict microbial movement and survival in soil and the subsurface (see Pavelic et al. (1996) for details on some of these equations). Most of the equations, however, were derived from information obtained from laboratory experiments using a limited number of soils and conditions. As described above, There are a wide range of conditions potentially influencing microbial movement and survival. Thus, the degree of movement and survival of microbial pathogens will always be highly site specific. This means that an assessment of the soil or groundwater conditions and their effect on introduced microbial pathogens will need to be done each time a wastewater treatment/disposal or reuse site is designed.

4.4 Treatment of Wastewater

Wastewater is almost always undergoes some level of treatment prior to reuse. Treatment is undertaken principally to remove microbial pathogens and nutrients from the wastewater. Other considerations for treatment can be the removal of toxic organic pollutants and heavy metals, altering the physical conditions of the water (e.g. pH, electrical conductivity etc), removing sediment loads, or the biochemical oxygen demand (BOD). There are several common treatment methods and some more novel methods which have been studied for their efficacy in treating wastewater.

The most sophisticated wastewater treatment method is the use of a modern tertiary treatment plant. Such plants usually employ the use of sedimentation, usually through the addition of chemicals, followed by passage through an activated sludge plant, a filtration process, and finally disinfection. These treatment plants are considered to be very efficient in the rapid removal of contaminating microorganisms and chemicals from large volumes of wastewater.

Tertiary treatment plants, however, have been found not to be absolute in their ability to treat wastewater. Rose et al. (1996) examined the efficiency of a tertiary treatment plant to remove indicator and pathogenic microorganisms. They found that the treatment plant was able to reduce total and faecal coliforms by > 7 $\log_{10}$, coliphage and enteroviruses by >5 $\log_{10}$, and protozoan by > 3 $\log_{10}$. However, they were still able to detect viruses and protozoan cysts in the final effluent post-treatment. Thus, the final effluent from this plant did not meet the US EPA guidelines for land application. A similar study by Aulicino et al. (1995) on the efficiency of a wastewater treatment plant to remove enteric viruses from wastewater found that up to 10^3 viruses/L could be detected in the final effluent. They were also able to determine that the treatment plant was less efficient at removing reoviruses than enteroviruses. The treatment plants in both studies had a very similar mode of operation.

Wastewater treatment plants are also expensive to establish and run, as well as requiring substantial amounts of energy and resources for operation. Thus, these plants are usually uneconomical for use by small communities which produce relatively small volumes of wastewater, and cannot be afforded by many developing countries. The high establishment and running costs would make many wastewater reuse ventures uneconomical if tertiary waste treatment plants were used as part of the recycling process.

Another of the common methods for treating wastewater is the use of waste stabilisation ponds. Waste stabilisation ponds clean wastewater by combining sedimentation, aeration, biodegradation and photosynthesis. These ponds are inexpensive to establish and run, and are ideal where large areas of land are available or in developing countries which cannot afford more expensive treatment processes. Waste stabilisation ponds operate through the use of several interconnected ponds, usually one or two small anaerobic ponds, followed by one or two facultative ponds, and then several maturation ponds.

Waste stabilisation ponds have been demonstrated to effectively reduce BOD, faecal coliform numbers (Jagals & Lues 1996, Rångeby et al. 1996), coliphage and helminth egg numbers (Jagals & Lues 1996), and Giardia cyst numbers (Grimason et .al 1996). Reductions in microbial pathogen numbers as high as 3 $\log_{10}$ units for faecal coliforms, almost 3 $\log_{10}$ units (100%) for coliphage, and 2 $\log_{10}$ units (100%) for helminth eggs have been demonstrated for waste stabilisation ponds (Jagals and Lues 1996). These systems have been shown to effectively treat wastewater to a level which makes the wastewater suitable for use for restricted irrigation (Juanico 1996, Shereif et al 1996) and recreational purposes (Jagals & Lues 1996). Examples of situations in which wastewater that had achieved these treatment levels could be used are given in Table 9.

Grimason et al. (1996), however, found that the complete removal of Giardia cysts by waste stabilisation ponds used in several countries was not achieved despite up to a 40 day retention time. This represents a serious problem as protozoan pathogens are a high risk group due to their low dose requirements for establishment of infection.

There is a wide range of waste stabilisation pond designs. The engineering and operation of the ponds has a large influence on the treatment efficiency of the different systems. Bahlaoui et al. (1997) compared conventional waste stabilisation ponds to high-rate oxidation ponds (HROP) for the removal of faecal coliforms and the opportunistic pathogens Pseudomonas aeruginosa and Aeromonas spp. The results obtained showed that the HROP ponds were the most efficient of the treatment ponds studied. The HROP ponds also have the advantage that they require a much smaller surface area than the conventional waste stabilisation ponds as they are able to operate with a much larger carbon oxygen demand (COD).

As stated above, environmental conditions can have a major influence on the survival and persistence of pathogenic microorganisms in the environment. The treatment efficiency of waste stabilisation ponds, therefore, can be affected by changes in the surrounding environment. El Hamouri et al. (1995) found that faecal coliform and enterococci reduction rates in a high-rate algal pond were significantly greater in the hotter seasons than in the colder seasons. The pond's ability to remove helminth eggs, however, was not significantly affected by reductions in temperature.

Another popular method for the disposal and/or treatment of wastewater is recharging the wastewater into groundwater, either by infiltration or direct injection (Bouwer 1996, Dillon & Pavelic 1995). The process uses the surface soil and/or vadose zone (the area of subsurface soil above the groundwater level) to remove nutrients and microbial pathogens through physical, chemical and biological processes. This treatment process is often termed soil aquifer treatment (SAT). SAT has been used to clean up wastewater prior to recovery and reuse in applications such as irrigation (Kanarek & Michail 1996, Oron 1996). The costs for operating a soil aquifer treatment system has been estimated to cost less than 40% the operational costs of equivalent in-plant treatment systems (Bouwer 1991). The efficiency of SAT methods often depend on the original condition of the wastewater and the level of pre-treatment required prior to application to the soil surface or injection into the subsurface.

Kopehynski et al. (1996) demonstrated that, using soil columns containing different soil types, the removal of nitrogen compounds and TOC varied depending on soil type, infiltration rates and dissolved oxygen levels. Infiltration rates and DO levels were often controlled by soil type. Wilsonet al. (1996) examined the ability of SAT to remove microbial pathogens, total organic halide (TOX) and dissolved organic carbon (DOC). Their findings indicated that Giardia was rapidly removed due to filtration, enteroviruses were completely removed from the wastewater during the 37 m travel to the groundwater, DOC was decreased by 92%, TOX by 85% and total nitrogen by 47%.

Another, land based method used for the treatment of wastewater is the use of artificial or constructed wetlands. In constructed wetlands, pollutants, both chemical and biological are removed by a complex variety of physical, chemical and biological processes. Wetlands are constructed to involve the separate and combined actions of sediments, water movement, macrophytic plants, phytoplankton and microorganisms. The efficiency of chemical and microbial pathogen removal also relies on the contact time of the wastewater with the sediments, and on the surface area of the wetlands. There are a number of wetland designs (Brix 1993), the efficiency of which are dependent on parameters such as wastewater effluent type, rainfall, land availability, construction and operation parameters, as well as seasonal and climatic conditions (Girts & Knight 1989).

