“Establishment of monitoring system for viral pollution of water”

A thesis
Submitted for the Degree of
Doctor of Philosophy in Science “Microbiology”

By

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Degree: Doctor of Philosophy in Science "Microbiology"

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Acknowledgments

First of all thanks to ALLAH for every blessing he gave me and for helping me finish this work.

I would like to express my deepest respect and sincere gratitude to **Prof. Dr. Ahmed Barakat** for the valuable advice, precious guidance since my undergraduate studies and until the progress of this work.

I am greatly indebted and expressing my sincere appreciation to **Prof. Dr. Nagwa EL-Esnawy** for providing all the facilities, endless help, innovation supervision, and suggestions, which were a great asset to this work.

My deepest thanks go to **Dr. Hussam A. Ghanem**, for his advice during my study. Appreciation also goes to my advisors in Virology lab, University of Arizona, USA; **Prof. Dr. Charles Gerba** and **Akrum Tamimi** for their support throughout my thesis.

I would like also to thank everyone who has provided me with assistance and advice in my virology labs in the National Research Centre and in University of Arizona, USA.

Dedication

To my parents, I thank you both, because you taught me how to be both brave and patience, thus enabling me to push forward. I have been blessed to have you as my parents. Thank you for all your love and care you giving to me and my brothers.

To my brothers for all your kindness and encouragement words that helped me during all my studies.

To each one of my precious and loving children Omar, Mohamed and Ali.

And finally to my lovely wife Asmaa, to whom I am so thankful for her love, care, support, encouragement and understanding.

This thesis has not been previously submitted for any degree at this or any other university

Signed

Sherif Abd-Elmaksoud

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List of Abbreviations

ANOVA	Analysis of variations
ATCC	American Type Culture Collection
Bp	Base pairs
BEG	beef extract-glycine buffer
BGM	Buffalo Green Monkey
BVDV	bovine viral diarrhea virus
CPE	Cytopathic effect
cDNA	Complementary DNA
CFU	Colony forming unit
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
FCSV	final concentrated sample volumes
HGV	hepatitis G virus
ICC/PCR	Integrated Cell Culture PCR
MA104	Rhesus monkey epithelial cell line
MEM	Minimum essential medium
MPN	Most Probable Number
NTU	Nephelometric turbidity units
NSF	National Sanitation Foundation
NLV	Norwalk like viruses
PLC	Primary Liver carcinoma
PFU	Plaque Forming Unit

PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PVC	polyvinylchloride
qPCR	Quantitative polymerase chain reaction
RT	Reverse transcriptase
RNA	Ribonucleic Acid
rpm	Revolutions per minute
TCVA	Total Culturable Viral Assay
$TCID_{50}$	50% Tissue Culture Infective Dose
TDS	Total dissolved solids
Tris	Hydroxymethyl amino methane
TOC	Total organic carbon
USEPA	United State Environmental Protection agency
UV	Ultraviolet
(v/v)	Volume per volume
WHO	World Health Organization
(w/v)	Weight per volume
xg	Relative centrifuge force

Chapter I

Introduction

Waterborne diseases are still a major problem in the world today, causing millions of deaths each year. While water treatment has significantly reduced waterborne disease in the United States, it is still estimated that 19.5 million cases occur annually (**Reynolds *et al*., 2008**). The majority of waterborne viruses are transmitted via the fecal-oral route, including the enteroviruses (e.g., echoviruses and Coxsackieviruses), adenoviruses (Types 40 and 41), human caliciviruses (noroviruses), and hepatitis A virus, among others. Illness is initiated upon ingestion of these microorganisms from contaminated water sources. Exposure to low numbers (1 to 10 infectious units) may lead to virus replication within the digestive tract and infection (**Haas *et al*., 1993**). A wide variety of clinical outcomes have been documented ranging from asymptomatic carrier states to life-threatening gastroenteritis, meningitis, febrile illness, paralysis, and acute hepatitis (**Moe, 2002**).

