Efficiency of glass wool adsorption-elution technique for the recovery of enteric viruses from water

by

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DECLARATION

I, Vurayai Ruhanya, declare that this work was not copied or repeated from any other studies either from national or international publications. Procedures were carried out in accordance with the ethical rules as prescribed by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

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Date: 14/02/2014

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EFFICIENCY OF GLASS WOOL ADSORPTION-ELUTION TECHNIQUE FOR THE RECOVERY OF ENTERIC VIRUSES FROM WATER

by

VURAYAI RUHANYA

SUPERVISOR: PROF MB TAYLOR

DEPARTMENT: MEDICAL VIROLOGY

DEGREE: MAGISTER SCIENTIAE (MEDICAL VIROLOGY)

SUMMARY

One of the major obstacles to human health relates to unsafe water and poor sanitation. Faecal contamination of source and drinking water introduces enteric pathogens which result in disease outbreaks. Therefore monitoring the occurrence of human pathogens in source water and drinking water is necessary in order to limit the prevalence of environmentally transmitted infectious diseases. Knowledge of pathogen loads in source waters provides the basis for establishing treatment requirements and health standards stipulated by water regulatory authorities and assists in determining the efficacy of water treatment plants. Water quality monitoring and public health assurance is performed routinely by enumerating faecal indicator bacteria. Studies have demonstrated that there is no relationship between current bacterial indicator detection and the presence of enteric pathogenic viruses in treated and source water. There is therefore a need to monitor the levels of pathogenic enteric viruses in surface waters, irrigation water, sewage effluent as well as treated drinking water for public health safety and quality assessment. However due to the low concentration of viruses in water matrices and presence of inhibitors, efficient concentration methods from large quantities of water are essential.

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The analysis of water for enteric viruses is a two stage process: the first step is to apply efficient viral recovery and concentration procedures from large volumes (10–1000 ℓ) of water followed by viral detection. Glass wool adsorption-elution is a cost-effective and practical viral recovery method for use in resource-limiting settings. The main objective of this study was to determine the efficiency of the glass wool adsorption-elution method for the recovery of viruses of different genera from large water samples (10 ℓ) of different quality by a step-by-step evaluation of its performance using seeding experiments. Standard curves were prepared using quantitative reverse transcription-polymerase chain reactions (RT-PCR)(for RNA viruses) and PCR (for DNA viruses). The efficiency of recovery (EOR) of glass wool between tap water and turbid surface water was compared for six enteric viruses by examining the recovery and loss of viruses at each stage of the process. The generalised linear statistical model was applied to compare the EOR of each virus in each water type and results clearly indicated that the EOR varied for each virus type and was higher for tap water than for turbid surface water for each virus. There was extensive loss of virus in the flow through and this was also higher for the turbid water than the tap water. In this study it was also demonstrated that mengovirus behaved similarly to the pathogenic enteric viruses and was therefore a suitable process control to monitor viral recovery and nucleic acid extraction when recovering and detecting enteric viruses from environmental matrices using glass wool adsorption method. It was also demonstrated that EOR of glass wool for turbid surface water was underestimated as the poor sample quality affected the quantitative molecular detection assays. Adenovirus was shown to be a suitable indicator for virus contamination of water. Modification of the glass wool column preparation did not result in significant difference in EOR but an increase in the amount of glass wool used resulted in reduction in EOR. There were no significant differences between the two polyethylene glycol/sodium chloride (PEG_{6000}/NaCl and PEG_{8000}/NaCl) precipitation methods applied to the secondary concentration of the viruses, but it should be noted that the former has the disadvantage of overnight incubation. The EOR of glass wool was shown to be influenced by pH of the sample. The optimal sample pH for the recovery of hepatitis A virus in turbid surface water was pH 6.0. The study provides valuable new data on the EOR of enteric viruses using the glass wool adsorption-elution technique where virus quantities could be traced from seeding to detection by molecular-based methods.

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PRESENTATIONS

Presentations

Ruhanya V, Olorunju S, Taylor MB. Efficiency of glass wool adsorption-elution technique for the recovery of selected enteric viruses from raw and treated water [Poster]. Faculty Day, Faculty of Health Sciences, University of Pretoria 28-29 August 2012: HW Snyman Building North, Pretoria.

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ABBREVIATIONS AND SYMBOLS

AdVs	Adenoviruses
AstVs	Astroviruses
ATCC	American Type Culture Collection
CAN-HEV	Canine hepatitis E virus
CAR	Coxsackievirus-adenovirus receptor
CCL	Candidate Contaminant List
CDC	Centres for Disease Control
CI	Confidence interval
Cl	Chlorine
CNS	Central nervous system
CP	Crossing point
CPE	Cytopathic effect
Cq	Cycle quantification
Ct	Cycle threshold
DAF	Decay accelerating factor
dsDNA	Double-stranded DNA
dsRNA	Double-stranded RNA
EBHSV	European brown hare syndrome virus
EIA	Enzyme immunoassay
EM	Electron microscopy
EPA	Environmental Protection Agency
EV	Enteroviruses
EOR	Efficiency of recovery
FDA	Food and Drug Administration
FCV	Feline calicivirus
g	Gram
GBEB	Glycine beef extract buffer
GLM	Generalised linear model
GI	Genogroup one

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GII	Genogroup two
GIII	Genogroup three
GIV	Genogroup four
GV	Genogroup five
h	Hour
HAdV	Human adenovirus
HAV	Hepatitis A virus
HCl	Hydrochloric acid
HuCV	Human caliciviruses
ICAM-1	Intracellular adhesion molecule 1
IEM	Immune electron microscopy
HEV	Hepatitis E virus
HFMD	Hand foot and mouth disease
ℓ	Litre
LRTI	Lower respiratory tract infections
M	Molar
min	Minute(s)
mℓ	Millilitre
mM	Millimolar
MPN	Most probable number
NaOH	Sodium hydroxide
NoV	Norovirus
NTR	Non-translated region
NLV	Norwalk-like virus
ORF	Open reading frame
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PV	Poliovirus
PVR	Poliovirus receptor
rt	Real-time
RT-PCR	Reverse transcription-polymerase chain reaction

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RV	Rotavirus
SA	South Africa
SaV	Sapovirus
sec	Second(s)
SLV	Sapporo-like virus
SMSV	San Miguel sea lion virus
+ssRNA	Positive-sense single-stranded RNA
$TCID_{50}$	50% tissue culture infectious dose
U	Unit
US	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
VDPV	Vaccine-derived poliovirus
VEVS	Vesicular exanthema virus of swine
RHDV	Rabbit haemorrhagic disease virus
RGD	Arginine-glycine-aspartic acid motif
WHO	World Health Organisation

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CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

In 2003, the World Health Organisation (WHO) noted that one of the major obstacles to human health relate to unsafe water, poor sanitation and inappropriate hygiene (Ashbolt, 2004). The world health authority also estimated that 1.1 billion people globally drink unsafe water, with 88% of diarrhoeal diseases attributable to contaminated water. It is estimated that 1.8 billion people die each year because of diarrhoeal diseases with 90% being children under five years mostly from developing countries (WHO, 2011a). Studies from North America suggest that 15-30% of gastroenteritis cases have been transmitted by water (Ashbolt, 2004; WHO, 2004). The WHO estimated that improved water supply can reduce diarrhoeal morbidity by 21% and additional improvement of drinking water quality like disinfection reduces diarrhoea episodes by 45%.

Breakdowns in water supply may lead to faecal contamination which results in outbreaks of waterborne diseases. Surface waters, municipal sewage and urban storm water flows and contaminated water from agricultural areas and septic tanks are major causes of pollution (Okoh et al., 2010). These sources of pollution release pathogens into recreational, source and drinking water reservoirs (Kocwa-Haluch, 2001; Maunula et al., 2004). For pathogens which are transmitted by faecal-oral route, significant epidemiologic evidence shows that waterborne exposure is an important vehicle of transmission (Maunula et al., 2005; Yoder et al., 2008; Rodríguez-Lázaro et al., 2012). Outbreaks of diseases have been associated with consumption of some viruses in drinking water (Schaub and Oshiro, 2000; Di Pasquale et al., 2010). In fact the most common cause of waterborne viral infections has been contamination by sewage of human origin (Maunula, 2007). Control of drinking water quality therefore correlates to the control of waterborne diseases and it is an important element of

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public health policy and an objective of water supply authorities (WHO, 2004). It is therefore very important to understand the occurrence, levels, exposure and health outcomes associated with human pathogens in source waters in order to limit the prevalence of environmentally transmitted infectious diseases (Guzmán et al., 2007). Knowledge of pathogen loads and concentration in source waters provides the basis for establishing treatment requirements to meet health standards stipulated by the water supply process (Dechesne and Soyeux, 2007). The knowledge also helps to understand the efficacy of the treatment plants which ensures the microbial reduction necessary to ensure water safety (WHO, 2004). Waterborne pathogens in natural waters are either adsorbed to sand, clay, naturally occurring suspended colloids, estuarine silts and sediments (Templeton et al., 2008). Conventional water and waste-water treatment processes such as coagulation, flocculation, sedimentation and filtration remove viruses and other pathogens adsorbed onto particulate matter and the efficiency of removal depends on the adsorptive affinities of viruses. The absorbance is affected by pH, redox potential and dissolved oxygen of the system (Okoh et al., 2010). Waste water treatment processes such as activated sludge process, oxidation ponds, activated carbon treatment, lime coagulation and chlorination only eliminate between 50% and 90% of viruses present in waste water, allowing a significant proportion of viruses to be released into the environmental waters (WHO, 2004; Okoh et al., 2010)

Water quality monitoring and public health assurance is performed routinely by enumerating faecal indicator bacteria in drinking water and water intended for human consumption should contain no indicator organisms (WHO, 2004). Faecal coliforms are used as indicators because of the assumption that the fate and transport of faecal coliforms reflect that of the waterborne pathogens (Payment and Locas, 2010). However, advances in the understanding of behaviour of pathogens in water and retrospective studies on waterborne disease outbreaks showed that absence of faecal coliform indicators does not entirely eliminate the possible presence of other pathogens with a potential to cause disease outbreaks (WHO, 2004; Ferguson et al., 2012). For example, some enteric viruses are resistant to many disinfectants and remain viable after disinfection (WHO, 2004). Studies have also demonstrated that

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there is no relationship between current bacterial indicator detection and the presence of viruses in treated water (Espinosa et al., 2009). There is therefore the need to have multiple or supplementary indicators which include enteric viruses and if possible to directly monitor the levels of viral pathogens in surface waters, irrigation water, sewage effluent as well as treated drinking water for public health safety and quality assessment (Gensberger and Kostic, 2012). However, due to the low concentration of viruses in water matrices and presence of inhibitors, efficient concentration methods from large quantities are essential (Mattison and Bidawid, 2009; Gensberger and Kostic, 2012).

1.2 WATERBORNE VIRUSES

Enteric viruses are major pathogens of concern in waterborne gastroenteritis and hepatitis (Bosch *et al.,* 2006) and a number of waterborne enteric viruses have been associated with sporadic and epidemic gastroenteritis worldwide (Wyn-Jones and Sellwood, 2001; Grabow, 2007). Enteric viruses from the families *Adenoviridae, Astroviridae, Caliciviridae, Hepeviridae, Picornaviridae* and *Reoviridae* have all been implicated in waterborne disease (Wyn-Jones and Sellwood, 2001; Carter, 2005; Grabow, 2007).

1.2.1 Human caliciviruses

Human caliciviruses (HuCVs) were first described in 1972 as small non-enveloped, single-stranded, positive-sense (+ssRNA) RNA viruses that are 27-40 nm in diameter, icosahedral in shape with 32 cup-shaped depressions called calici or calyx which means cup in Latin (Oehmig et al., 2003; Clarke et al., 2012). They are the most common viral cause of food and waterborne gastroenteritis (Atmar et al., 2008). The family *Caliciviridae* is divided into five genera namely, *Norovirus, Sapovirus, Nebovirus, Lagovirus* and *Vesivirus* (Clarke et al., 2012). There are two calicivirus genera, namely norovirus (NoV) and sapovirus (SaV), that affect humans (Oehmig et al., 2003). Noroviruses and SaVs are recognised as the leading causes of gastroenteritis worldwide (Haramoto et al., 2004; Hansman et al., 2005; Harada et al., 2009).

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1.2.1.1 *Noroviruses*

Noroviruses are the leading cause of acute nonbacterial epidemic and sporadic gastroenteritis worldwide (Glass et al., 2000a, 2009). Norwalk virus is the prototype calicivirus which was first described by electron microscopy (EM) in 1972 during an epidemic of gastroenteritis in a school in Norwalk, Ohio (Clarke and Lambden, 2000). Morphologically similar viruses are known as "small round structured viruses" or Norwalk-like viruses (NLV) are named according to the location of the outbreak (Glass et al., 2009). Phylogenetic analyses of NoV further subdivided viruses into genogroups (Gerba and Kayed, 2003; Zheng et al., 2006). The prototype NoV, Norwalk virus, and the Southampton strain, Desert shield and Cruise ship virus belong to genogroup I while the Snow Mountain agent, Hawaii, Toronto and White River viruses belong to genogroup II (Ando et al., 1995; Hall et al., 2011).

a) Virus characteristics

Noroviruses are non-enveloped viruses with a +ssRNA genome approximately 7.5-7.7 kilobases (kb) polyadenylated at the 3' end and composed of three overlapping open reading frames (ORFs) (Glass et al., 2009) (Figure 1.1).