Wetlands, predominantly artificial wetlands, have been used to treat wastewater from livestock operations, petroleum refineries, textile and paper mills, acid mine drainage, aquaculture effluent, urban

stormwater runoff, food processing factories, landfill leachate, and domestic wastewater systems (Bastian & Hammer 1993, Brodie 1993, Ferlow 1993, Hammer et al. 1993, Hunter et al. 1993, Litchfield 1993, Martin et al. 1993, 1993, Sansanayuth et al. 1996, Steiner & Combs 1993, Thut 1993). Studies have shown that wetlands are capable of significantly reducing the number of faecal coliforms, faecal streptococci, (Karpiscak et al. 1996, Vrhovsek et al. 1996), Giardia cysts, Cryptosporidium oocysts, viruses (Karpiscak et al. 1996), and helminth eggs (Mandi et al. 1996). Removals of up to 100 % of faecal coliforms, 96% of Salmonella spp., 99% of bacteriophage, and 100% of enteroviruses by constructed wetlands have been observed (Gersberg et al. 1989, Scheuerman et al. 1989).

Other wastewater treatment methods which have either been used or studied include infiltration-percolation disinfection of secondary effluent (Salgot et al. 1996), soil filtration (Jayawardane 1995), microfiltration (Joliset al. 1996), the use of soil as a biofilter using subsurface drip irrigation systems (Oron 1996), submerged flow biofilters for the treatment of aquaculture wastewater (Abeysinghe 1996), and lime or coagulation-flocculation using ferric chloride and polymers addition (Grambrill et al. 1989, Nacheva et al. 1996). Nieuwstad et al. (1988) also demonstrated that the combination of flocculation and filtration was very effective in removing pathogens from wastewater.

Many countries, for example the USA, require the disinfection of the wastewater even after treatment using one of the processes mentioned above (US EPA 1992). The most common disinfection method used is chlorination. Chlorine, however, is well known to react with organic compounds in the water to form reactive chlorinated organic compounds which are considered a health hazard. Chlorine also requires a relatively long contact time and has poor virucidal activity (US EPA 1992).

Chloramination is another widely used, chlorine-based disinfection method. Both chlorine and chloramine act as an oxidising agent. However, the oxidation potential of chlorine is much greater than chloramine and is thus, is a much more efficient disinfectant. For example the contact time required to inactivate 99% of E. coli cells using chlorine (HOCl) is 0.02 minutes compared to 50 minutes for chloramine (NH_2Cl). Chlorine has been shown to be much more effective at inactivating poliovirus and the protozoan E. histolytica ($c.t_{99}$ 1.0 and 20 minutes respectively) than chloramine ($c.t_{99}$ 500 and 150 minutes respectively (Hamilton 1996). Chloramination has the

advantage, however, that it produces significantly less toxic substances than free chlorine (Lykins et al. 1992). The toxic substances are produced from the interaction of free chlorine with nitrogen containing organic compounds. This makes chloramination popular to many authorities responsible for wastewater treatment, especially where the wastewater is high in organic compounds

Other non-chlorine disinfection methods have been studied for replacement of chlorination. The two most common disinfection methods examined for their efficacy to replace chlorination are the use of ozone (ozonation) and ionizing radiation using ultraviolet (UV) light. Both UV light treatment and ozonation have very good bactericidal and virucidal activities. UV light requires only a short contact time to inactivate microorganisms in wastewater, while ozonation needs a moderate contact time. Both require much shorter contact times than chlorination. Both of these methods, however, can only be used on secondary treated wastewater whereas chlorination can be used on raw wastewater, partially or fully treated wastewater. Ozone treatment is most suitable for medium to large scale treatment process while UV treatment is best suited for small to medium scale treatment processes. Chlorination can be used for any sized treatment process (US EPA 1992). Chlorination also has a long residual time which can prevent regrowth of microorganisms, particularly in treated wastewaters. Neither UV light or ozonation maintain a residual in the water following treatment. Thus there is a chance that microbial regrowth could occur following treatment with either of these methods.

Oppenheimer et al. (1997) compared the efficacy of chlorine and UV light for the disinfection of wastewater. They found that UV light could produce a disinfection level in the wastewater (i.e., inactivation of bacteria, bacteriophage and poliovirus) which was as efficient as chlorination, but did not have the problems of toxic byproduct formation which occurred with chlorination. The disinfection efficiency of UV light is affected by the amount of turbidity, colour dissolved organic and inorganics in the wastewater with increases in any of these factors decreasing the disinfection efficiency (Wolfe 1990).

Ozonation is a very efficient method for disinfecting wastewaters and does not have the problems with suspended solids, colour etc. that effect the efficiency of UV light. Despite these advantages, however, ozonation is an expensive and energy intensive process which is more

complex to operate and maintain than the other systems available (USEPA 1992).

4.5 Regulations and Guidelines for the Microbiological Quality of Recycled Wastewater

Concerns for the health of workers and the general public due to contact with recycled wastewater has led a number of countries to establish guidelines and/or criteria for wastewater reuse.

The World Health Organization established a set of guidelines in 1989 and the recommended microbiological quality guidelines are outlined in Table 5. Many of the countries which have established wastewater reuse guidelines have used the WHO recommendations as a blueprint for their own criteria or have established much stricter guidelines. Examples for the microbiological guidelines and criteria established by several countries are given in Table 9.

The World Health Organization (WHO) (1989) has placed a large emphasis on the presence of helminth eggs in wastewater and has deemed that these pathogens are the greatest microbiological health risk associated with wastewater reuse. As a result, the WHO guidelines have a recommended limit of less than 1 helminth egg per litre of wastewater for the irrigation of crops which are likely to be eaten uncooked and for sports fields and public parks. Further study by Ayers et al. (1992) suggested that the WHO guidelines could be relaxed for restricted irrigation. They did add, however, that the health risk for works from ascaris and trichuriasis from soil acquisition still needed to be established. Blumenthal et al. (1996), however, found that while the WHO guidelines protected food consumers from infection, farm workers and their families were still at risk, particularly from helminth infections. They suggested that the detection limits in the WHO guidelines for helminths be increased to 0.5 nematode eggs/L of wastewater to ensure the safety of these people.

The WHO guidelines considered the health risks associated with wastewater reuse in developing countries as the basis for their guidelines. Many developed countries which have established guidelines have criteria which more reflect their own situation.

Of the countries cited in Table 9, only Tunisia specifies a detection limit for nematode eggs as part of its criteria. The remaining countries, all of which can be considered to be developed nations, use some level of faecal coliform numbers as the standard for wastewater

reuse in different situations. It can be considered that treatment regimes required to achieve these levels of coliform numbers will remove the virus and helminth risk from the wastewater (USEPA 1992).

All of the cited guidelines require some form of treatment of the wastewater prior to reuse except for Israel which permits the use of wastewater with no mandatory treatment levels (with no corresponding microbiological limits) for industrial and fodder crops, and fruit trees. All of the other countries require at least primary and secondary treatment before the wastewater can be used for any irrigation purposes. It should be noted that the US EPA guidelines are recommendations only and that legislation regarding wastewater reuse is left to the individual US states, thus there is some variation in criteria from state to state (US EPA 1992).

Marecos do Monteet al. (1996) stated that it is necessary for the European nations in the mediterranean region to also establish guidelines for wastewater reuse The recommendations from this study determined that microbiological hazards were the greatest risk in the reuse of wastewater and that application restrictions and faecal coliform and helminth egg number limits should be applied. Microbiological limits and application restrictions would also be dependant on the treatment method, with greater restrictions applying to irrigation with primary and secondary treated effluents than to wastewater which been treated in a facultative pond. The method of application would also be dependent on the microbiological limit used, (i.e., surface and spray irrigation would be allowed for edible crops, sports fields, playgrounds and parks only if the wastewater had a limit of less than 10^3 faecal coliforms/100 mL and less than 1 helminth egg/L)

5 Conclusions

A number of general conclusions can be made from this review of the literature.

- Adverse effects of pathogenic microorganisms are the major risk associated with the recycling of wastewaters.

- There is a wide range of microbial pathogen types which can occur in wastewater, with the type and number present being highly dependent on the socioeconomic conditions and customs of the communities creating the wastewater.