As these viruses are excreted in high numbers within fecal matter by infected persons (10^6 per gram of excreta), they are subsequently present in elevated numbers in raw sewage water (**Gerba *et al*., 1978**). These viruses cannot always be effectively eliminated by current methods of sewage treatment (**Van den**

Berg *et al*., 2005) and consequently cause viral contamination of the environment from treated as well as untreated wastewater. Other examples of indirect routes are run-off from manure used in agriculture. There is also direct fecal contamination of the environment from humans and animals, for example by bathers or by defecation of free-range or wild animals onto soil or surface waters. The resulting viral contamination of sea and coastal water, rivers and other surface waters, groundwaters, and irrigated vegetables and fruit is associated with subsequent risks of reintroduction of the viral pathogens into human and animal populations (**La Rosa *et al*., 2007**). In the latter scenario, however, viruses are often present in lower numbers, making their detection difficult (**Farrah *et al*., 1976**). Therefore, a concentration step must be implemented whereby large volumes of water ranging from hundreds to thousands of liters are passed through filters to capture the microorganisms.

A variety of filter devices have been evaluated over the course of several decades for their capacity to concentrate enteric viruses and include electronegative (**Fuhrman *et al*., 2005**) and electropositive pleated filters (**Chapron *et al*., 2000**), glass wool (**Lambertini *et al*., 2008**), ultrafilters (**Polaczyk *et al*., 2008**), and ultracentrifugation (**Mehnert *et al*., 1997**).

While these devices have proven effective for the capture of viruses from water, several are either impractical for application with more turbid natural surface waters (e.g., ultrafilters), or are costly (e.g., 1MDS electropositive filter, CUNO, Meriden, CT,

~$180 each). Both electronegative and electropositive filters are widely used today; however, electronegative filters are not ideal for large-scale water sampling as they require acidification of the water and the addition of mutivalent cationic salts to the water prior to filtration (**Wallis *et al*., 1972**).

Development of an inexpensive and efficient system for concentrating, eluting, and re-concentrating pathogenic viruses from drinking water and surface waters is very important for accurate detection and identification of human enteric viruses in environmental waters and help in proper monitoring of microbial quality in order to avoid health hazards of infection by these viruses.

Sodocalcic glass wool offers a promising alternative as an adsorptive material for virus concentration. Glass wool, held together by a binding agent and coated with mineral oil, provides both hydrophobic and electropositive sites for adsorption of microorganisms. Viruses are usually negatively charged in water at near neutral pH and readily adsorbed to the positively charged glass wool fibers (**Environment Agency, 2000**). The fibers are inexpensive, require no water conditioning outside of pH adjustment in some circumstances and can be applied in the field for on-site filtration and concentration (**Wyn-Jones and Sellwood, 2001**). Glass wool has been shown to be effective for the concentration of human enteric viruses from wastewater (**Gantzer *et al.,* 1997**), drinking water (**Lambertini *et al.,* 2008**), groundwater (**Ehlers *et al*., 2005**), river water (**Albinana-**

Gimenez *et al.,* 2009), and reservoirs (**Deboosere, *et al.,* 2011**). However, only a handful of studies have attempted to quantify how effective glass wool is for concentrating viruses (**Environment Agency, 2000**), and these examined only enteroviruses and rotavirus.

Cell culture is the most commonly use method for the isolation of infectious viruses from water (**Pepper *et al*., 2000**). The Buffalo green monkey kidney (BGM) cell line is very sensitive to infection by poliovirus and coxsackievirus B and is the most common cell line used for the detection of such viruses in water (**Dahling, 1991**). The BGM cell line recommended for the total culturable viral assay (TCVA) is the standard method for the enumeration of viruses from water (**US.EPA, 1995**). The TCVA depends on the ability of the viruses to propagate in BGM cells and create morphological changes or cytopathic effects (CPE) that can be identified and quantified. The combination of the BGM cell line with other cell lines such as A549, human colon adenocarcinoma (Caco- 2), and primary liver carcinoma (PLC/PRF/5) lines for the monitoring of viruses in water has been reported to increase the sensitivity for the detection of adenoviruses, coxsackie A viruses, echoviruses and astroviruses, or other viruses that do not grow effectively on BGM cells (**Sedmak *et al*., 2005**). PLC/PRF/5 is a more efficient cell line for the propagation of adenoviruses 40 and 41 than Graham 293, Chang, KB, and A549 (**Grabow *et al*., 1992**). In addition, the