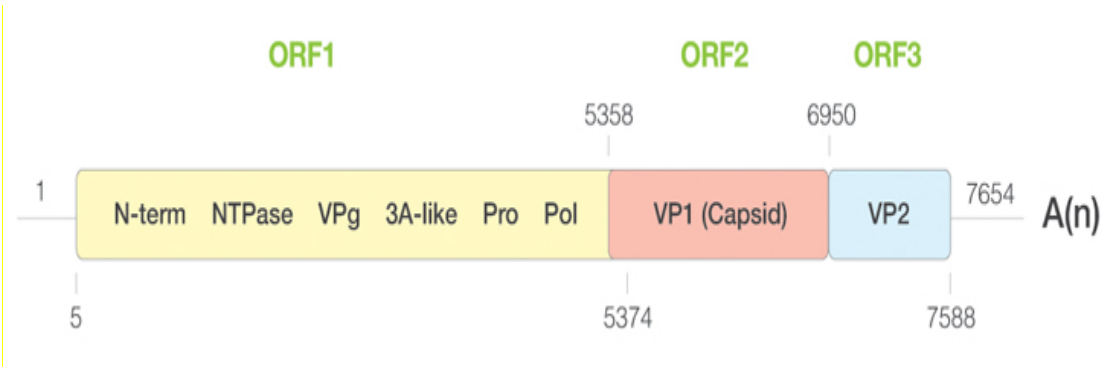

Figure 1.1: The norovirus genome (kcdc.labkm.net; accessed 2014-01-22)

The first open reading frame (ORF1), beginning at the 5'-end, encodes a non-structural polyprotein needed for replication (Glass et al., 2000b). The polyprotein is proteolytically cleaved to produce the N-terminal, NTPase, VPg, 3C-protease and 3D-polymerase proteins (Oliver et al., 2007). The second reading frame (ORF2) encodes the major structural protein (viral protein 1 [VP1]) which determines antigenicity and a small third ORF (ORF3) near the 3'-end of the genome encodes a basic minor structural

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protein (VP2) (Glass et al., 2000b). Recent findings showed that murine NoV has an additional ORF, ORF4, which overlaps ORF2 (Thackray et al., 2007). The current classification puts the NoV into five genogroups (Zheng et al., 2006; Patel et al., 2009). In each genogroup are 'clusters' or 'genotypes', of which there are eight clusters or genotypes in GI and 17 genotypes in GII, two in GIII, and one each in GIV and GV (Zheng et al., 2006). The VP1 major capsid protein encoded by ORF2 is considered very important as it is involved in recognition of the pathogen by the host. The protein has a protruding domain P which is made up of subunits P1 and P2 of which the P2 is critical in binding to the receptor (Marshall and Bruggink, 2011). It is suggested that genetic recombinations and mutations in the P2 domain can result in variants that can reinfect individuals thereby evading the herd immunity (Bull et al., 2007). It has also been discovered that a small change in the NoV capsid gene can have a big influence on the virulence of the pathogen and changes in the polymerase region may influence GII.4 virulence (Bailey et al., 2008; Bruggink and Marshall., 2008; Bull et al., 2010).

Studies with human volunteers showed the virus to be heat stable, ether resistant, acid stable and to remain infectious for more than 3 hours (h) at pH 2.7 at room temperature and after heating at 60°C for 30 min (Gerba and Kayed, 2003). No cell culture or animal infectivity assays exist for human NoV, therefore certain animal caliciviruses like feline caliciviruses (FCV) are grown in cell culture as surrogates or models for HuCVs (Lopman et al., 2012). Investigations using FCV as a surrogate for survival of the virus showed that the virus can survive for at least 60 days (d) at 4°C with minimum loss of infectivity. The virus survived for 14-21 d in cell culture medium and 21-28 d in a dried state (Marshall and Bruggink, 2011). Studies using purified NoV and reverse transcription-polymerase chain reaction (RT-PCR) detection suggests that the virus was more resistant than poliovirus (PV) type 1 to free chlorine and a variety of disinfectants (Gerba and Kayed, 2003; Satter et al., 2011). It has been argued that the appropriateness of FCV as a surrogate is subject to debate because NoV is an enteric pathogen and FCV is a respiratory pathogen (Gerba and Kayed, 2003). Therefore, the clinical and epidemiological implications of the survival characteristics are difficult to assess directly although the laboratory findings generally agree with NoV association with weather variables (Lopman et al., 2012).

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b) Clinical features

Norovirus gastroenteritis is characterised by self limiting vomiting which is projectile (present in more than 50% of cases), stomach cramps, watery non-bloody diarrhoea, abdominal pain and low grade fever which last between 24-48 h (Clarke and Lambden, 2000). Infection with NoV can be asymptomatic in patients (as many as 30%) or mild with non-specific symptoms such as nausea, abdominal cramps, myalgia and fever (Gerba and Kayed, 2003; Patel et al., 2009). Studies have shown shedding of the virus in stool during the incubation period and up to 44 d following onset of symptoms in naturally infected people, with prolonged excretion well documented in immunocompromised patients (Ludwig et al., 2008). Noroviruses are shed at approximately 10^6- 10^9 particles per gram (g) of stool and studies with volunteers have shown that 10 - 100 particles or less are needed to cause infection (Gerba and Kayed, 2003; Teunis et al., 2008; Glass et al., 2009).

c) Epidemiology

Noroviruses are spread predominantly by the faecal-oral route, directly from person-to-person or aerosol or indirectly via contaminated food or water (Yoder et al., 2008). Outbreaks are likely to occur in any situation where people gather in groups of various magnitudes, like in children's home care centres, hospitals, home care centres and cruise ships (Marshall et al., 2005; Bruggink and Marshall, 2008). Food sources can be contaminated from irrigation water or from infected food handlers during preparation and service (Scallan et al., 2011). Drinking and recreational water contaminated by sewage from septic tank leakages and burst sewer pipes or breakdowns in chlorination of municipal waters result in large community outbreaks of NoV infection (Yoder et al., 2004, 2008; Seitz et al., 2011). Data from the United States of America (US) and Europe show that NoV is responsible for 50% of all reported cases of gastroenteritis (Patel et al., 2009). A review of 31 community, outpatient and hospital-based studies in both developed and developing countries show that NoV was responsible for 10-15% of severe gastroenteritis in children under the age of five years and for 9-15% of mild and moderate diarrhoea among people of all age groups (Patel et al., 2009).

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1.2.1.2 *Sapoviruses*

Human SaV are becoming more prevalent worldwide and an emerging pathogen associated with gastroenteritis (Harada et al., 2009; Svraka et al., 2010). They are included in the US Environmental Protection Agency (EPA) contaminant candidate list (CCL3) which identifies pathogens in aquatic environments that have a potential risk to public health (USEPA, 2009; Kitajima et al., 2011). The virus was named after its discovery in Sapporo, Japan in 1977 in an outbreak of gastroenteritis in a home for infants.

a) Virus characteristics

Sapovirus is a non-enveloped +ssRNA virus with a genome of approximately 7.5 kb. The genome consists of two open reading frames encoding the non structural proteins and the structural (capsid) proteins VP1 encoded in ORF 1 and VP2 encoded in ORF 2 which is located at the 3' end of the genome (Fullerton et al., 2007). On the basis of the VP1 nucleotide sequences SaV was initially divided into five genogroups, GI, GII, GII, GIV and GV which further subdivided into genotypes (Hansman et al., 2005). Genogroup I, GII, GIV and GV infect humans while GIII infects porcine species (Farkas et al., 2004; Katayama et al., 2004; Chan et al., 2006). Two new genogroups have been proposed namely GVI and GVII (Wang et al, 2005, Yin et al., 2006) and it has been predicted that genogroups GI, GIV and GV have a third open reading frame (ORF3) which encodes for an unknown protein (Koopmans et al., 2002; Hansman et al., 2007).

b) Clinical features

The infectious dose of SAV ranges from 10 to 100 virus particles (Schaub and Oshiro, 2000). The viruses infect the intestinal brush border preventing the proper absorption of nutrients and water, leading to diarrhoea, vomiting, abdominal cramps, nausea, headache, myalgia and fever. The clinical manifestations of SaV are less severe than NoV. Self-limiting diarrhoea and vomiting are the most common symptoms associated with SaV infections (Green et al., 2000; Mikula et al., 2010). The symptoms last between 24- 48 h and with diarrhoea occurring between 72-97% of cases and vomiting 56-73% of cases (Green et al., 2000).

c) Epidemiology

Sapoviruses are transmitted via the faecal-oral route (Atmar and Estes, 2006; Hansman et al., 2007). A study conducted in Europe to determine the epidemiology and

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genotypes in emerging SaV-associated infections showed that 4% of acute gastroenteritis outbreaks in the Netherlands were caused by SaV, of which 63% occurred in nursing homes, 26% in hospitals and 11% in child day care centres. Genetic characterisation of the SaVs from the these outbreaks and other sporadic cases showed that 11 out of 19 outbreaks (57%) were caused by genotype I.2 which was also responsible for outbreaks recorded in Sweden, Slovenia, and Hungary (Svraka et al., 2010).

1.2.2 Human adenoviruses

Adenoviruses (HAdVS), first isolated in 1953 from adenoids and tonsils surgically removed from children, are non-enveloped double-stranded (ds) DNA iscosahedral viruses with a diameter of 70-90 nm (Ruuskanen et al., 2009; Harrach et al., 2012 (Figure 1.2).

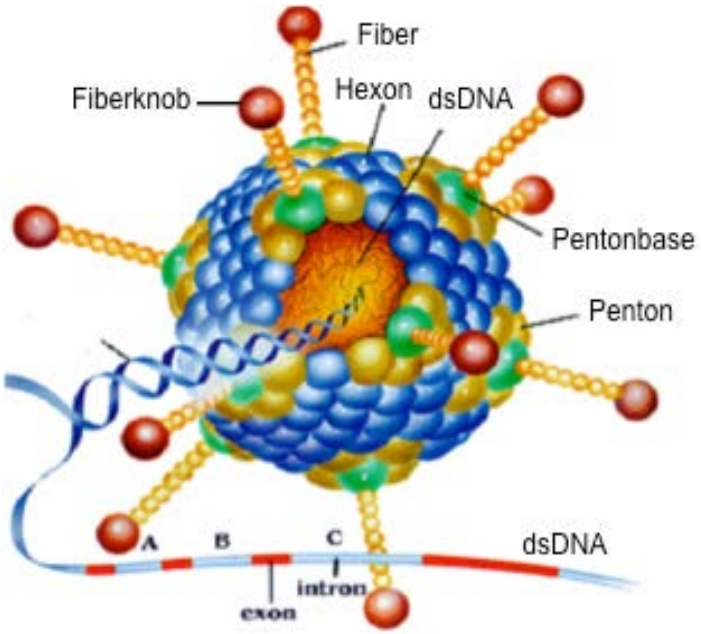

Figure 1.2: The adenovirus particle (Rauschhuber, 2011)

Viruses of this family were classically divided into two genera, *Mastadenoviruses* (mammal host) and *Aviadenoviruses* (avian host). Available data from DNA sequencing, phylogenetic analyses and genome arrangement have added two additional genera

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Atadenovirus and *Siadenovirus* which includes serotypes from bovine, reptiles and amphibians (Hundesa et al., 2006; Ruuskanen et al., 2009; Harrach et al., 2012). Based on neutralisation assays HAdVs have been classified into 51 antigenic serotypes which are further classified into 6 species, A to F, which is based on their ability to agglutinate various types of erythrocytes (Sarantis et al., 2004). The classification of HAdVs into species using agglutination is supported by other viral properties such as antigenicity, oncogenicity, fibre length, percentage of DNA homology, G+C content, the cleavage patterns by S*ma*1 and the molecular weights of some proteins which are generally in concordance within species (Shenk, 2001). Different DNA genomic patterns can be identified by restriction enzyme analysis. The different restriction fragment patterns vary from different geographical locations and space and have been used as markers in epidemiological studies (Ruuskanen et al., 2009). Enteric AdVs (AdV40 and AdV41), which are important aetiological agents of paediatric gastroenteritis, have been found to be more stable in the water environment than PV type 1 or hepatitis A virus (HAV).

a) Virus characteristics

In 1991 the first adenoviral particle was visualised by cryo-EM, upon which morphological knowledge is based (Stewart et al., 1993). As described earlier, AdVs are non-enveloped viruses containing a 25-45 kb ds DNA genome (Harrach et al., 2012). The capsid consists of 252 subunits and seven structural proteins (II, III, IIIa, IV, VI, VIII, and IX) and 10 copies of the AdV protease enzymes (Harrach et al., 2012).

The genome consists of overlapping transcriptional units which are located on both strands of the dsDNA (Figure 1.3). Coding regions of the genome are transcribed by cellular RNA polymerase II. Gene expression is divided into three phases, the early 5 genes (E1A, EIB, E2, E3 and E4), two early delayed transcription units (pIX and IVa2) and five late five transcription units (LI-L5) (Harrach et al., 2012). E1 proteins regulate cellular metabolism to make the cell more susceptible to virus replication, host cell defence mechanisms and activate the expression of E1, E2, E3 and E4. The E2 proteins are the machinery for replication of adenoviral DNA and ensure transcription of late genes. E3 facilitates apoptosis and release of progeny virions and E4 facilitates viral mRNA metabolism and shuts off host protein synthesis (Hay et al., 1995; Tollefson et al., 1996, Weigel and Dobbelstein, 2000). After E4 gene expression, the late genes

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which are mostly structural are then expressed followed by encapsidation and maturation of adenovirus particles (Ruuskanen et al., 2009; Harrach et al., 2012).

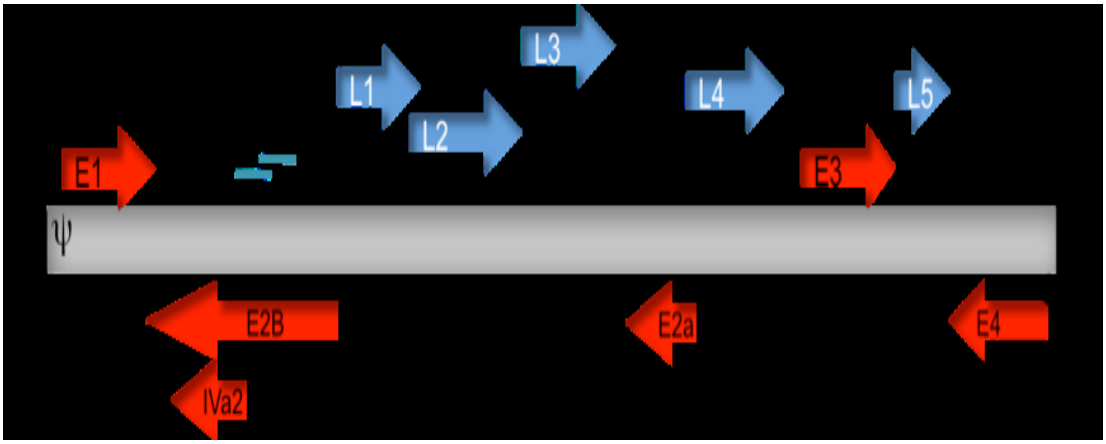

Figure 1.3: The adenovirus genome indicating transcriptional units (Rauschhuber, 2011)

b) Clinical features

According to the tropism HAdVs can infect a wide range of host cells which results in a diversity of diseases, including gastroenteritis, lower and upper respiratory tract infections and keratoconjuctivitis (Echavarria et al., 2003). It is responsible for 5-10% of lower respiratory tract infections (LRTIs) in infants and children (Jiang, 2006). Serotypes which are associated with LRTIs are AdV type 1 (AdV1), AdV2, AdV3, AdV5 and AdV7 (Kim et al., 2003). Severe acute respiratory illnesses have been associated with AdV1-AdV8, AdV19, AdV21 and AdV35 and others but outbreaks of infections in children are frequently reported to be caused by Ad7, followed by Ad3 and Ad21 (Hong et al., 2001). The virus is also associated with severe pneumonia, haemorrhagic cystitis and hepatitis in immunocompromised patients (Akiyama et al., 2001).

c) Epidemiology

The epidemiologic characteristics of HAdVs vary by type but all are either transmitted through direct contact, respiratory droplets, faecal-oral transmission or water. Some types cause asymptomatic persistent infection in the tonsils, adenoids and intestines of infected hosts. Other types (AdV8, AdV19 and AdV37) cause sporadic infection and outbreaks of keratoconjuctivitis. Conjunctivitis and febrile illness caused by AdVs is

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usually transmitted through water in swimming pools. Adenoviruses have been reported as the second most significant causes of viral outbreaks in recreational waters in the US and have been included in the EPA CCL (Sinclair et al., 2009). Enteric AdV types 40 and 41 are associated with infantile gastroenteritis. Human adenoviruses have been shown to be more prevalent in water and shellfish than enteroviruses, to be highly stable in the environment and to be highly resistant to different disinfectants including ultraviolet (UV) (Pina et al., 1998; Gerba et al., 2002; Thurston-Enquarez et al., 2005, Bofill-Mas et al., 2006). Human adenoviruses can be shed in faeces for months or years after infection. Adenoviruses have therefore been proposed as indicators of the presence of human faecal pathogens in the environment (Pina et al., 1998).