- Risk assessment is still an area which requires intensive research. There are many factors which can influence the risk factors associated with a proposed wastewater reclamation project, many of which are, at present, difficult to accurately determine.

- The World Health Organization's guidelines for wastewater reuse for agricultural purposes appears to be directed more towards developing nations and thus, may not be as applicable to more developed nations which have higher standards of living and greater sanitation regimes.

- Methods for the detection of pathogenic and indicator microorganisms are improving, but further research and ratification of new methods is still required.

- There is a range of options available for the treatment and use/application of wastewater. Many factors can influence choices of treatment processes including the type of microbial pathogens present, their resistance to treatment and environmental processes, the intended use for the recycled wastewater, and the potential for contact with workers and the general public.

One of the major gaps in the knowledge of pathogenic microorganisms in wastewater is a thorough understanding of the survival and persistence of the different microbial types (i.e., viruses, bacteria, protozoa, and helminths) in different conditions and environments (e.g., water, soils, and groundwater). Almost as important is a detailed knowledge of their resistance to various forms of treatment. Integral to these requirements is the need for a detailed knowledge on the

movement and behaviour of these microorganisms in wastewaters, soils, and in the subsurface (both subsurface soils and in groundwater).

Developed nations such as Australia have potentially different concerns regarding microbial pathogens in wastewater than those covered by the WHO guidelines. The Australian population, for example, as a whole, has little contact with debilitating diseases such as poliomyelitis and hepatitis A. Thus, no community wide immunity has developed, despite the availability of immunisation against many of these diseases. There is much less contact with helminths in Australia, thus reducing their incidence in wastewaters, and therefore reducing their potential risk to the general community.

The presence of these viral, some bacterial, and protozoan pathogens in wastewater could put the Australian public at risk if they are not effectively removed from the wastewater prior to reuse. However, extensive risk analyses need not be done for all microbial pathogens. Several of the viral pathogens, for example coxsackie virus and echovirus are common in the Australian community, in particular in the larger cities. The tracing of these pathogens in wastewater and attempts to develop an epidemiological risk analysis based on their incidence in wastewater would be extremely difficult, expensive, and would probably provide little valuable information.

As indicated in the review, viruses can be present in large numbers in faecally contaminated wastewater (see Table 6 for details). It has been established that viruses are more resistant to treatment and environmental conditions than the other types of microbial pathogens. Having a low dose requirement to cause infection, they pose a large potential risk. However, there is still a great deal of debate regarding the actual risk posed by viruses. Some of the uncertainty can be linked to the inability to effectively detect and enumerate the viruses in the environment.

Much more research needs to be focused on assessing virus survival and to some degree movement in soils and the subsurface. Questions which need to be answered include: what effect do the environmental conditions at a site have on microbial pathogen survival? How can combinations of these factors be used to improve removal of viruses?; does the non-detection of microbial pathogens such as viruses mean that they have be inactivated or just removed from the system?; how do indigenous microorganisms inactivate introduced microorganisms?

The recycling of wastewater should also be monitored closely for the emergence of unforseen incidences of disease. Much of the concern with wastewater reuse has focused in the common viral, bacterial, protozoan, and helminth pathogens. However, there is a range of other, less common pathogenic microorganisms which can be present in wastewaters. The presence of these other microbial pathogens could create situations of increased infection rates not normally observed in a healthy population. The epidemiological evidence which provided a suggested link between an increase in Mycobacterium ulcerans infections and the use of wastewater for irrigation of a golf course (Johnson et al. 1996) highlights this risk. Other causes of potential increases in uncommon infections include Legionella and Nagleria fowleri and the opportunistic pathogens such as Aeromonas and Pseudomonas species.

Much of the lack of knowledge has resulted from the inability to adequately detect and trace the microorganisms, in particular viruses. The rapid development of new, efficient detection methods, particularly PCR, should lead to more efficient study of the processes and microbial interactions affecting pathogenic microbe survival in the environment.

Technology based on PCR is developing rapidly in a number of biologically based industries. Improvements are occurring in quantitation, primer design for individual organisms, and detection of results. PCR is also a method which lends itself to potential automation. There are several instruments currently on the market which enable the semi- or complete automation of PCR for specific applications. As this technology further improves, it is likely that PCR could become a valuable tool for the water industry, delivering an accurate, relatively cheap means for the detection and enumeration of microbial pathogens in water.

Another major gap in understanding the issues relating to wastewater recycling is an effective risk management strategy. However, such a program or tool requires quantitative knowledge of all of the factors mentioned above, along with information on pathogen infection rates and health consequences.

The recycling of wastewater presents an effective means of managing the world's precious fresh water supplies, for the survival and well being of both the human population and the natural environment. Much still needs to be learnt, however, particularly with regard to pathogenic microorganisms in wastewaters and the risk they pose.

Greater knowledge will lead to the ability to control and manipulate the processes which can efficiently remove these risks.

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EARLY WATER AND SANITATION SYSTEMS: 300 BC to 1850

Minoan Civilisation

- 97 AD - - Water supply commisioner for city of Rome - Sextus Julius Frontius
- Sewage farms in Germany
- Sewage farms in UK
- Legal use of sewers for human waste disposal: London (1815), Boston (1833), Paris (1 860)
- Cholera epidemic in London (aslso 1848-49 & 1854)
- Sanitary status of Great Britain labor force: Chadwick report "The rain to the river and the sewage to the soil"

3000 BC 1550 1600 1650 1700 1750 1800 1850

GREAT SANITARY AWAKENING: 1850 to 1950

- Cholera epidemic linked to water pollution control by Snow (London)
- Typhoid fever prevention theory developed by Budd (UK)
- Anthrax connection to bacterial etiology demonstrated by Koch (Germany)
- Microbial pollution of water demonstrated by Pasteur (France)
- Sodium hypochlorite disinfection in UK by Down to render the water "pure and wholesome"
- Chlorination of Jersey City, NJ water supply (USA)
- Disinfection kinetics elucidated by Chick (USA)
- Activated sludge process demonstrated by Ardern and Lockett in UK
- First regulations for use of sewage for irrigation purposes in California

1850 1870 1890 1910 1930 1950

ERA OF WASTEWATER RECLAMATION, RECYCLING AND REUSE: POST 1960

- California legislation encourages wastewater reclamation, recycling and reuse
- Use of secondary effluent for crop irrigation in Israel
- Research on direct potable reuses in Windhoek, Namibia
- US Clean Water Act to restore and maintain water quality
- Pomona Virus Study, Pomona, California
- California Wastewater Reclamation Criteria (Title 22)
- Health Effect study by LA County Sanitation Districts, California
- Monterey Wastewater Reclamation Study for Agriculture, California
- WHO Guidelines for Agricultural and aquaculture reuse
- Total ResourcesRecovery Health Effects Study; City of San Diego, California
- US-EPA Guidelines for Water Reuse
- Potable water reuse demonstration plant; Denver, Colorado: Final report (plant began operation in 1984)

1960 1965 1970 1975 1980 1985 1990 1995 2000

FIGURE 1. Milestone events in the evolution of wastewater reclamation, recycling and reuse SourceAsano & Levine (1996)

Pollution worry in bore zone

REPORTS BY GERALDINE CAPP

WATER contaminated with faecal bacteria and nutrients may be polluting a ground water body which supplies Perth's drinking water.

But authorities have failed to remove the foul-smelling water, which has filled a long ditch on a public road at Pinjar on the Gnangara water mound north of Perth.

A piggery has two big effluent ponds next to the ditch but the owners have denied the water is from their land. They claim horses and cattle may be the source of faecal matter.

Pinjar is in a ground water protection zone and the WA Water Corporation has signs in the area urging people to report pollution.