1.2.3 Hepatitis A virus

Hepatitis A virus was first identified in 1973 by immune EM (IEM) of faecal suspension samples from infected human volunteers (Martin and Lemon, 2006). It is a non-enveloped spherical, +ssRNA virus within the genus *Hepatovirus* and family *Picornaviridae* (Lemon, 1997; Hollinger and Emerson, 2001; Spradling et al., 2009). It is the only member of the genus *Hepatovirus.*

a) Virus characteristics

The RNA genome is packaged within an icosahedral protein capsid composed of 60 copies of each of the 3 major structural proteins VP1, VP2, and VP3. The genome is 7500 nucleotides and contains a single large ORF flanked by 5' and 3' end non-translated regions (NTR) (Konduru et al., 2009). The NTR is a complex structure that contains an internal ribosome entry site required for viral translation (Spradling et al., 2009). Hepatitis A viruses recovered from different parts of the world are antigenically similar indicating that there is only one serotype for HAV (Hollinger and Ticehurst, 1996). There are seven HAV genotypes, two major human HAV genotypes (I and III) and two minor genotypes (II and VII) documented so far (Spradling et al., 2009) according to the commonly used VP1-2A junction phylogenetic analysis (Spradling et al., 2009, Pintó et al., 2010). The most common genotype world wide is genotype 1

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which has the most commonly used cell culture-adapted Australian strain HM175 (genotype 1B) and the German strain GBM (genotype 1A)(Dotzauer, 2008).

Many features of HAV replication distinguish it from PV and other picornaviruses, for example, protracted slow course, low virus yields, and the propensity to establish persistent infections in cell culture as well adaptation to replicate in non-hepatic and non-primate origins (Feigelstock et al., 2005; Martin and Lemon, 2006). Growth of wild type HAV in cell culture is generally poor and the virus must undergo a process of adaption before efficient replication. Adaptive mutations that permit HAV to replicate efficiently have been extensively characterised (Martin and Lemon, 2006). Although there are a few highly cell-culture adapted HAV strains that are cytopathic that appear to cause cell death by inducing apoptosis, there is no cytopathic effect (CPE) in most HAV infected cells (FRhK-4 and MRC-5 cells)(Lemon et al., 1991; Brack et al ., 2002)

Hepatitis A virus is exceptionally stable at ambient temperatures and at low pH (Spradling et al., 2009), which explains why the virus survives in the environment and its transmission by contaminated foods and drinking water. Food and water have been identified as important vehicles for HAV transmission worldwide with outbreaks linked to faecally contaminated treated and untreated water (Koopmans et al., 2002). Hepatitis A virus has been shown to be resistant to free residual chlorine of 0.5-1.5 mg/ℓ and can survive for months in contaminated fresh water, seawater, sediments, wastewaters and soils (Hollinger and Emerson, 2001).

b) Clinical features

Hepatitis A was formerly called infectious hepatitis, epidemic hepatitis, epidemic jaundice, or catarrhal jaundice (Stapleton and Lemon, 1994; Dotzauer, 2008). After infection via the gastrointestinal tract HAV replicates in the liver for several (2-7) weeks and produce high titers of the virus in the hepatocytes, bile, stool, and to a lesser extent in the blood (Spradling et al., 2009). The clinical course of hepatitis is extremely variable with some patients showing no symptoms or jaundice (asymptomatic) and the patients are only recognized by biochemical or serological changes in their blood (Lemon, 1994; Hollinger and Ticehurst, 1996; Dotzauer, 2008). Generally the disease is divided into four clinical phases, which are preclinical or

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incubation period of about 50 d, where the patient is asymptomatic, followed by prodomal or pre-icteric phase which is characterised by loss of appetite, fatigue, abdominal pain, fever and diarrhoea and dark urine (Pintó et al., 2010). The third phase is called the icteric phase in which jaundice develops and total bilirubin levels rise to 20-40 mg/ℓ (Pintó et al., 2010). At this phase the disease can ultimately resolve, but occasionally there is extensive hepatic necrosis, with high fever, abdominal pains, jaundice and the development of hepatic encephalopathy associated with coma and seizures, symptomatic of fulminant hepatitis (Stapleton and Lemon, 1994). The convalescent period is where the hepatitis disease slowly resolves and the patient recovers. Faecal shedding of the virus which takes 3-6 weeks during the incubation period reaches its maximum just before the onset of hepatocellular injury where the patient is most infectious.

c) Epidemiology

Hepatitis A is generally acquired via the faecal-oral route by person-to-person contact or ingestion of contaminated food or water (Pintó et al., 2010). The highest prevalence of HAV transmission occurs where there are poor standards of water supply and sanitation particularly in developing countries where there are high patterns of endemicity, more than 90% of naturally acquired immunity before the age of 10 (Lemon, 1994; WHO, 2000; Spradling et al., 2009). Infections are also very common in places or institutions which are overcrowded like refugee camps. Although there is a lack of updated data on the global epidemiology of HAV, statistics from different regions of the world show a marked change in the epidemiology of hepatitis A with highest percentage of infections in their early childhood (Poovorawani et al., 2002; Spradling et al., 2009). A seroepidemiological study showed that at the age of 11-15 years 90% of people in Mexico and 91% in Dominican Republic have been infected compared to 54% in Argentina, 62% in Venezuela, 60% in Brazil and 70% in Chile (Tanaka, 2000), and by the age of 31-40 years over 80% of all the population in these six countries been exposed to HAV. The improvement in living standards and environmental hygiene in South-East Asia and China resulted in the lowering of HAV prevalence from high endemicity (85%-95%) to moderate and from moderate to low endemicity (Poovorawan et al., 2002; Spradling et al., 2009). Therefore endemicity is low in developed regions and high in underdeveloped regions (Pintó et al., 2010). A

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study to investigate the association of HAV seroprevalence and socioeconomic conditions in Turkey showed a seropositivity of 57.2% which is medium phase of HAV endemicity (Kaya et al., 2007). In the United States approximately 20 000 acute hepatitis A infections and approximately 100 deaths are occurring every year (Holmberg, 2012). The infectious dose of HAV is given to be one virion (Grabow, 1997), but the US Food and Drug Administration (FDA) suggests that the infectious dose of HAV is 10 to 100 viral particles (USEPA, 2005). This implies that very low levels of faecal contamination of water could pose a risk of infection.

1.2.4. Enteroviruses

Human enteroviruses (EVSs) can cause a variety of clinical illnesses including acute haemorrhagic conjunctivitis, aseptic meningitis, undifferentiated rash, acute flaccid paralysis (AFP), myocarditis, and neonatal sepsis-like disease (Bauer et al., 2002). Human EVs belong to the family *Picornaviridae*, which consists of non-enveloped viruses containing 7.5 kb +ssRNA genome enclosed in an icosahedral capsid (Nasri et al., 2007) which comprises 60 identical subunits each of which contains four of the structural proteins (Solomon et al., 2010). They are called EVs because they replicate in the human gastrointestinal tract (GI) (Romero, 2008). There are more than 80 serotypes of human EVs based on neutralisation tests, but with the use of molecular biology techniques like sequencing new strains are being discovered (Oberste et al., 2006).

Traditional classification of EVs according to their pathogenicity in humans and cytopathic characteristics in tissue culture studies has been replaced by a new classification which applies molecular biology (phylogenetic analysis of multiple genome regions) and biological properties which recognises at least 90 subtypes divided into four species (A-D)(Palacios and Oberste, 2005), which include echoviruses, coxsackieviruses A (CV-A) coxsackievirus B (CV-B) and three types of PVs and numbered serotypes of EVs. The international Committee on Taxonomy of Viruses (ICTV) current criteria for species demarcation of EVs requires that members of EV species share greater than 70% amino acid identity in the VP1 coding region and 2C

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and 3CD, share limited range of natural hosts and host receptors, and not more than 2.5% variation in guanine-cytosine (G-C) content as well as compatibility in replication and genetic recombination (Romero, 2008). This current criterion has resulted in the reclassification of the EVs into new groupings like human EV-D (68, 70) as opposed to the old EV-D (68-71) and recombinants like the vaccine-derived polioviruses (VDPVs) (Kew et al., 2005; WHO, 2008; Romero, 2008).

a) Virus characteristics

The lack of an envelope confers stability to EVs to harsh gut conditions like gastric acid, and environmental conditions as found in surface and groundwater (Romero, 2008). Studies have shown that EVs are resistant to organic solvents like chloroform and ether, alcohol and can be inactivated at temperatures higher than 56°C (Romero, 2008). The EV genome encodes four different capsid or structural proteins known as VP1, VP2, VP3 and VP4 (Figure 1.4)(Kew et al., 2005; Okoh et al., 2010) and seven non-structural proteins involved in replication and maturation.

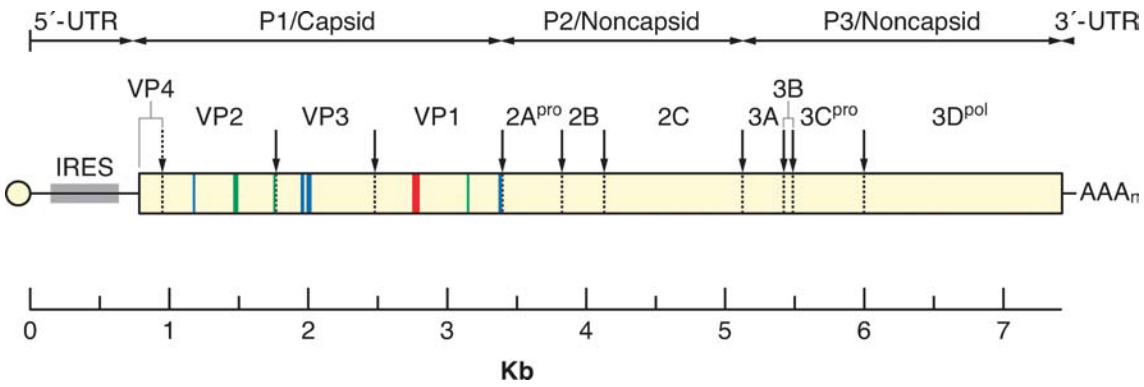

Figure 1.4: Schematic diagram of poliovirus, a typical EV (Kew et al., 2005).

Enteroviruses use arginine-glycine-aspartic acid (RGD) motif on the capsid proteins for attachment to receptors of human cells. Seven different receptors for different EVs have been identified so far, namely PV receptor (PVR called CD155), three integrins, decay accelerating factor (DAF called CD55), the coxsackievirus-adenovirus receptor (CAR) and intracellular adhesion molecule 1 (ICAM-1) (Palacios and Oberste, 2005). The primary site of infection is the epithelial cells of the respiratory or gastrointestinal tract, where they initially replicate without any detectable pathology (Hovi et al., 2007; Romero, 2008). From the primary sites in the mucosae, the virus may enter the

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cervical and mesenteric lymph nodes and then into the blood stream resulting in a brief or transient viremic phase. In the case of PV, target tissues of secondary infection include the motor neurons of the central nervous system (CNS), resulting in paralytic symptoms (Hovi et al., 2005). However, more than 99% of PV infections do not result in paralysis. Other EV infections result in aseptic meningitis, myocarditis and generalised neonatal infection (Kim, 2010).

b) Clinical features

Enteroviruses are a leading cause of aseptic meningitis in Europe (Ortner et al., 2009). Hand, foot and mouth disease (HFMD) is frequently caused by coxsackievirus type A16 and enterovirus 71 (EV71) (Rhoades et al., 2011). Hand, foot and mouth disease is mild and self-limiting rash-associated illness (Wang et al., 2012). Enterovirus 71 is considered the most dangerous EV after the eradication of PV from most parts of the world (Chan et al., 2003; Ortner et al., 2009; Rhoades et al., 2011). Enterovirus 71 has been reported to cause many outbreaks worldwide, including the outbreaks in Shandong and Fuyang in China in 2007 and 2008 which were characterised by severe neurological disease such as acute flaccid paralysis, pulmonary oedema, myocarditis, and fatal encephalitis (Wang et al., 2012). Enterovirus 68 (EV68) is unique in the genus *Enterovirus* as it shares clinical characteristics with rhinoviruses such as the infection of the respiratory tract (Lauinger et al., 2012). Apart from respiratory tract infections EV68 has been implicated in some rare cases of fatal central nervous system infection (Oberste et al., 2004).

c) Epidemiology

In the temperate Northern Hemisphere EVs are commonly referred as summer viruses because they occur during the warmer months, but in the tropical regions they occur all year round without seasonal variation (Zaoutis and Klein, 1998; Ortner et al., 2009). Humans are the only known natural hosts for EVs. The faecal-oral route is the most common mode of transmission but respiratory and oral-to-oral transmission is also possible. The most important risk factor in the transmission of EVs is poor water supply and sanitation, crowded lower socioeconomic living conditions (WHO, 2010). A study to investigate the distribution of EVs causing neurological disease in Austria showed that more than 90% of cases were children under the age of 14 years (Ortner et al., 2009).

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1.2.5 Human rotaviruses

Rotaviruses (RVs) are the leading cause of severe dehydrating diarrhoea in children under the age of five worldwide and is estimated to be responsible for approximately 527 000-611 000 deaths annually (Carvalho-Costa et al., 2011; Ogilive et al., 2011). More than 85% of the deaths occur in low income countries in Asia and Africa and above two million children are hospitalised every year with pronounced dehydration (Parashar et al., 2009; Ogilive et al., 2011; WHO, 2011b). Recently vaccines have been developed and licensed for immunisation against the virus. Data from clinical trials which evaluated the vaccine efficacy has led the WHO Strategic Advisory Committee on Immunisation to recommend inclusion of rotavirus in the routine national immunisation programmes (WHO, 2011b).

a) Virus characteristics

Rotavirus is a non-enveloped virus which has a triple layer capsid which encloses a genome composed of 11 segments of double-stranded RNA (dsRNA). The virus particle is 100 nm in diameter and genome organised into six structural genes (VP1-4 and VP6-7) and non-structural genes (NSP1-NSP6). Two structural proteins VP7 (the glycoprotein called G protein and VP4 (the protease-cleaved protein/protease sensitive protein or P protein) make up the outer shell and are important for vaccine development (Figure 1.5) (Carvalho-Costa et al., 2011; Kargar et al., 2011). These outer coat proteins (VP7 and VP4) resulted in the classification of rotavirus into subtypes G and P types (Alam et al., 2009) which gives the genotypic designation of rotavirus a binary system (Carvalho-Costa et al., 2011). The virus has substantial diversity with a possibility of 132 G-P separate combinations. Currently seven groups designated A-G have been distinguished in the rotavirus strains based on the antigenicity of the VP6 protein (Gosh et al., 2010).

Rotaviruses are shed in very large quantities in the faeces of infected individuals, with quantities as high as 10^{11} virus particles/g stool (Gratacap-Cavallier et al., 2000).

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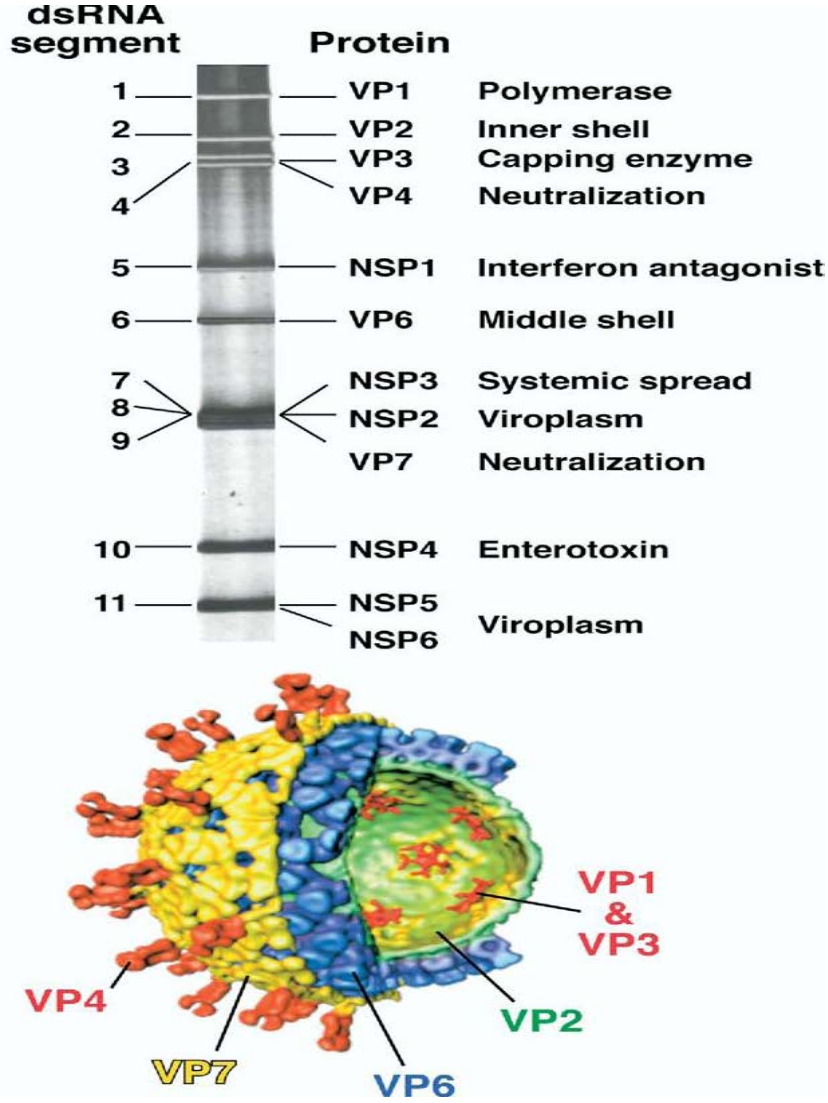

Figure 1.5: Structure of rotavirus (Greenberg and Estes, 2009)

The virus has key properties that are important to the survival as gastrointestinal pathogens. For example, they have a triple-layer capsid, which is very stable and well suited for faecal-oral transmission. The stability of the virus allows it to persist for long periods in the environment; therefore large amounts are present in wastewaters and environmental waters (Greenberg and Estes, 2009). Due to their physicochemical characteristics, there is suspicion that certain treatments of water for human consumption may not be completely effective against RV.

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b) Clinical features

Infection by RVs can be symptomatic or asymptomatic depending on the age of the host. In neonates rotavirus infection is rarely symptomatic due to transplacental transfer of maternal antibodies, but severe rotavirus-induced disease ranges from 3 months to 2 years. Although rotavirus is shed in faeces in large quantities limited infectivity studies have indicated that ten or fewer particles can cause infection (Glass et al., 2001). Virulence is multigenic and has been shown to be associated with genes, 3, 4, 5, 9 and 10 (Greenberg and Estes, 2009). Diarrhoea is the main clinical manifestation of RV in infants and young children. The virus primarily infects intestinal villus enterocytes, resulting in malabsorptive diarrhoea emanating from the destruction of absorptive enterocytes, virus-induced down regulation of the expression of absorptive enzymes and functional changes in tight junctions between enterocytes leading to cellular leakage (Greenberg and Estes, 2009).

c) Epidemiology

Recent worldwide surveillance data from CDC showed that 40% of the 62 584 hospitalisations for diarrhoea were caused by rotavirus infection. It is also estimated that rotavirus causes 114 million diarrhoeal episodes, 2.4 million hospitalisations and 600 000 deaths in children under the age of 5 years (Grimwood and Buttery, 2007; Parashar et al., 2006). Studies from Western Europe found that 50% of cases of gastroenteritis in children younger than 5 years of age treated in an emergency department were caused by RVs (van Damme et al., 2007). In the US RV is estimated to cause 20-60 deaths, 55 000-70 000 hospitalisations and 410 000 physician visits annually. An estimated 110 000 to 150 000 children younger than 5 years of age on the African continent die annually due to RV infection (Parashar et al., 2003).

1.3 ROLE OF WATER IN THE TRANSMISSION OF VIRUSES

Waterborne viruses are responsible for several outbreaks of gastroenteritis, respiratory diseases neurological diseases, hepatitis, paralysis and skin infections (Wolfaardt et al., 1995; Griffin et al., 2003; Lindsay et al., 2008). Their presence and persistence in environmental water has become a public health hazard (Dongdem et al., 2009; Okoh et al., 2010). Human infection by waterborne enteric viruses occur

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through contaminated food, drinking and recreational waters (Taylor et al., 1995; Okoh et al., 2010). Enteric viruses replicate in the gut of human beings and then they are shed into the sewage and they disperse into the environment if sewage is not adequately treated (Dongdem et al., 2009). Research has shown that enteric viruses are more resistant to common disinfectants than bacterial indicators like *Escherichia coli* and *Enterococcus faecalis* (Cho et al., 2000; Pusch et al., 2005). Most of the epidemiologic studies of risks of recreation water such as swimming focused on indicator bacteria, rather than the pathogens associated with waterborne illness as predictors of health outcome (Aslan et al, 2010). The bacterial indicators are limited as predictors of viral pathogens in recreational water due to lack of correlation with viral persistence and their growth in sediments, sand and algae (Byappanahali and Fujioka, 1998, Miagostovich et al., 2008, Verhougstraete et al., 2010).

The USEPA recommends the use of an additional criterion for the evaluation of water disinfection based on viral inactivation (Thurston-Enriquez et al., 2005). Depending on the level of treatment virus levels in treated waste waters measured by cell culture range from 1.0×10^{-3}-1.0×10^{2}/ℓ (Griffin et al., 2003). The release of inadequately treated waste water containing viruses has a direct impact on the microbiological quality of surface waters and consequently the drinking water derived from it and this poses a risk of infection to consumers (Okoh et al., 2010). Ehlers et al., (2005) found that 29% of river water samples and 19% of drinking water samples in South Africa (SA) were positive for EVs. Due to their small size enteric viruses can infiltrate soils from sources of contamination such as burst sewage pipes and septic tanks and contaminate groundwater sources like aquifers and wells (Okoh et al., 2010). In one study in the US human enteric viruses were detected in 72% of groundwater sites and about 80% of waterborne of gastroenteritis (Griffin et al., 2003).

The most well documented waterborne outbreaks of viral gastroenteritis are related to NoV (Hewitt et al., 2007) and from 1995 to 2000 NoV gastroenteritis was responsible for more than 85% of all nonbacterial gastroenteritis reported in Europe (Lopman et al., 2003). The NoV is robust and will survive in water and shellfish where it may cause a public health problem (Lees et al., 1995; He et al., 2010). The virus has been found to

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be infectious to humans after remaining in water for two months and intact virus capsid can be detected for over three years (Seitz et al., 2011). Numerous NoV outbreaks have originated from sewage polluted drinking water and recreation water (Hewitt et al., 2007; Sartorius et al., 2007). Unintentional ingestion of contaminated recreational water during swimming can lead to gastrointestinal illness (Pruss, 1998). Surveillance of AdVs and NoVs in European recreational waters showed that almost 40% of bathing water samples were virus positive, implying a possibility of public health risk from bathing (Wyn-Jones et al., 2011). Seafood from filter feeders such as oysters that are harvested from contaminated waters become sources foodborne transmission of NoV (Rutjes et al., 2006; La Rosa et al., 2007). In a three year study carried out in Gauteng SA, NoV contamination was detected in 66% of Klip River samples, 83-100% of Rietspruit samples and 12.5–33% of the Suikerbosrant River (Mans et al., 2013). This data showed a high degree of NoV environmental pollution as a result of sewage contamination. In a study done on the Llobregat River in Spain, a source for drinking water for the Barcelona urban area, high numbers of NoV genome copies of up to 10^6 were detected (Peréz-Sautu et al., 2011). Epidemiological and molecular analyses indicated that a large scale outbreak of NoV gastroenteritis in a school in Guangdong, China was caused by consumption of contaminated well water (Zhou et al., 2012). From 1999 to 2002 waterborne viruses were responsible for 14% of outbreaks and 38% of illnesses-associated with drinking water in the US, and NoVs were responsible for 6% outbreaks and 17% illnesses associated with recreational water (Lambertini et al., 2008). Norovirus RNA has also been detected in commercially available bottled natural mineral waters (Beuret, 2003). A study carried out in Japan showed that 100% of influent and 57% of effluent wastewater samples were positive for SaV (Kitajima et al., 2011). Sapoviruses have also been detected in water sources in Spain (Sano et al., 2011) and SA (Murray et al., 2013). The study carried out in five provinces of SA showed that 37/51 (72.5%) of wastewater samples were positive for SaV with concentrations ranging from $4.24 \times 10^3 – 1.31 \times 10^6$ copies/ mℓ (Murray et al., 2013).

In North Carolina, an outbreak of acute hepatitis A was linked to drinking water from shallow contaminated spring water by phylogenetic analyses of HAV genomic

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sequences (Lindsay et al., 2008) and contaminated river water was identified as a source of hepatitis A infection in canoeists in SA (Taylor et al., 1995). Hepatitis E virus (HEV) is the second most important cause of acute clinical hepatitis in adults throughout Asia, the Middle East and Africa (Ashbolt, 2004). A study in India indicated that 70% of HEV infections are transmitted through contaminated water and 20% are due to foodborne transmission (Gerba and Rose, 2003). Hepatitis E virus is principally the result of waterborne infection in developing countries and is thought to be spread zoonotically in industrialised countries (Purcell and Emerson, 2008). It is therefore important for water authorities to have a sensitive and rapid method for the detection of enteric viruses in water, including bottled waters, as a virological water quality monitoring tool for public health (Brassard et al., 2005). For outbreaks linked to drinking water for example, detection of the virus in water is useful to provide timely information on prevention and control measures (Lindsay et al., 2008). Other enteric viruses such as RVs, astroviruses (AstVs) and SaVs have occasionally been implicated in waterborne outbreaks of gastroenteritis (Villena et al., 2003).

The virological analyses of water plays an important role in research on the incidence and behaviour of viruses in the water environment and the data is used for the evaluation on the efficiency of water treatment and disinfection processes (Dechesne and Soyeux, 2007), routine monitoring to test the compliance with guidelines and specifications (WHO, 2004). In addition the information is necessary to study the public health impact of waterborne viral infections and for the identification of strains isolated from water as a tool to study epidemiology of viral infections (Bosch et al., 2008). The preservation of water quality and prevention of waterborne disease is a complicated task (Meinhardt, 2006) with the detection and quantification of viral pathogen levels in water rarely performed because of the technical challenges involved in viral assays (Bosch et al., 2008).

1.4 RECOVERY OF VIRUSES FROM WATER

The analysis of viruses in food and environmental samples is complex (Wyn-Jones, 2007; Mattison and Bidawid, 2009; Bosch et al., 2011). One of the major difficulties

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encountered in the detection of viruses in water sources is the occurrence of low virus particle numbers in water (Soule et al., 2000; Rutjes et al., 2005). The analysis of water for enteric viruses is a two stage process: the first step is to apply efficient viral recovery and concentration procedures from large volumes (10–1000 ℓ) of water (Köster *et al.,* 2003; Gerba, 2007), thereafter, a range of isolation and/or detection methods may be applied (Wyn-Jones and Sellwood, 2001; Wyn-Jones, 2007; Mattison and Bidawid, 2009; Bosch et al., 2011). Important criteria to be considered when concentrating viruses from water are: i) the technique should be easy to complete in a short time; ii) have high virus recovery rate; iii) should concentrate large range of viruses; iv) be less costly; v) be capable of processing large volumes of water; and vi) be repeatable within a laboratory. For this reason, different methods have been used for the recovery of viruses from water in different studies. Each method has its own variations, advantages and disadvantages (Wyn-Jones, 2007). The cost of virus recovery technologies is a very important issue, particularly in resource poor settings like Africa and other low-income countries. The recovered and concentrated viruses are then detected using different methods like conventional cell culture techniques, conventional qualitative molecular-based assays such as nucleic acid hybridisation and polymerase chain reaction (PCR), and qualitative or quantitative real-time (*rt)* RT-PCR/PCR. In order to monitor the virological quality of water an efficient combination of techniques has to be assessed for optimal recovery and detection of low virus titres in water (Soule et al., 2000; Wyn-Jones, 2007).