On September 26, *The West Australian* examined the site and made a formal pollution report to the corporation.

Two weeks later, the water had not been removed and *The West Australian* took water samples for analysis. This has revealed high levels of ammonia, nitrogen and phosphorus.

The water also has extremely high levels of faecal coliforms and faecal streptococci.

Coliforms are from the bowels of warm-blooded mammals and may indicate the presence of other dangerous bacteria such as E. coli, which can cause severe infections.

The government departments and the Wanneroo City Council have been sent the analysis results.

Corporation managing director Jim Gill said the water in the ditch was not polluting water bores.

"The fact that the drain has some bacteria in it probably means that the bacteria, after passing through the sand, will rapidly die off and cause no pollution problem," he said.

Piggery director Richard John Hill, 52, of Ocean Reef, said his business was a possible source of ground water contamination.

But there was no proof the water in the ditch was from his land. Other landowners let cattle and horses graze on the road.

Mr Hill said he started talks with the State Government three years ago to move off the mound because planning laws stopped him expanding.

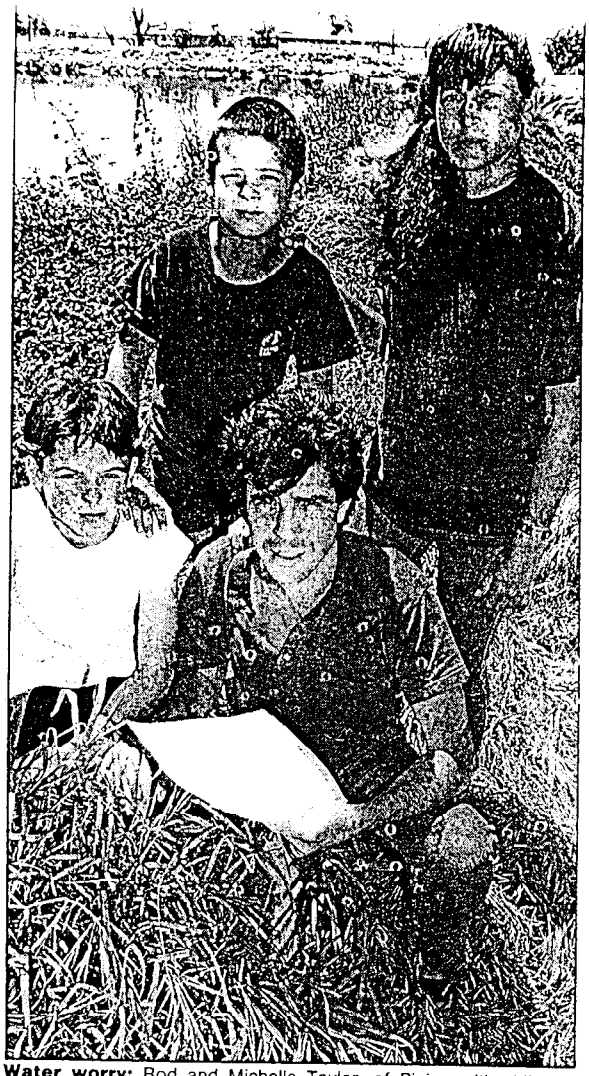
"It is very frustrating for us because the Government has not offered a fair price and talks have stalled," he said.

The business is licensed by the Department of Environmental Protection as a potential source of water pollution.

Its licence expired last month. The piggery is operating under its old conditions until the department issues a new licence.

DEP pollution prevention licensing manager Fred Tromp said ground water monitoring would be required as part of the new licence.

The DEP, with the Water and Rivers Commission, was concerned about the long-term suitability of the piggery's location and "would be addressing this as a matter of priority".



Water worry: Rod and Michelle Taylor, of Pinjar, with children Michael, 11, and Jevan, 9, inspect the ditch. PICTURE: DIONE DAVIDSON

...dete... fire b... actio...

KALAMUNDA r... with the Activ Fo... ting new fire bre... of bushland in Hi...

Last week, the ... cleared understore... sia woodland wh... wants to clear t... homes for people...

The Environm... Authority is cons... bush for protecti... System Six report... land.

Activ Founda... munda Shire C... Department of Er... lection have talk... save the bush but... the conflict.

Activ Foundati... ray Blanchard s... fire breaks compl... Fires Act.

The fire breaks ... foundation's Hav... lage where peopl... worked and lived...

"Two years ago ... and we nearly los... said. "There has ... some time from ... there could be a ... and we have don... it."

DEP conservat... Whisson said the ... legal but the DEI... ferred no clearing...

Tony Fowler, ... Reserves Preserv... the fire breaks w...

"Presumably u... tidying up fire br... been cut through ... land with every ... tree churned ... smoothed over."

...rice stalls piggery move

GERALDINE CAPP

...land sale negotiations ...een the WA Planning ...mmission and Lake Pinjar land ... had made protection of the ...ara water mound more ...ficult, the Department of ...vironmental Protection said ...ay.

...irector of pollution prevention ...drew Baker said potential ...on sources could be removed ... if the commission and land ...ners could agree on land sales.

... he could not push the ...ssion to act faster.

"To some extent it is making my job more difficult because we would like to see relocation much sooner than later," he said.

"But I am not going to be drawn into criticism of the Planning Commission. We cannot push them to act faster than the system allows them to move."

Mr Baker hoped the commission would arrange a fair deal with a piggery owner so he could move his business off the mound within a year.

Water contaminated with faecal matter and nutrients has filled a ditch near the piggery but Mr Baker said there was no evidence that the water was from the piggery or that it was contaminating ground water.

But no ground water samples had been taken to prove there was no pollution.

University of WA microbiology senior lecturer Brian Mee said whether faecal bacteria in the ditch had reached ground water depended on bacterial concentrations, soil type and temperature, and depth to ground water.

Piggery owner Richard John Hill, of Ocean Reef, said he started negotiations with the commission to move off the mound three years ago. But he had not been offered a fair price.

Lake Pinjar is 19 sq. km of privately owned rural land which the commission has been trying to buy since the State Government imposed a planning control in 1994.

The control prevents activities with the potential to pollute ground water such as piggeries, chicken farms, dog kennels, market gardens and turf farms.

In 1992, the former Water Authority identified Lake Pinjar as a priority one zone for water protection, which also made most of the land uses incompatible with preserving ground water.

But the zone has no statutory status and the authority did not object to many new developments on the mound.

The Pinjar Landowners Group, which represents about 60 landholders, said yesterday it was unhappy with many of the commission's offers.

Group members Rod Taylor and Jenny Hawson claimed the commission had been secretive and misleading by withholding valuations of properties.

About 20 landowners had accepted offers but some were well below market prices, they said.

Mrs Hawson's Freedom of Information request to get the commission's valuation of her 60ha block was refused.

A commission spokesman said it was not policy to release valuations but it would pay for the land owners to have an independent valuation.

Olympian for ...trial over

HURRY

Figure 2. Examples of public concern relating to wastewater. Source: *The Western Australian* October 29 1996 and October 30 1996.

Table 1. Examples of quantities of wastewater reused.

Country	Quanity of wastewater reused
Brazil (San Paulo)[a]	80 m^3/day (1989)[b]
Chile [a]	70-80% (1992)
Isreal [a]	70% of wastewater produced, 10% of Isreal's water supply (1987)
Japan [a]	270 x 10^3 m^3/day (1986)
Kuwait [a]	100% (1992)
Mexico (Mexico City)[a]	50000 L/s (90%) (1992)
Saudi Arabia [a]	1100 L/s (1978)
Tunisia [a]	2850 L/s (1992)

[a] Source: USEPA 1992.

[b] Dates in parenthesis are quates dates from which data was obtained.

Table 2. Examples of microbial pathogens detected in untreated wastewaters.