A number of concentration methods have been used in viral recovery studies with recovery efficiencies as high as 100% (Hill et al., 2007). Based on the different properties of the viruses four main principal approaches are used for viral recovery and concentration. These include ultrafiltration, ultracentrifigation, and two-phase separation with polymers or flocculation, and adsorption-elution using filters, membranes, glass wool or glass powder (Vilaginés et al., 1993; Wyn-Jones, 2007; Albinana-Gimenez et al., 2009).

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1.4.1. Entrapment by ultrafiltration

Entrapment by ultrafiltration recovers viruses from water samples based on particle size (Wyn-Jones and Sellwood, 2001; Wyn-Jones, 2007). More recent technologies involve passing the water sample through the capillaries, or membranes, or hollow fibres with pore sizes that permit passage of water and low molecular mass solutes but exclude viruses and macromolecules (Mattison and Bidawid, 2009). The advantages of ultrafiltration are principally that the sample requires no preconditioning and that a wide range of viruses, including bacteriophages, can therefore be recovered (Wyn-Jones and Sellwood, 2001; Wyn-Jones, 2007) and the efficiency of recovery is usually good (Hill et al., 2007; Jones et al., 2009). The main disadvantage of the method is the high cost of the equipment and that turbid waters take a relatively long time to process (Huang et al., 2000).

1.4.2. Ultracentrifugation

Ultracentrifugation is capable of concentrating all viruses in a sample provided sufficient gravitational force is applied. It is sometimes described as a catch-all method (Wyn-Jones and Sellwood, 2001, Wyn-Jones, 2007). Ultracentrifugation has been used for recovery of viruses from water, fruits, meat and shellfish (Mattison and Bidawid, 2009). However, the method has not been widely used because it co-concentrates inhibitors that interfere with the efficiency of detection, but works well when the sample is pure (La Rosa et al., 2009). Another disadvantage of the method is that the equipment is highly specialised, expensive and only a limited volume of water can be processed.

1.4.3. Two-phase separation, flocculation and immuno-capture

Two-phase separation, flocculation, hydro-extraction or immune-capture methods can be applied (Wyn-Jones, 2007). None of these methods meet all the necessary requirements for the efficient recovery of viruses from large volumes of water.

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Immuno-capture, however, has the benefit of removing RT-PCR inhibitors (Wyn-Jones, 2007).

1.4.4. Adsorption-elution

The adsorption-elution method, based on the ionic properties of the viruses, is widely applied in the primary concentration of enteric viruses from water. Due to the unique surface properties of viruses, they readily adsorb to a number of substances including starch, minerals, fabrics alumina gel and various resins (Powell et al., 2000). The adsorption-elution theory stems from the work of Wallis and Melnick in 1967 who reported the first application of virus adsorbing filters when they described a method for concentrating viruses from sewage (Wallis and Melnick, 1967; Powell et al., 2000; Wyn-Jones, 2007). The principle is that virus containing sample is brought in contact with solid matrix to which virus will adsorb under specific pH and ionic strength. When the virus is adsorbed, the residual water is discarded. Thereafter the virus is released from the matrix by elution into a smaller volume, usually one tenth of the original volume. The choice of the adsorbing matrix, eluting fluid and processing conditions are influenced by the nature of the sample. Adsorption and elution using charged membranes or filters is applied worldwide for the concentration of different enteric viruses (EVs, PVs, RVs, HAV, NoVs, AdVs and bacteriophages) (Lambertini et al., 2008; Albinana-Gimenez et al., 2009).

a) Electronegative membranes and filters

The virus is bound to the filters by electrostatic forces and not by size exclusion. The virus containing sample is passed under positive pressure or vacuum through a cellulose nitrate membrane. Since the viruses and filter materials are both negatively charged at neutral pH, the water samples have to be preconditioned by adjusting the pH to pH 3.5 with the addition of aluminium or magnesium ions to allow electrostatic binding (Wyn-Jones, 2007). Viral recoveries using negatively-charged membranes range from 60% to 70% with river water (Wyn-Jones, 2007), they are less expensive and can be obtained in a sterile cartridges in a disposable form. The membranes however are prone to clogging and cannot be used for turbid waters (Gerba, 1987).

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b) Electropositive membranes and cartridges

These membrane and cartridges are commonly used for the recovery of enteric viruses from water and other materials without prior conditioning of the sample because most enteric viruses are negatively charged at ambient pH (Haramoto et al., 2004; Gassilloud et al., 2007). The other advantage of the electropositive membranes is the ability to recover RVs and coliphages which are sensitive to the low pH needed for electronegative membranes (Kim et al., 2008). Viral recoveries from the electropositive filters are comparable to the electronegative filters (Sobsey and Jones, 1979), but they have low recovery rates for viruses from marine waters and they clog easily (Lukasik et al., 2000).

c) Glass wool

The use of glass wool for the recovery and concentration of enteric viruses from water is an economic alternative to the microporous filters and charged membranes. The technique was pioneered in France by Vilaginés and colleagues, 1993 who applied it to the concentration of a range of viruses from drinking, surface and waste waters (Gantzer et al., 1997; Wyn-Jones, 2007). Oiled sodocalcic glass wool, packed in holders at a density of 0.5 g/cm^3, is washed with 1 M hydrochloric acid (HCl), sterile distilled water, 1 M sodium hydroxide (NaOH) in sequence and finally with sterile distilled water again to bring the glass wool to neutral pH before the sample is passed through the filter (Vilaginés et al., 1993; Wyn-Jones et al., 2011). Elution is done using a solution of buffered beef extract or skimmed milk, both at high pH (pH 9.5) so that the negatively charged viruses adsorbed on the matrix would be displaced by the buffer. The only pre-treatment necessary is dechlorination for the recovery of viruses from treated drinking water (Lambertini et al., 2008). Glass wool has been used to recover viruses from wastewater (Gantzer et al., 1997), treated and untreated drinking water (Vilaginés et al, 1997, Vivier et al., 2004, Ehlers et al., 2005, van Heerden et al., 2005), groundwater (Powell et al., 2000, van Zyl et al., 2004, 2006; Ehlers et al., 2005) and river water (Taylor et al., 2001; van Heerden et al., 2005). Compared to charged membranes glass wool is cheap with a good efficiency of recovery (EOR) for a wide range of enteric viruses, e.g. 29% for NoV from drinking water (Lambertini et al., 2008), 56% for NoV in treated wastewater (Gantzer et al., 1997), 3.4% for NoV from acidified fresh water. The data available show that the highest recoveries using glass wool were

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obtained using seeded EVs in drinking water, with 102.5% for PV (Gassilloud et al., 2003), 75% for coxsackie B4 (Vilaginés et al., 1993). An EOR of 87% was reported for the recovery of simian RV SA11 from estuarine waters and while the EOR for human RV (WA) from fresh water was 77% (Lewis and Metcalf, 1998). These studies however, used different quantities of glass wool, different volumes of water matrices, different volumes of elution buffer, as well as different virus quantification methods (Lambertini et al., 2008). There is therefore a need for more standardised data on the EOR of enteric viruses from both surface and drinking waters for quantitative virus monitoring and risk and exposure analyses.

The glass wool recovery of viruses from large volumes of water is a primary process and the volume is too large for direct detection and also contains some inhibitors which compromise viral assays such as cell culture-based plaque assays and molecular-based assays. Therefore there is need for secondary concentration which reduces the volume of the eluate as well as getting rid of the possible inhibitors which may interfere with the detection methods. Many methods for reconcentration/secondary concentration of viruses from various eluates are available. Most are based on acid precipitation (Mattison and Bidawid, 2009) and organic flocculation (Wyn-Jones, 2007). One disadvantage for these two methods is operating pH range for the procedures (pH 3.0-4.5). The low pH compromises the viability of viruses like RVs (Mattison and Bidawid, 2009). Polyethylene glycol (PEG_{6000}) precipitation was found to be an effective secondary concentration method for detection of human enteric viruses (Lewis and Metcalf, 1998; Vilaginès et al., 1997; Mattison and Bidawid, 2009). The advantage of PEG is that it is simple and inexpensive, non destructive to viruses (Mattison and Bidawid, 2009) and has been applied to concentrate many different enteric viruses, namely PVs, RVs, HAV and NoVs from water (Huang et al., 2000). One of the best advantages of PEG is the ability to obtain precipitation at neutral pH and high ionic concentration (Wyn-Jones, 2007). Part or all of the secondary concentrate may be used to detect the virus.

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1.5 DETECTION OF VIRUSES IN WATER

1.5.1 Viral isolation

There are several approaches to detection of viruses from water and other environmental samples (Wyn-Jones, 2007; Mattison and Bidawid 2009, Bosch et al., 2011). Isolation in cell culture and enumeration by plaque assay is the gold standard for infectious virus quantification (Mattison and Bidawid, 2009). Tissue culture infectious dose ($TCID_{50}$) or most probable number (MPN) units may also be used to quantify the viruses (Wyn-Jones, 2007). Viruses that do not produce CPE can be detected by immunofluorescence staining. However viral culture is costly, labour intensive and time consuming, taking several days or weeks making it not very suitable for routine monitoring of viruses in environmental samples (Rutjes et al., 2005). Cell culture can be used in conjunction with molecular-based methods such as integrated cell culture-PCR/RT-PCR with consequent increase in molecular detection (Bosch et al., 2011).

1.5.2 Direct detection

Recovered and concentrated viruses can be detected by EM, immunological- or molecular-based assays.

1.5.2.1 *Electron microscopy and immunological-based detection assays*

Viruses are usually present in low numbers in water (Soule et al., 2000), therefore direct detection by EM, IEM or enzyme immunoassay (EIA) is limited by the sensitivity of these assays. Their detection limit is too high (10^5 particles or more per mℓ) to be used for detection of viruses from water (Mattison and Bidawid, 2009).

1.5.2.2 *Molecular-based detection assays*

Molecular methods have permitted faster detection and have become very useful for viruses that cannot be propagated in cell culture (Dubrou et al., 1991). Molecular methods have the highest degree of sensitivity and retain a good specificity and are mostly applied in food and environmental samples (Mattison and Bidawid, 2009). The increased sensitivity of molecular-based assays enables the detection of low numbers

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of enteric virus pathogens in the water. The PCR assay is now widely used to monitor the presence of virus contamination in water samples (Huang et al., 2000; Rodríguez-Lázaro et al., 2012). Quantitative real-time (*rt)* PCR which is faster, more reliable and more efficient than conventional PCR is used more often for monitoring viruses in environmental samples (Beuret, 2004; Gregory et al., 2006; Bosch et al., 2011; Rodríguez-Lázaro et al., 2012).

First introduced in 1992 by Higuchi and co-workers, *rt* RT-PCR/PCR allows precise quantification of nucleic acids in a complex mixtures even if the starting amount of nucleic acid is in very low concentrations (Fraga et al., 2008). The *rt* PCR measures the amount of amplicon produced at each cycle of amplification using fluorescence based technology (Guo et al., 2009; Rodríguez-Lázaro et al., 2012). The amplicon is measured in 'real time' or as it is being produced by labelling and detecting the accumulating product with a fluorescently tagged substrate during amplification procedure (Kricka, 2002; Forlenza et al., 2012). In *rt* RT-PCR/PCR the amplicon is measured early in the reaction during the exponential phase of PCR when amplification is proceeding most efficiently (Fraga et al., 2008). Therefore *rt* PCR has increased speed due to reduced cycle number, is more sensitive due to higher sensitivity of fluorescent dyes used for the detection of the amplicon and more specific by use of DNA/RNA strand specific probes like Taqman or molecular beacons (Gregory et al., 2006; Fraga et al., 2008; Rodríguez-Lázaro et al., 2012). Quantitative *rt* RT-PCR/PCR (*rt* qRT-PCR/PCR) provides numerical data on the presence of enteric viral genomes which is important in estimating the public health risks of low levels of enteric viruses in environmental samples (Rutjes et al., 2005).

Real- time RT-PCR/PCR is a multi-stage protocol that requires high quality nucleic acid extraction/purification of RNA/DNA, optimal conversion of RNA to cDNA and accurate detection of PCR products. One of the most important obstacles to successful PCR-based analysis of environmental samples is co-purification of the nucleic acid with inhibitory compounds like polysaccharides, humic acids, fulvic and tannic acid present in environmental samples (Gregory et al., 2006; Gibson and Borchardt, 2013). The presence of these organic acids in purified nucleic acids inhibits RT and or polymerase

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reactions (Dubrou et al., 1991). It is therefore important that nucleic acid extraction systems applied must get rid of all possible inhibitors. A variety of 'home-brew' and kit-based extraction systems are available which rely on ethanol to precipitate nucleic acids (Kim et al., 2008). Some of the more modern extraction methods are based on the Boom principle of binding nucleic acids to silica beads for additional purification (Boom et al., 1990) and removal of inhibitors (Dubois et al., 2007). However there is no nucleic acid extraction method that removes all the inhibitors in the environmental samples. As a result the *rt* PCR/RT-PCR analyses for environmental samples which are easily affected by inhibitors have to find ways to mitigate the effect of inhibitors on the assays (Bosch et al, 2011). Several methods for the removal or relief of *rt* RT-PCR/PCR inhibition of virus detection in water sample concentrates have been evaluated (Opel et al., 2009; Gibson et al., 2012; Rodríguez-Lázaro et al., 2012). The primary way is to improve sample concentration and nucleic acid extraction and purification techniques to avoid co-concentrating inhibitors and ablate extracted inhibitors (Gibson et al., 2012). Gel filtration resins like Sephadex or Sepharose are generally effective in removing inhibitors but there is loss of nucleic acids in the process (Gibson et al., 2012; Rodríguez-Lázaro et al., 2012). To avoid loss of nucleic acids that can arise from chromatographic techniques and binding resins, bovine serum albumin (BSA) has been added to PCR mixtures to reduce inhibition by binding to inhibitors (Rutjes et al., 2005; Bosch et al., 2011; Gibson et al., 2012). Methods including the use of internal or external controls are also available for identifying and monitoring inhibition (Fraga et al., 2008; Lambertini et al., 2008; Albinana-Gimenez et al., 2009; Gibson et al., 2012). Another approach to identify inhibition is to analyse shifts in the amplification efficiency of *rt* qRT-PCR/PCR reactions (Rodríguez-Lázaro et al., 2012). The presence of inhibitors is shown by a shift or increase in the quantification cycle (Cq) which is the cycle at which target nucleic acid has been amplified enough to reach a defined threshold (Gibson and Borchardt, 2013). In some situations it may be adequate to identify samples containing inhibitors from their flat curves or from the complete absence of amplification products (Saunders, 2008). Inhibition makes interpretation of public health risk of enteric virus pathogens difficult, because inhibition can result in underestimating exposure and consequently health risk (Gibson et al., 2012).

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Since enteric viruses are present in low quantities in water samples, effective quality assurances and quality control are required to avoid false positives and false negatives (Bosch et al., 2011). To generate useful quality, reliable and comparable data with *rt* RT-PCR/PCR it is important to monitor the efficiency and accuracy of the critical steps in the recovery and detection assays which are acceptable to regulatory authorities (Bosch et al., 2011). In order to monitor the critical steps in the recovery and molecular detection of enteric viruses in water and other environmental samples, process controls were introduced (da Silva et al., 2007; Comelli et al., 2008; Mattison et al., 2009; Pintó et al., 2009). The process control should have similar features to the target virus, it must not be associated or naturally present in water or food samples and must not be infectious to human beings and must be added to the sample prior to processing (Mattison and Bidawid, 2009). The process control is therefore co-recovered, concentrated, co-extracted with the target enteric virus and then detected in the same nucleic acid extract (da Silva et al., 2007; Mattison and Bidawid, 2009). Feline calicivirus and mengovirus have been proposed as process controls for recovery, concentration and detection of enteric viruses from food and environmental samples (Costafreda et al., 2006; Pintó et al., 2009).

To ensure accuracy of data generated by *rt* qRT-PCR/PCR quality control has to be done on all critical reagents including optimisation. Primers are critical reagents in PCR analyses, hence appropriate concentrations need to be ensured to enhance detection of PCR products (Fraga et al., 2008). Too high concentrations lead to non-specific products such as primer-dimers. Tests are usually done with varying amounts of each primer where optimal concentrations which may not be the same for both primers are established. The primer concentrations with the lowest cycle threshold (Ct)/crossing point (CP) values should be selected since lower Ct values correspond to efficient production of the amplicon (Fraga et al., 2008; Saunders, 2008). To verify the specificity of the primers in the optimisation process, PCR products are run on the gel where one band will be visible when primers are specific. Using *rt* thermocyclers, amplification specificity is verified by melting point analysis. This is done using the melting curve analysis feature, where target amplicons are distinguished from PCR

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artefacts like primer dimers and misprimed products. Melting point analysis is also done to optimise primer annealing temperature if it becomes necessary.

When *rt* RT-PCR tests are performed, quality control steps are essential to exclude false positive and false negative results (Bosch et al., 2011). Negative and positive controls are included in each run. Depending on the analyses, at least one negative control must be included. A negative control, where a run includes the master mix without template, confirms that PCR reagents are not contaminated when the result is negative (Saunders, 2008). If the no template control becomes positive, it means that one or more reagents are contaminated with template or previously amplified products (Fraga et al., 2008). A control with no reverse transcriptase helps the detection of contaminating DNA in the RNA. A positive control makes sure that all reagents are working properly. The positive control could be quantified DNA or RNA containing the target sequence (Fraga et al., 2008).

Quality and reliability of *rt* qRT-PCR/PCR for enteric viruses from environmental samples depends on the correct setting of thresholds for detection. When setting thresholds, the level of fluorescence signal that is sufficiently above background/ noise to be considered reliable is determined (Fraga et al., 2008). The cycle at which the threshold is met or exceeded is called the Ct value and is used to make comparison between samples (Bernard and Wittwer, 2002; Watzinger et al., 2004; Fraga et al., 2008; Saunders, 2008). The point at which the product's detected fluorescence crosses the threshold is called Ct or CP value. It is important that thresholds are set to allow detection of amplicons when amplification is in the exponential phase (Bernard and Wittwer, 2002; Fraga et al., 2008; Saunders, 2008; Forlenza et al., 2012). If the thresholds are too low, it results in pre-mature 'detection' of the amplicon and too high thresholds results in detection of the product above the exponential phase leading to inaccurate quantification of the virus genome copies (Saunders, 2008).

There are two methods of *rt* qRT-PCR/PCR, namely absolute quantification and relative quantification (Forlenza et al., 2012). Absolute quantification results in a measure of the amount of target sequence in the sample expressed as genome copies (Saunders,

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2008; Martinez-Martinez et al., 2009). Relative quantification provides a relative value expressed as ratio of initial target sequence between control and experimental treatments (Bernard and Wittwer, 2002; Saunders, 2008; Forlenza et al., 2012). Absolute quantification requires generating a standard calibration curve using a standard that has been carefully quantified as to the copy number (Fraga et al., 2008; Forlenza et al., 2012). This approach is more accurate but is more labour intensive. Accurately quantified standard is serially diluted in increments of 3-10 fold and with a range sufficient to cover the quantities of viruses expected in experimental samples. The standard curve generated from average Ct values of each dilution plotted against the absolute amount of the standard is used for comparison with experimental Ct values (Saunders, 2008). This produces an estimate of the amount of target present in the experimental sample (Forlenza et al., 2012). Quality of standard curves should be monitored for accurate quantification, like wide dynamic range, limit of detection which determines sensitivity as well as the efficiency (Bernard and Wittwer, 2002; Forlenza et al., 2012).

1.6 MOTIVATION FOR INVESTIGATION

Currently most water quality standards including WHO guidelines do not specify the level of viruses considered acceptable for drinking water, irrigation water, recreational or treated waste water. As result the burden of infectious diseases emanating from water polluted by enteric viruses, particularly in resource limited settings like developing countries have not documented, reported or properly investigated (Okoh et al., 2010). The main challenge in the surveillance and monitoring of enteric viruses in water is the recovery/concentration of low titre viruses from large volumes to small volumes which can be used for either cell culture or molecular detection. Available methods are either too costly for routine water quality monitoring or limited to small volumes. The sodocalcic glass wool adsorption-elution technique is a cost effective and easy-to-use method to recover and concentrate viruses from water but more data is required on its reliability and efficiency.

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1.7 HYPOTHESIS

The hypothesis of this project is:

The glass wool adsorption-elution technique for the recovery of enteric viruses from water sources has a higher efficiency of recovery for treated tap water than turbid raw water.

1.8 AIM OF INVESTIGATION

The aim of this study is to assess the efficiency of the glass wool adsorption-elution technique and the secondary concentration procedure for the recovery of enteric viruses from water sources of different quality and turbidity.

1.9 SPECIFIC OBJECTIVES

The specific objectives for this study are to:

a) Use seeding experiments with different enteric viruses in order to determine the efficiency of recovery of the glass wool adsorption-elution technique for river (turbid) and treated tap water;
b) Assess the effect of pre-treatment of water on the recovery of viruses from turbid water;
c) Assess the effect of modifications to the glass wool column on the efficiency of recovery of viruses using mengovirus and HAV as representative enteric viruses;
d) Assess modifications to the PEG/NaCl precipitation method on the efficiency of recovery of viruses using mengovirus and HAV as representative enteric viruses.

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CHAPTER 2

MATERIALS AND METHODS

2.1 VIRUS STOCKS

Enteric viruses used to assess the efficiency of recovery of the glass wool adsorption-elution method:

a) Adenoviruses (Type 2 ATCC VR-846): The strain of unknown passage from stored material was propagated and titrated for this study.

b) Coxsackie virus B6: CV-B6 ($TCID_{50}$ 1.5 X 10^7 /mℓ) A laboratory strain received from National Institute of Virology (now National Institute for Communicable Diseases, Sandringham) and propagated and titrated at the Dept of Medical Virology, University of Pretoria, was available for this investigation.

c) Hepatitis A virus: HAV ($TCID_{50}$ 1 X 10^8/mℓ) The cytopathic cell culture-adapted HM-175 43c variant (referred to as pHM-175) of the HM-175 strain used for this investigation was propagated and titrated from a culture kindly provided by Prof A Bosch, Department of Microbiology, Faculty of Biology, University of Barcelona, Barcelona, Spain.

d) Mengovirus: (p5: $TCID_{50}$ 1X 10^7 mℓ) The stock culture was propagated and titrated from a culture kindly provided by Prof A Bosch, Department of Microbiology, Faculty of Biology, University of Barcelona, Barcelona, Spain.

e) Noroviruses GII: NoV GII (1.1X10^{10} copies /mℓ) The viral stock originated from a clinical strain detected in a stool specimen from a patient after an outbreak of gastroenteritis on a cruise ship. Dr M Wolfaardt from the Enteric and Environmental Research Group, Department of Medical Virology, University of Pretoria typed the virus as NoV GII.4. The virus was quantified by Prof A Bosch and co-workers, Department of Microbiology, Faculty of Biology, University of Barcelona, Spain.

f) Rotavirus (Simian rotavirus SA11: ATCC VR-899) ($TCID_{50}$ 4.7 X 10^4 mℓ) A RV-SA11 stock propagated and titrated in an African green monkey kidney cell line MA104 by Prof MB Taylor was used for seeding experiments and construction of standard curve.

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2.2 WATER SAMPLES FOR SEEDING EXPERIMENTS

2.2.1 Treated drinking water

Treated drinking water was drawn from a laboratory tap. This water has been treated by conventional means by Rand Water and subsequently the Tshwane Metropolitan Council. For each seeding experiment three 10 ℓ samples were drawn. Sodium thiosulphate was added, to a final concentration of 0.065 mM, to dechlorinate the water and the sample was left overnight at room temperature before use.

2.2.2 Turbid surface water

Samples (10 ℓ) of turbid water were drawn by the Rand Water Scientific Services, Vereeniging samplers from the Klip River K19 sampling site and forwarded to the laboratory in cooler bags. The water samples were stored at 4°C until use. For each experiment three 10 ℓ samples were drawn simultaneously. The samples were used without dechlorination and pH adjustment.

2.2.3 Acidified untreated turbid surface water

Samples (10 ℓ) of turbid water were drawn by the Rand Water Scientific Services, Vereeniging samplers from the Klip River K19 sampling site and forwarded to the laboratory in cooler bags. Three water samples were acidified with 1 M HCl to levels of pH 3.5 and pH 6.0.

2.3 SEEDING OF WATER SAMPLES

Water samples (10 ℓ) were seeded individually with 1 mℓ of a known titre of the virus under investigation. The seeded water samples were thoroughly shaken to ensure uniform distribution of viruses in the water samples. Three replicate water samples were seeded for each experiment. After thorough mixing a 1 mℓ aliquot of each

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seeded water sample was drawn aseptically for the determination of the input virus concentration.

2.4 PRIMARY RECOVERY OF VIRUSES FROM WATER SAMPLES

2.4.1 Standard glass wool preparation

The method for preparing the glass wool filters was derived from procedures described by Vilaginés et al. (1993) and Vivier et al. (2004) and modified by Venter (2004). The glass wool columns, 20 cm in length and with an internal diameter of 30 mm were packed with 15 g of oiled sodocalcic glass wool (Glass wool Bourre 725 QN, Ouest Isol, Alizay, France). Briefly, three portions of 5 g of glass wool were teased and compressed into a column at a different angle to each other to a final density of 0.5 g/cm^3, with two steel sieve grids (pore size of ~1 mm^2) inserted between the glass wool sections. Glass wool was positively charged by initially soaking in sterile distilled water and pre-treated consecutively with 40 mℓ 1 M HCl (Merck KGaA, Darmstadt, Germany), 100 mℓ sterile distilled water, and 40 mℓ 1 M NaOH (Merck). The charged glass wool was then treated with 100 mℓ sterile distilled water to adjust the pH to pH 7.

2.4.2 Modified glass wool preparation

Two modifications to the preparation of the glass wool filters were tested:

a) The glass wool column was prepared using 3 x 5 g (15 g) of glass wool as described previously in 2.4.1 but without the inclusion of the gauze grids;

b) The glass wool column was prepared using 4 x 5 g (20 g) of glass wool as described previous in section 2.4.1 but without the inclusion of the gauze grids.

2.4.3 Filtration procedure

Filtration or adsorption of viruses from water samples was done by passing a seeded water sample from a 10 ℓ container through the positively charged glass wool columns

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by negative pressure at a rate of 10 ℓ/h. The negatively charged viruses which adsorbed to the glass wool, were eluted with 100 mℓ of sterile glycine-beef-extract buffer pH 9.5 (GBEB: 3.754 g/ℓ glycine [Merck]; 5 g/ℓ beef extract powder [BBL™, Becton, Dickinson and Company, Sparks, MD]), which reverses the ionic charge of the viruses and releases them from glass wool. The elution buffer (GBEB) was left in contact with the glass wool for 15 min before being passed through the filter under pressure, and thereafter the pH of the eluate was adjusted to pH 7.0 with 1 M HCl (Merck) as described by Vivier et al. (2004). After elution three replicate samples of 1 mℓ were drawn from the eluate for determination of the percentage recovery in the elution stage. The same number of replicate samples were drawn from the filtrate or flow through which were used to determine the percentage of viruses lost in the flow through.

2.5 SECONDARY CONCENTRATION OF RECOVERED VIRUSES

2.5.1 PEG/NaCl precipitation

The secondary concentration of viruses was done using a modification of PEG_{6000}/NaCl precipitation methods described by Vilaginés et al. (1997) and Minor (1985). Three replicate 1 mℓ samples were drawn from these final concentrates which were used to determine the final percentage recovery for the glass wool adsorption-elution process.

2.5.2 Modified liquid PEG/NaCl precipitation

The PEG/NaCl precipitation method as described by the European Committee of Standardisation (CEN) Technical Committee (CEN/TC 275/WG6/TAG4) was applied.

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2.6 DETECTION AND QUANTIFICATION OF VIRUSES FROM WATER

2.6.1 Nucleic acid extraction

Viral nucleic acids were extracted from 1 mℓ of the recovered virus suspensions using the MagNA Pure LC Total Nucleic Acid Isolation Kit (large volume) (Roche Diagnostics, GmbH, Mannheim, Germany) in the robotic MagNA Pure LC v2.0 instrument (Roche Diagnostics). The nucleic acid was aliquoted and stored at -70^{o}C until use.

2.6.2 Virus detection

2.6.2.1 *Primers and Probes*

Published sets of primers and Taqman probes (Table 1) were used for the detection of the selected enteric viruses. The NoV GII, HAV and mengovirus primer sets and probes were those recommended by the CEN/TC 275/WG6/TAG4 Technical Committee for the detection of these viruses in food and water samples.