Microbial type	Major disease(s)[¶]	Concentration in wastewaters	Infectious dose[†]
Viruses			
Enteroviruses			
Poliovirus	Poliomylitis		
Enterovirus	Gastroenteritis, heart anomalies, meningitis		
Echovirus			
Coxsackievirus			
Hepatitis A virus[§]	Hepatitis		
Adenovirus	Respiratory disease, conjunctivitis	Medium to High	Low
Reovirus	Not clearly established		
Calicivirus			
Norwalk agent	Gastroenteritis, diarrhoea, vomiting, fever		
SSRV			
Rotavirus	Gastroenteritis		
Astrovirus	Gastroenteritis		
Bacteria			
Vibrio cholerae	Cholera		High
Salmonella typhi	Typhoid, Salmonellosis		High
Enteropathogenic E. coli	Gastroenteritis	Medium to High	High
Campylobacter jejunei	Gastroenteritis		High
Shigella dysinterae	Dysentery		Low
Yersinia enterocolitica	Yersiniosis		High
Protozoa			
Giardia intestinalis	Giardiasis	Low to Medium	Low
Cryptosporidium parvum	Diarrhea, fever		Low
Entamoeba histolytica	Amoebic dysentery		Low
Helminths			
Ascaris lumbricoides (Round worm)	Ascariasis	Low	Low
Ancylostoma spp. (Hook worm)			Low
Trichuris trichiura (Whip worm)	Trichuriasis		Low
Strongiloides stercoralis	Strongyloidasis		Low

[¶] A number of the pathogens listed are capable of causing other infections in some situations

[†] Low indicates only a few viral particles/cells/cysts/eggs required to cause infection. High indicates many required to cause an infection.

[§] The positioning of the Hepatitis A virus in the enterovirus group is still to be confirmed.

Table 3. Survival times of selected excreted pathogens in soil and on crop surfaces at 20 - 30 ^{o}C

	Survival time	
	In soil	On crops
Viruses		
Enteroviruses	< 100 but usually < 20 days	<60 but usually < 15 days
Bacteria		
Faecal coliforms	<70 but usually < 20 days	<30 but usually < 15 days
Salmonella spp.	<70 but usually < 20 days	<30 but usually < 15 days
Vibrio cholerae	<70 but usually < 20 days	<5 but usually < 2 days
Protozoa		
Entamoeba histolytica cysts	<20 but usually < 10 days	<10 but usually < 2 days
Helminths		
Ascaris lumbricoides eggs	Many months	<60 but usually < 30 days
Hookworm larvae	<90 but usually < 30 days	<30 but usually < 10 days
Taenia saginata eggs	Many months	<60 but usually < 30 days
Trichuris trichiura eggs	Many months	<60 but usually < 30 days

Source: Feachem et al. (1983)

Table 4. Percentage of human and animal faecal samples positive for somatic coliphages, male-specific bacteriophage, and Bacteroides fragilis bacteriophage

Faeces Sources	Percent positive faecal specimens		
	Somatic	Male-specific	B. fragilis
Human	54	26	13
Higher Primates	53	63	0
Primates	57	76	0
Domestic Animals	70	60	0
Birds	48	36	0

Source: Adapted from Grabow et al. (1995)

Table 5. Recommended microbiological quality guidelines for wastewater use in agriculture[a]

Category	Reuse conditions	Exposed group	Intestinal nematodes (arithmetic mean no. of eggs per litre[c])	Faecal coliform (geometric mean no. per 100 ml[c])	Wastewater treatment expected to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks[d]	Workers, consumers, public	≤ 1	≤ 1000[d]	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees[e]	Workers	≤ 1	No standard recommended	Retention in stabilization ponds for 8 -1 0 days or equivalent helminth and faecal coliform removal
C	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

[a] In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account, and the guidelines modified accordingly.

[b] Ascaris and Trichuris species and hookworms.

[c] During the irrigation period.

[d] A more stringent guideline (≤ 200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public m come in direct contact.

[e] In the case of fruit trees, irrigation should be two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irriga should not be used.

Source: WHO (1989).

Table 6. Possible out put of selected pathogens in the faeces and sewage of a tropical community of 50,0(in a developing country.

Pathogen	Average number of organisms/g of faeces [a]	Total number excreted daily/infected person	Concentration/L in town sewage [a]
Viruses			
Enteroviruses	10^6	10^8	5000
Bacteria			
Pathogenic E. coli	10^8	10^{10}	?
Salmonella spp.	10^6	10^8	7000
Shigella spp.	10^6	10^8	7000
Vibrio cholerae	10^6	10^8	1000
Protozoa			
Entamoeba histolytica	15×10^4	15×10^6	4500
Helminths			
Ascaris lumbricoides	10^4 [b]	10^6	600
Hookworms [c]	800 [b]	8×10^4	32
Schistosoma mansoni	40 [b]	4×10^3	1
Taenia saginata	10^4 [b]	10^6	10
Trichuris trichiura	2×10^3 [b]	2×10^5	120

Note: this table is hypothetical, and the data are not taken from any actual, single town. Fore each pathogen, however, the figures are reasonable an congruous with those found in the literature. The concentrations derived for each pathogen in sewage are in line with higher figures in the literature, but is unlikely that all these infections at such relatively high prevalence would occur in any one community.

? = uncertain

[a] It must be recognised that the pathogens listed have different abilities to survive outside of the host and that the concentrations of some rapidly decline after the faeces have been passed. The concentration of pathogens/L in the sewage of the town were calculated by assuming 100 L of sewage are produced daily/capita and that 90 % of the pathogens do not enter the sewers or are inactivated in the first few minu after the excretion.

[b] The distribution of egg output from people infected with these helminths is extremely skewed; a few people excrete very high egg concentrations.

[c] Anclylostoma duodenale and Necator americanus

Source: Adapted from Feachem et al. (1983)

Table 7. Movement of viral particles and bacteria in soil and groundwater

Factor	Virus	Bacteria
Soil type	Pore size has an influence. Iron oxides increase the adsorptive capacity of soils. Muck soils are generally poor adsorbents. The presence of clays can retard movement	Pore size is important for filtration of bacterial cells. Clay particles retard movement
pH	Adsorption increases as pH decreases	Adsorption increases as pH decreases
Cations	Adsorption increases as cation concentration increases	Adsorption increases as cation concentration increases
Soluble organics	Increasing concentration of organic matter decreases viral adsorption	Increases in organic matter can retard bacterial cell movement. Organic matter may also compete for adsorption sites
Flow rate	Increased flow rates decrease viral adsorption	Increased flow rates decrease bacterial adsorption
Saturated vs. unsaturated flow	Viral movement decrease under unsaturated flow conditions through increased adsorption	Bacterial movement decrease under unsaturated flow conditions due to loss of water in larger pore spaces.
Microbial factors	Adsorption to soils varies with viral species. Different viruses may have different isolelectric points	Motile bacterial cells move faster than non-motile cells. The possession of appendages can increase adsorption capacity. Size and shape of the bacterial cell.

Sources: Gerba & Bitton (1994), Yates & Yates (1988) and Roper & Marshall (1979).

Table 8. Survival of viral particles and bacteria in soil and groundwater

Factor	Virus	Bacteria
Moisture content	Increased virus reduction in drying soils although reduction rates varies between viral types	Bacteria survive longer in moister soils
Moisture holding capacity	Viral dependant. Some viruses more susceptible to drying	Survival is less in sandy soils with lower water-holding capacity
Soil Type	Adsorption to surfaces can increase survival times	Clay coatings can inhibit predation and parasitism effects. Adsorption can increase survival times.
pH	Indirect effects through effects on adsorption. Most enteric viruses stable between pH 3 and 9	Shorter survival times in acidic soils
Cations	Generally increased cations increases virus survival. The opposite has also been observed	Increased cations increases adsorption which tends to increase survival rates
Soluble organics	May protect viral particles from inactivation. Some evidence to suggest may reversibly decrease infectivity	Increased survival and possible regrowth when sufficient amounts of organic matter are present
Temperature	Increased temperature decreases virus survival	Lower temperatures increase survival rates.
Sunlight	Minor influence at the soil surface.	Bacterial survival is least at the soil surface where the light is most intense
Microbial factors	The presence of indigenous microorganisms has been shown to decrease virus survival times. Survival varies between virus types	Indigenous microbes tend to out compete introduced microorganisms
Type of organism	Different viruses vary in their ability to with stand environmental conditions	Varies depending on bacterial physiology, metabolism, spore formation, ability to form biofilms etc.

Source: Gerba & Bitton (1994), Yates & Yates (1988), and Roper & Marshall (1979).

Table 9. Guidelines and criteria for wastewater reuse in irrigation in various countries

Country	Crop type	Treatment required	Microbiological criteria (max.)
USA	Sod farms and silviculture sites with restricted access	Secondary + disinfection (1 mg/L chlorine residual (min.)	≤ 200 faecal coliforms/ 100 mL
	Any food crops not commercially processed (including crops eaten raw).	Secondary + Filtration + disinfection (1 mg/L chlorine residual (min.)	0.0 faecal coliforms/ 100 mL
	Commercially processed food crops, orchards and vineyards	Secondary + disinfection (1 mg/L chlorine residual (min.)	≤ 200 faecal coliforms/ 100 mL
	Non food crops (seed and fibre crops), pasture for milking animals	Secondary + disinfection (1 mg/L chlorine residual (min.)	≤ 200 faecal coliforms/ 100 mL
South Africa	Irrigation of dry fodder crops, seed crops, trees, non-recreational parks, nurseries (restricted access)	Primary and secondary	<1000 faecal coliform/ 100 mL
	Food crops not eaten raw, cut flowers, orchard and vineyards, pasture, parks	Primary, secondary and tertiary; oxidation pond system	<1000 faecal coliform/ 100 mL
	Pasture for milking animals	Standard - primary, secondary and tertiary	0.0 faecal coliform/ 100 mL
	Food crops eaten raw, lawns, nurseries (unrestricted access)	Advanced (general drinking water standards	Drinking water standards
Israel	Cotton, sugar beets, dry fodder, seeds, forest irrigation	None	None
	Green fodder, olives, peanuts, citrus,bananas, almonds, nuts, etc.	None	None
	Deciduous fruits, conserved vegetables, cooked and peeled vegetables.	Chlorination of 60 minutes (min) contact time	250 Coliforms/100mL
	Unrestricted crops, including vegetables eaten uncooked (raw), parks and lawns	Sand filtration required plus chlorination of 120 minutes (min) contact time	12 coliforms/ 100mL (80%) 2.2 coliforms/ 100 mL (50%)

Table 9. Guidelines and criteria for wastewater reuse in irrigation in various countries

Country	Crop type	Treatment required	Microbiological criteria (max.)
Japan	Landscape Irrigation	≥0.4 mg/L of combined chlorine residual	No E. coli colonies detected / 100mL
Kuwait	Fodder, food crops not eaten raw, forest land	Advanced water treatment. 1 mg/L chlorine residual after 12 hrs @ 20 ^{o}C	10 000 coliforms/ 100 mL
	Food crops eaten raw	Advanced water treatment. 1 mg/L chlorine residual after 12 hrs @ 20 ^{o}C	100 coliforms/ 100 mL
Saudi Arabia	All irrigation purposes (unrestricted)	Advanced wastewater treatment	2.2 coliforms/ 100 mL
Tunisia	All irrigation purposes	Treatment plants and waste settling ponds	< 1 intestinal nematode egg/ L
Australia (South Australia only)[a]	Residential use, municipal irrigation, unrestricted crop irrigation	Full secondary plus tertiary filtration plus disinfection	<10 thermotolerant coliforms/100 mL
	Ornamental ponds with public access, restricted crop irrigation, irrigation of pasture and fodder crops for diary animals, fire fighting	Full secondary plus disinfection	<100 thermotolerant coliforms/100 mL
	Municiple irrigation with restricted access, restricted crop irrigation, irrigation of pasture and fodder crops for grazing animals	Primary sedimentation plus lagooning, or full secondary. Disinfection if required	<1000 thermotolerant coliforms/100 mL
	Irrigation for turef production, silivculture, non food chain aquaculture	Primary sedimentation plus lagooning, or full secondary	<10000 thermotolerant coliforms/100 mL

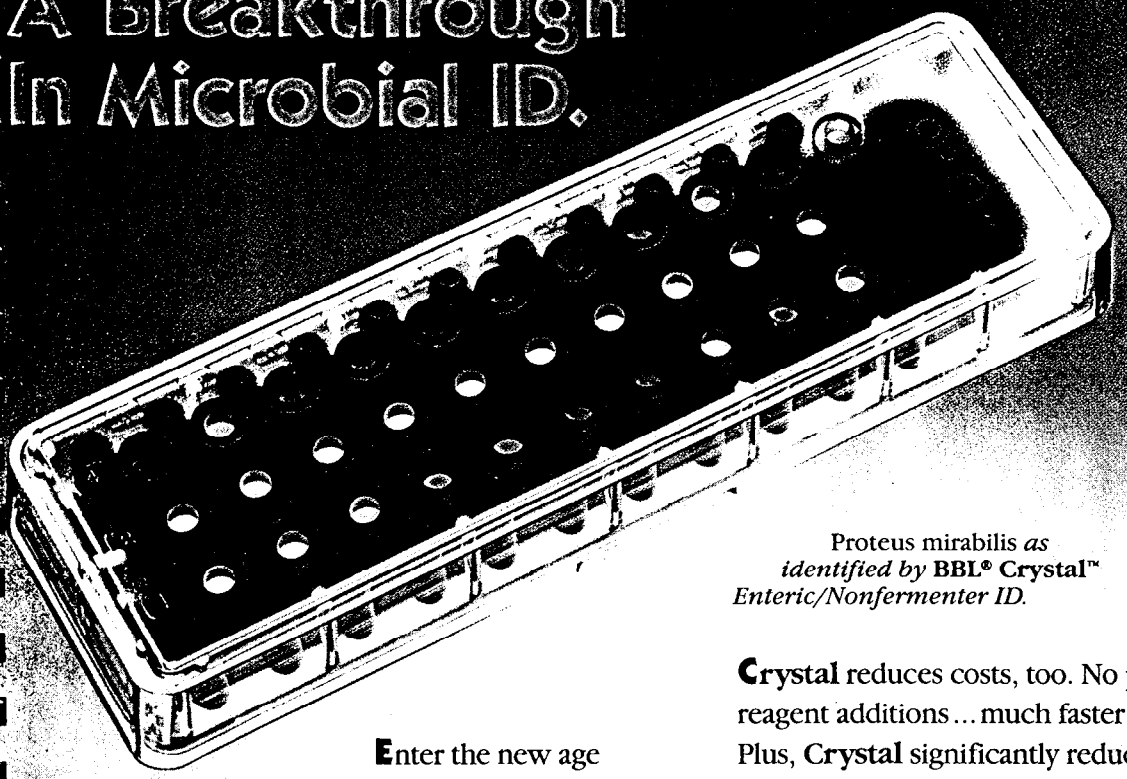
[a] Draft only as of 10/1/97.
Source: Adapted from US EPA (1992) and SAEPA (1997).

Attachment 1.

Examples of commercial rapid detection and identification kits.