Table 2.1: References to primer and probe sets applied in this study.

Virus	Forward primer	Reverse primer	Probe	Reference
Hepatitis A virus	HAV 68	HAV 240	HAV 150	Costafreda et al., 2006
Human adenovirus	AQ1	AQ2	AP	Heim et al., 2003
Human rotavirus	RotaF	RotaR	Rota probe	Zeng et al., 2008
Mengovirus	MENGO 110	MENGO 209	MENGO 147	Pintó et al., 2009
Enterovirus	EV1	EV2	Probe EV	Fuhrman et al., 2005
Norovirus GII	QNIF2	COG2R	QNIFS	Loisy et al., 2005 & Kageyama et al., 2003

2.6.2.2 *Real-time PCR/RT-PCR assays*

All assays were performed on the carousel-based LightCycler® 2.0 instrument (Roche Diagnostics).

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a) Hepatitis A virus: The RNA Ultrasense™ One-step qRT-PCR system (Invitrogen, Carlsbad, CA) was used. Five microlitres (5 µℓ) of RNA was added to the Master Mix containing, 10 pmol of the forward primer, and 18 pmol of the reverse primer and 5pmol of the labelled probe. The RT-PCR cycling conditions used were as follows: reverse transcription at 50^{o}C for 45 min, DNA polymerase activation at 95^{o}C for 15 min and then 50 cycles of amplification with denaturation at 95^{o}C for 15 sec, annealing at 60^{o}C for 1 min and extension at 65^{o}C for 1 min.

b) Human adenoviruses: The *rt* PCR Taqman Environmental Master Mix 2.0 (Applied Biosystems™, Foster City, CA) was used. Five microlitres of DNA were added to the Master Mix containing 10 pmol of the forward primer, 10 pmol of the reverse primer and 10 pmol of the labelled probe. The cycling conditions used were as follows: DNA polymerase activation at 95^{o}C for 10 min and then 45 cycles of amplification with denaturation at 95^{o}C for 3 sec, annealing at 55^{o}C for 10 sec and extension at 65^{o}C for 1 min.

c) Human rotavirus: For the *rt* RT-PCR for amplification of HRV the Quantitect Probe® RT-PCR kit (Qiagen GmbH, Hilden, Germany) was used. Five microlitres of RNA was added to the QuantiTect Probe Master Mix, containing 8 pmol each of forward primer, reverse primer and 4 pmol labelled Taqman probe. The RT-PCR cycling conditions used were as follows: reverse transcription at 50^{o}C for 45 min, DNA polymerase activation at 95^{o}C for 15 minutes, and then 50 cycles of amplification with denaturation at 95^{o}C for 15 sec, annealing at 60^{o}C for 1 min and extension at 65^{o}C for 1 min.

d) Mengovirus: The *rt* RT-PCR for amplification of mengovirus was done using Quantitect Probe® RT-PCR kit (Qiagen). Five microlitres of viral RNA was added to the QuantiTect Probe Master Mix, containing 10 pmol each of forward primer, 18 pmol of reverse primer and 5 pmol of labelled TaqMan probe. The cycling conditions used were the same as those described for HAV.

e) Enteroviruses: A two step *rt* RT-PCR was used in the detection and quantification of enteroviruses,. The Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics) was used for the synthesis of complementary DNA from the extracted RNA. Ten microlitres of RNA was added to the primer mix containing 10 pmol dNTPs and 1200 pmol of random primers. Denaturing of RNA was done at 96^{o}C for 2 minutes in a thermocycler (PTC-100™ Programmable Thermal Controller MJ Research, Inc). The

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denatured RNA was then put on ice for 2 min to avoid renaturing. Five microlitres of the reverse transcription mix was added to RNA-primer mix, containing 20 U of RNase inhibitor and 10 U of Transcriptor reverse transcription enzyme. Reverse transcription was performed on a Px2 Thermal Cycler at cycling conditions as described by the manufacturer. The LightCycler® TaqMan® Master Kit (Roche Diagnostics) was used for amplification and detection. Five microlitres cDNA was added to master mix containing 5 pmol forward primer, 5 pmol reverse primer and 2 pmol labelled probe. The cycling conditions used were as follows: DNA polymerase activation at 95^{o}C for 15 minutes and then 45 cycles of amplification with denaturation at 95^{o}C for 15 sec, annealing at 60^{o}C for 1 min and extension at 65^{o}C for 1 min.

f) Norovirus GII: Molecular amplification and *rt* RT-PCR detection of NoV GII was done using the QuantiTect Probe® RT-PCR kit (Qiagen). Five microlitres of extracted viral RNA was added to the QuantiTect Probe Master Mix, containing 4 pmol each of the forward primer, reverse primer and labelled TaqMan probe. The cycling conditions were used were as follows: reverse transcription at 45^{o}C for 45 min, DNA polymerase activation at 95^{o}C for 10 min and then 45 cycles of amplification with denaturation at 95^{o}C for 15 secs , annealing at 60^{o}C or 1 min and extension at 65^{o}C for 1 min.

2.6.3 Preparation of standard curves

Standard curves were available for enterovirus (CV-B6), NoV GII and mengovirus. Standard curves were therefore prepared for HAV, HAdV and HRV. The nucleic acid for the construction of standard curves were extracted from 1 mℓ of a cell culture suspension of each virus using the QIAamp® Ultrasens® virus kit (Qiagen) and eluted in 100 µℓ elution buffer. Ten-fold serial dilutions of the nucleic acid were prepared in nuclease-free water (Promega Corp., Madison, WI). Real-time RT-PCR (HAV and RV-SA11) and *rt* PCR (HAdV) reactions were performed in triplicate for each dilution of nucleic acid by adding 5 µl RNA/DNA of each of the dilutions to the master mix of the appropriate kit as described in 2.6.2.2. The standard curve was constructed by plotting the log of the number of viral genome copies added to a *rt* RT-PCR/PCR reaction against their corresponding crossing point (CP)/cycle threshold (Ct) values and was done automatically by the LightCycler® 2.0 instrument software (Roche Diagnostics).

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The CP/Ct value is defined as the PCR cycle at which an increase in the fluorescence above the base-line is first detected. Using the standard curves the viruses in each sample type was quantified to calculate the quantities of virus lost and recovered at each stage of the glass wool adsorption-elution technique.

a) Hepatitis A virus: A stock suspension of HAV strain pmH175 of known viral titre (1 x 10^5 copies per mℓ) was used for the construction of the standard curve. Triplicate aliquots of the diluted nucleic acid were amplified to obtain a standard curve. The detection assay and cycling conditions were as described in section 2.6.2.2. A factor of X60 was applied in order to estimate the physical number of genomes and the standard curve was adjusted accordingly (Costafreda *et al*., 2006).

b) Rotavirus SA11: A stock suspension RV-SA 11 (ATTC VR-899), propagated and titrated in an African green monkey kidney cell line MA104 (10^4 copies / mℓ), was used for the construction of the standard curve. The detection assay and cycling conditions were as described in section 2.6.2.2.

c) Human adenovirus: A stock suspension of AdV type 2 (ATCC VR-846) with a known titre (1 x 10^6 copies/mℓ) was used for the construction of standard curve. The detection assay and cycling conditions were as described in section 2.6.2.2.

2.7 CALCULATION OF EFFICIENCY OF RECOVERY

The number of genome copies per mℓ of virus in the seeded water, in the flow through, in the eluate and in the final viral concentrate were used to calculate the EOR and percentage virus lost at each step in the recovery process. Percentage recovery was calculated as the genomic copy number of the virus recovered after filtration of water sample divided by the genomic copy number of the virus seeded in the 10 ℓ water samples multiplied by 100 as shown in the calculation below.

a) The final EOR percentage was calculated as follows:

$$\frac{\text{Genome copy numbers of the virus in the final virus concentrate}}{\text{Genome copy number of the virus in the seeded 10 ℓ water sample}} \times 100$$

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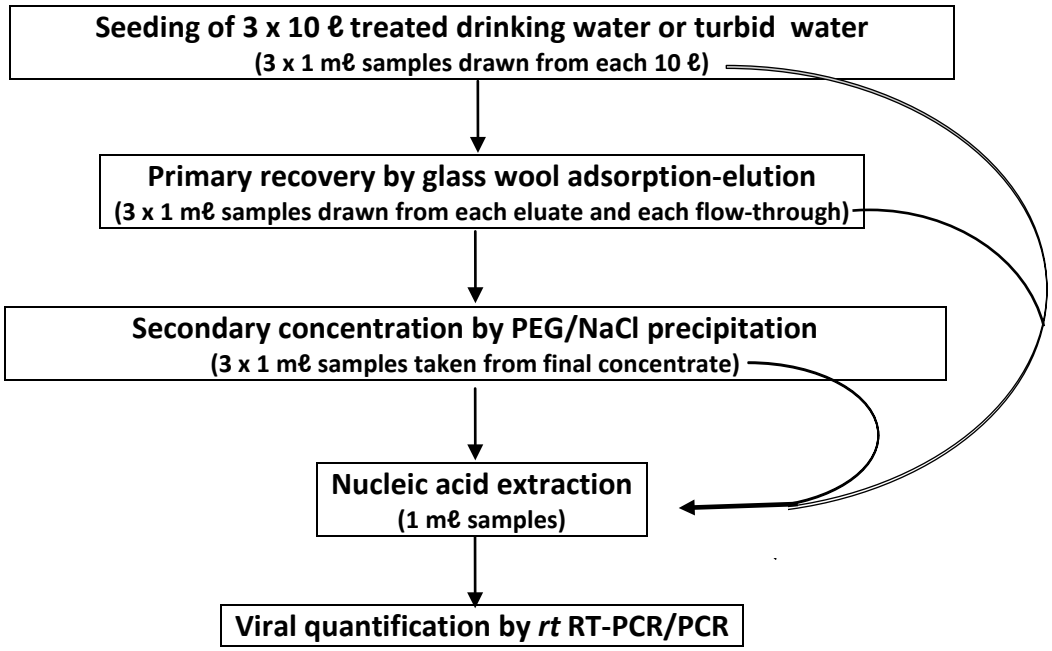
b) The percentage recovery at each stage of the glass wool adsorption-elution method was calculated as follows:

$$\frac{\text{Genome copy number of the virus after the step}}{\text{Genome copy number of the virus in the seeded 10 ℓ water sample}} \times 100$$

2.8 EXPERIMENTAL PROCEDURE



2.8.1 Comparison of the RT-PCR/PCR amplification and detection of enteric viruses in tap and turbid water

The efficiency of amplification and rapidity of detection was analysed for each virus and compared between the two water matrices, namely tap water and turbid water. Amplification and detection was noted at each stage of the glass wool adsorption-elution process and compared between the two water matrices. This was done by comparing the CP values and the corresponding amount of fluorescence.

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2.8.2 Assessment of the effect of modifications to the glass wool column on the efficiency of recovery

Recovery experiments were done using the three methods of glass wool column preparation, namely the standard glass wool column as described in section 2.4.1 and modified glass wool columns as described in section 2.4.2. The EOR data obtained for each glass wool modification was compared to the standard column and to each other to see effect of the changes of column preparation on the EOR.

2.8.3 Comparison of the effect of liquid and solid PEG/NaCl precipitation on the efficiency of recovery

To assess the effect of PEG/NaCl precipitation on the recovery of viruses, two methods of preparing PEG/NaCl namely the solid PEG_{6000}/NaCl and the liquid PEG_{8000}/NaCl precipitation methods were compared on their ability to precipitate viruses. Known titres of mengovirus were seeded into the two types of PEG/NaCl. Three replicate samples were taken from each seeded type of PEG/NaCl for quantification of input viruses. The viruses were precipitated according to procedures described in sections 2.5.1 and 2.5.2, respectively. Three replicate 1 mℓ samples were drawn for the determination of the percentage recovery of viruses using the two different methods of virus precipitation.

2.8.4 Assesment of the effect of water pH on the efficiency of recovery

To assess the effect of pH on efficiency of recovery, turbid water adjusted to three pH levels of 3.5, 6.0 and 7.0 as described earlier in 2.2.3, seeded with HAV as described in 2.3 was run through the standard glass wool columns as described in 2.4.3. Viruses were precipitated as described in 2.5.1. Viruses recovered and lost at each stage of the glass wool adsorption elution procedure were determined and compared between the three pH values

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2.9 QUALITY ASSURANCE AND CONTROL

To minimise risks of contamination each stage of the glass wool adsorption-elution process was performed in separate designated rooms with dedicated equipment, consumables and protective clothing. Precautions were taken to avoid cross contamination between samples as well as contamination of the laboratory environment. All viral recovery equipment was disinfected or sterilised before and after use and laboratories were decontaminated after each experiment. Separate rooms and equipment were also designated for nucleic extraction, master mix preparation and amplification. To avoid cross contamination by viruses and amplicons during the viral recovery and viral detection processes a step-wise unidirectional flow of procedures was strictly adhered to.

Positive controls, containing predetermined concentrations of viral RNA/DNA (standards), negative controls (nuclease-free water; Promega Corp.) and a master mix control were included in each quantitative *rt* RT-PCR/PCR assay. The standards were aliquoted and stored at -80^{o}C to avoid the degradation of the nucleic acids. The positive controls were also used to validate the use of standard curves. Tests where the negative controls tested positive or positive controls tested negative were considered to be invalid and were repeated.

2.10 STATISTICAL ANALYSIS

A generalised linear model (GLM) was applied using Stata 12 statistical software package (StataCorp LP, College Station, TX) to test if there was an association between the viruses under investigation and the EOR. The same model was applied to determine whether there was association between recovery efficiencies and the types water. The model was applied because it is able to handle different data cases, i.e. continuous or discrete. It also allows comparison of mean or group outcomes to different exposures or treatments using predictive margins. Two-sample mean comparison t-test was applied using Stata 8.1 statistical software (StataCorp) to assess

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the effect of glass wool column modification/preparation, the effect of PEG/NaCl precipitation and the effect of water on the EOR.

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CHAPTER 3

RESULTS

3.1 GENERATION OF STANDARD CURVES FOR THE QUANTIFICATION OF SELECTED VIRUSES

The amplification curves for the 10-fold serial dilutions and standard curves generated from these amplification curves for RV SA11, HAV and HAdV are shown in the Figures 3.1, 3.2 and 3.3, respectively.