BECTON DICKINSON
MICROBIOLOGY SYSTEMS

BBL® Crystal™ A Breakthrough In Microbial ID.



Proteus mirabilis *as identified by* **BBL® Crystal™** *Enteric/Nonfermenter ID.*

Enter the new age with **BBL® Crystal™**. The first microbial ID system that links automatic inoculation and one-step convenience with unmatched safety and accuracy.

Crystal is so easy to use. You simply pour your inoculum suspension directly into the **Crystal** device, rock the base and snap on the lid. Automatically, you've created a comprehensive, accurate and closed microbial system!

Crystal reduces costs, too. No pipettes... no reagent additions... much faster to prepare and use. Plus, **Crystal** significantly reduces your QC time and materials, saves incubation time and greatly simplifies your lab workflow. **Crystal** even gives you the convenience and efficiency of a computerized codebook.

For exceptional ease of use, safety, and time and money savings, use **BBL® Crystal™** for Rapid Stool/Enteric and Enteric/Nonfermenter ID. Available now from your Becton Dickinson Microbiology Systems distributor.

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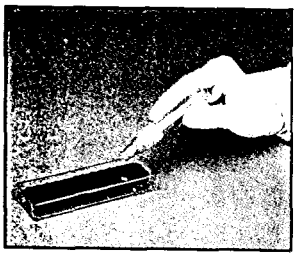
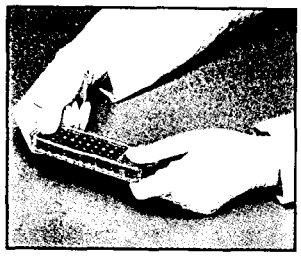
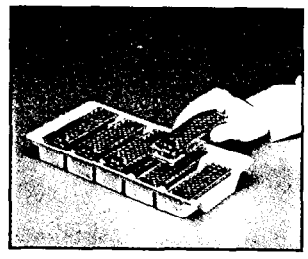
The BBL® Crystal™ ID System — Exceptionally Easy to Use, With Proven Time and Money Savings.



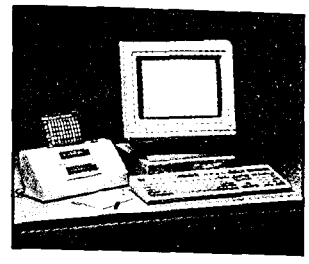
Pour inoculum directly into **BBL® Crystal™** *device.*



Rock to fill wells automatically; then snap on the lid.



Convenient stackable 10-device **Crystal** *trays save incubator space.*



Distinct **Crystal** *reactions generate a profile number that you key directly into your computer for instant ID – you never have to phone in for an answer!*

18 Hour Enteric/Nonfermenter And 3 Hour Rapid Stool/Enteric ID

BBL® Crystal™* employs modified conventional and chromogenic substrates to identify clinically significant aerobic gram-negative bacteria of the family *Enterobacteriaceae*. The Enteric/Nonfermenter system also identifies some of the more frequently isolated glucose-nonfermenting gram-negative bacilli of human origin; the Rapid Stool/Enteric system also identifies most pathogens isolated from stool specimens.

	Catalog No.
BBL® Crystal™ Enteric/Nonfermenter ID Kit Contains sufficient lids, bases, inoculum fluid and incubation trays for 20 determinations.	4345000
BBL® Crystal™ Rapid Stool/Enteric ID Kit Contains sufficient lids, bases, inoculum fluid and incubation trays for 20 determinations.	4345050
BBL® Crystal™ Light Box	4345026
BBL® Crystal™ Electronic Codebook Includes a 3 1/2" disk, works on any IBM-compatible computer with MS-DOS 3.1 or higher, can run off hard drive or floppy disks.	4345001

*U.S. Patent No. 5,182,082

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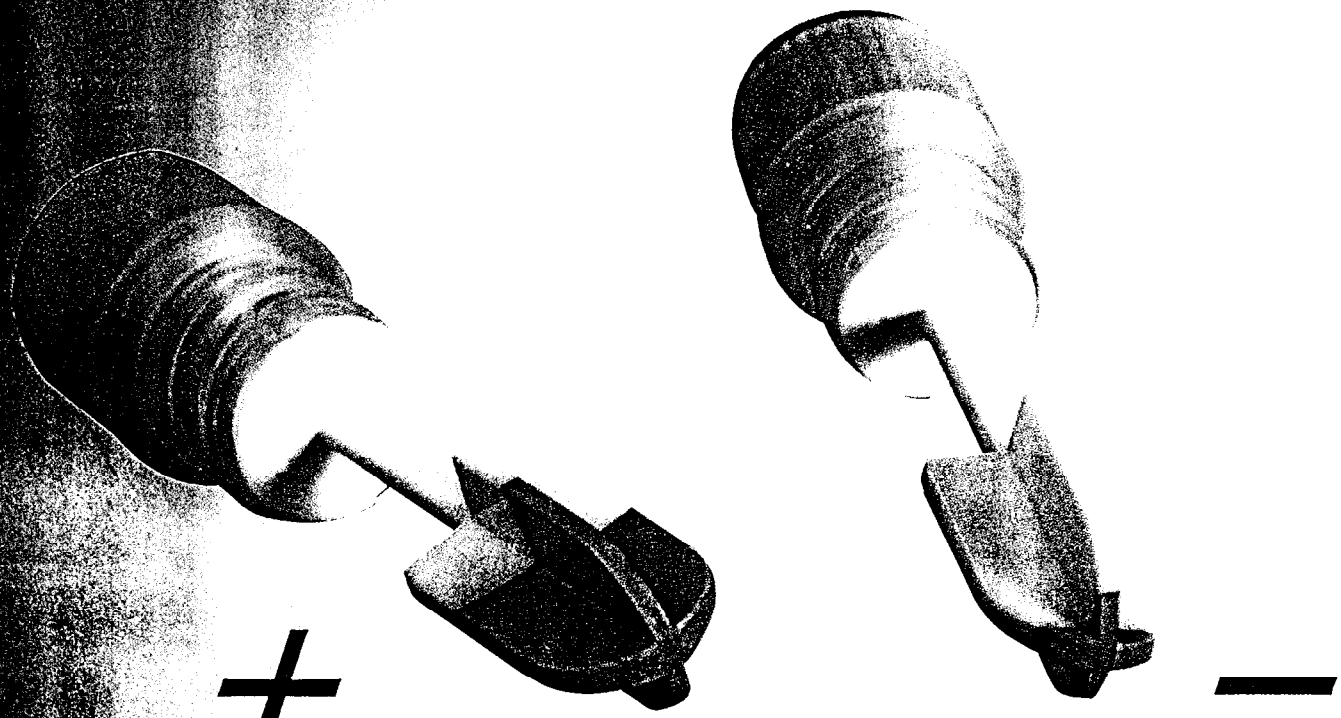
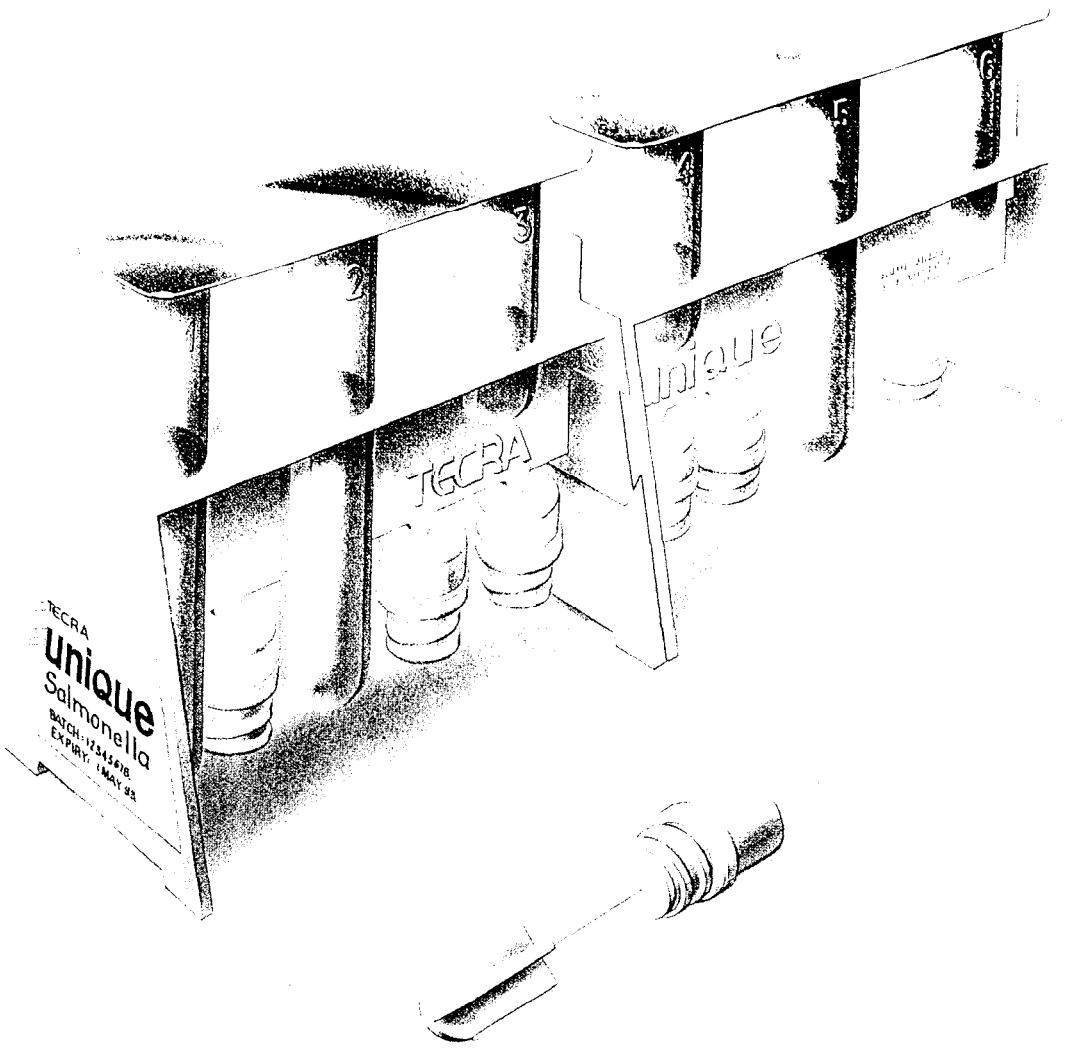


SALMONELLA SCREENING

From Sample to Result in less than 22 HOURS



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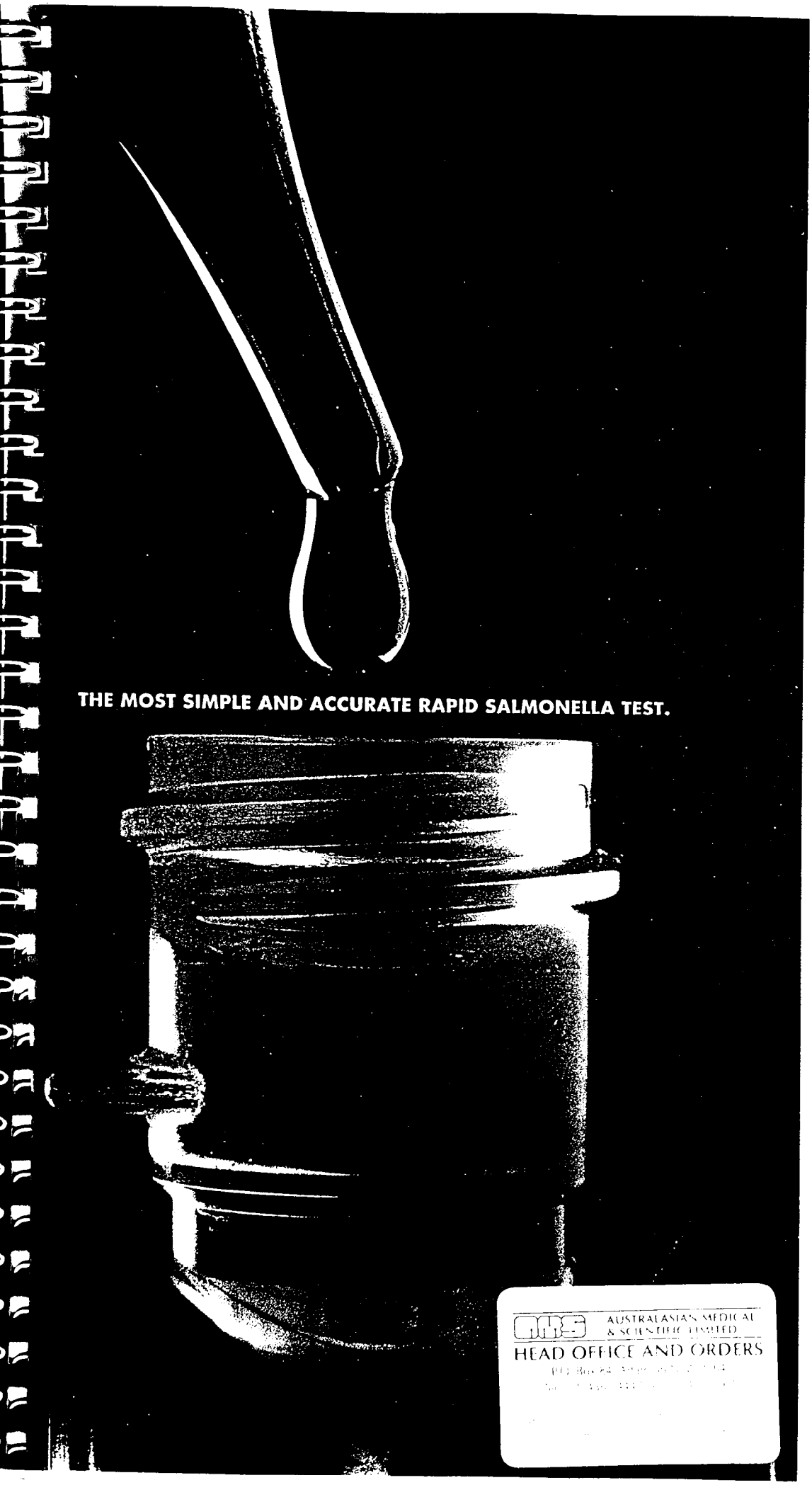
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Why analysts like you prefer the 1-2 Test.

SPECIFIC. The highly purified 1-2 Test antibody preparation, proprietary to BioControl, has been developed to capture a wide range of *Salmonella* and also to screen out common cross-reactors.

SENSITIVE. The 1-2 Test detects living cells of *Salmonella*, so even a low level (1-5 cells) of *Salmonella* will trigger the immunodiffusion process.

ACCURATE AND RELIABLE. The 1-2 Test works, whether it is used for testing food products, raw ingredients, or environmental samples.

RAPID RESULTS. Just enrich samples, inoculate, and incubate for at least 14 hours. Results are available days earlier than with the standard culture method. This means your products can be released earlier.

EASY TO USE AND SELF-CONTAINED. The 1-2 Test takes only 2 or 3 minutes of hands-on time. Reading the test is easy, too. The single-use format makes the test easy to store and easy to discard.

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FULL TECHNICAL SUPPORT. BioControl staff scientists are always available to provide solutions to microbiological problems.

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