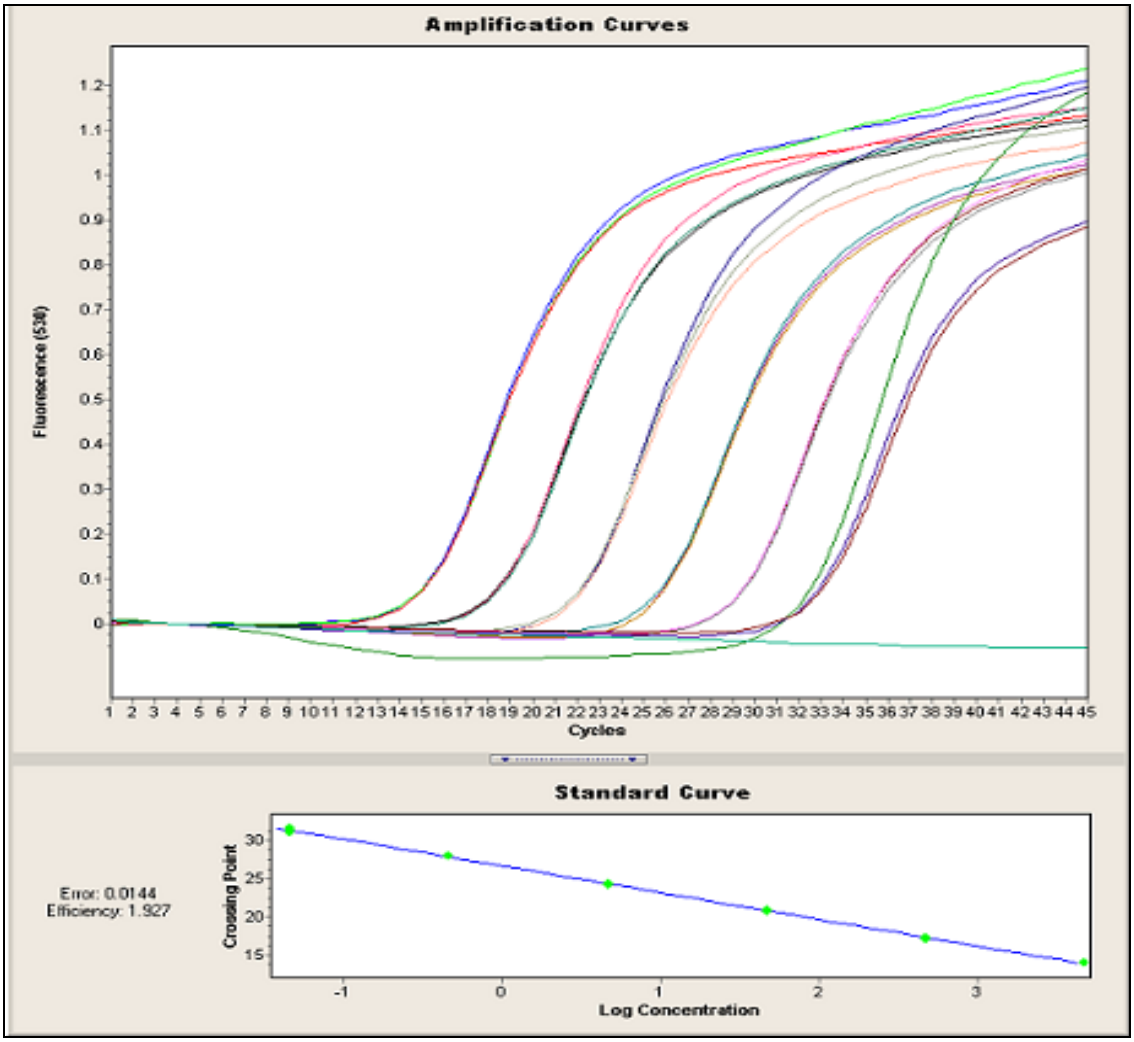

Figure 3.1: Standard curve and associated amplification curves prepared from a 10-fold dilution series of RNA from a known titre of rotavirus SA11.

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From Figure 3.1, where the amplification curves and standard curve generated from serial log dilutions of RV SA11 RNA is shown, a high efficiency of 1.927 and an error of 0.0144 which was within acceptable limits. Based on the slope of the curve in relation to the CP values the PCR efficiency was calculated automatically by the LightCycler 2.0 software (Roche). The limit of detection was calculated to be 4 genome copies per reaction.

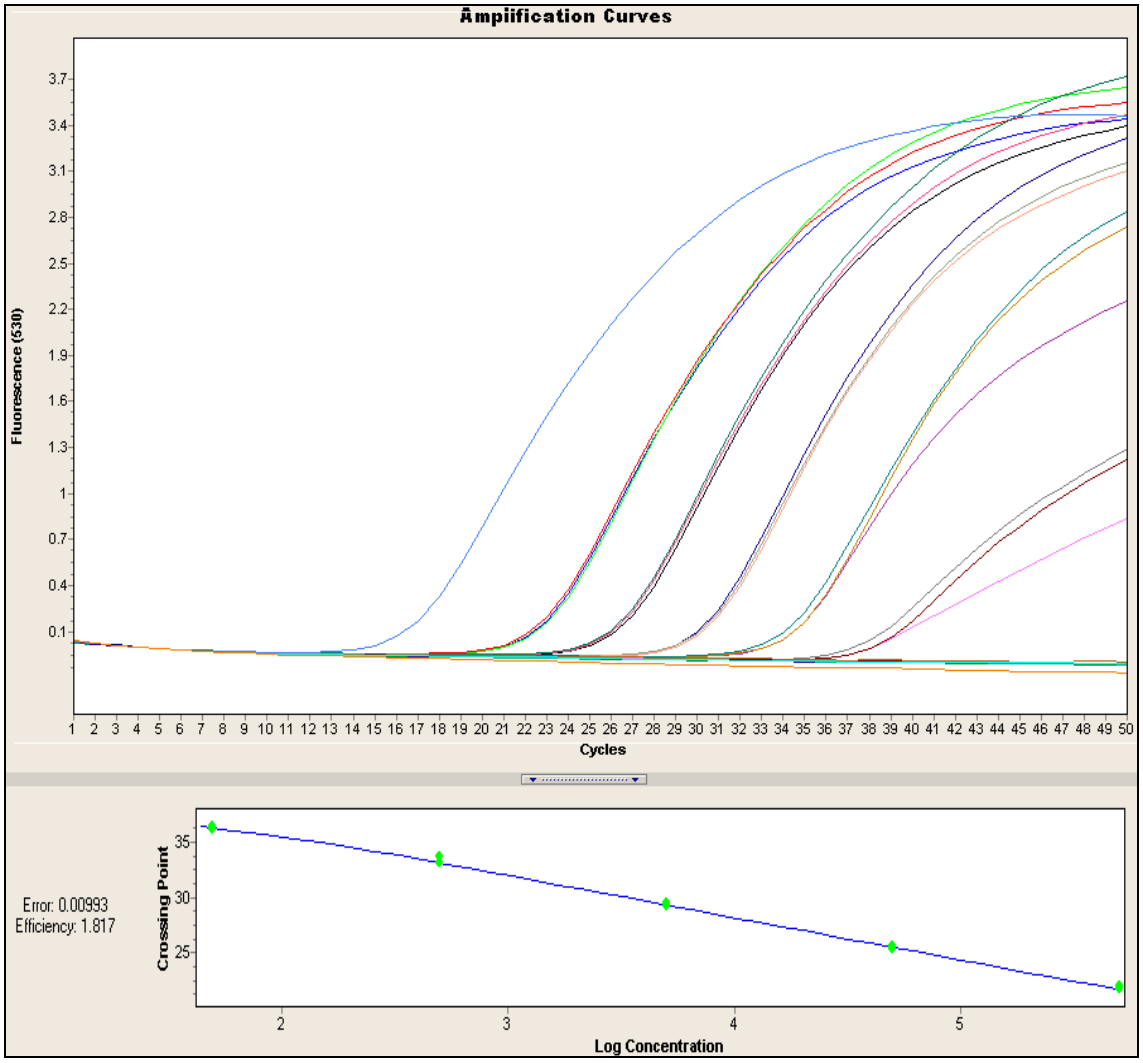

Figure 3.2: Standard curve and associated amplification curves prepared from a 10-fold dilution series of RNA from a known titre of hepatitis A virus.

In Figure 3.2 the amplification curves and standard curve generated from serial $\log_{10}$ dilutions of RNA from a known titre of HAV is presented. The curve displays high efficiency of 1.817 and a small error of 0.00993, within acceptable limits, based on the

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slope of the curve in relation to the CP values. The PCR efficiency and the error were calculated automatically by the LightCycler® 2.0 software (Roche). The detection limit was approximately 50 genome copies per reaction.

Figure 3.3 shows the amplification curves and standard curve generated from serial log dilutions of DNA from a known titre of HAdV. The curve displays a high efficiency of 1.866 and an acceptable error of 0.0166 which, based on the slope of the curve in relation to the CP values, was calculated automatically by the LightCycler® 2.0 software (Roche). The detection limit was one genome copy per reaction.

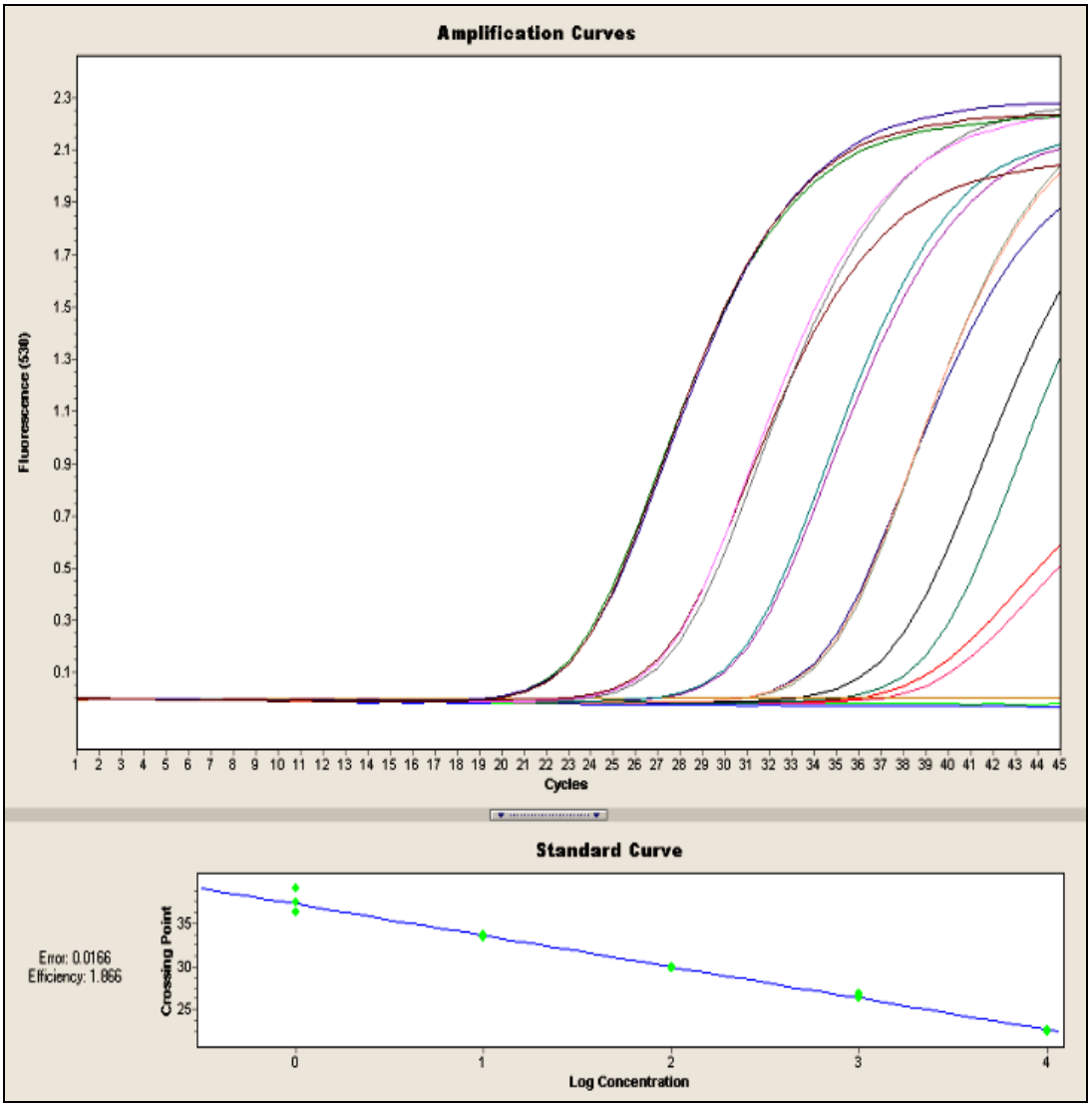

Figure 3.3: Standard curve and associated amplification curves prepared from a 10-fold dilution series of DNA from a known titre of human adenovirus type 2.

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3.2 COMPARISON OF AMPLIFICATION CURVES FOR THE DETECTION OF SELECTED ENTERIC VIRUSES AND PROCESS CONTROL IN TAP AND TURBID WATER

When the amplification of the different viruses in the two water matrixes were compared, it was noted that except for mengovirus, amplification was more rapid in tap water than in turbid water as shown by the higher CP values for the turbid water (Table 3.1) and the corresponding lower levels of fluorescence. The efficiency of amplification was higher in tap water than turbid water as shown by the slopes of the amplification curves. This pattern was more noticeable in NoV GII than for the rest of the viruses (Appendix A). In both tap water and turbid water, amplification of RNA/DNA from recovered viruses at each of the different steps occurred 1-3 cycles earlier in the final concentrate than in the eluate. In all the experiments, except for HAV, the RNA/DNA amplification of the viruses recovered from the seeded water and flow through occurred 2-3 cycles later than noted for the elution and final concentration steps.

Table 3.1: Average CP values for amplification of RNA/DNA viruses recovered at different stages of process

Virus	Seeded water	Flow through	Eluate	Final concentrate
Human adenovirus: Tap	29.31	29.86	25.01	22.00
Turbid	35.06	39.84	28.07	25.56
Hepatitis A virus: Tap	32.97	33.71	28.31	25.57
Turbid	33.78	34.67	32.02	31.48
Rotavirus SA11: Tap	27.00	26.90	23.36	20.58
Turbid	31.00	31.05	30.97	30.50
Mengovirus: Tap	29.73	30.13	28.15	29.07
Turbid	32.29	33.45	25.78	23.05
Norovirus GII: Tap	37.52	37.16	34.42	33.80
Turbid	33.66	34.29	35.78	36.33
Coxsackievirus B6: Tap	33.77	34.75	28.36	25.22
Turbid	40.10	38.19	37.78	36.40

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3.3 EFFICIENCY OF RECOVERY OF SELECTED VIRUSES FROM TAP AND TURBID WATER

3.3.1 Efficiency of recovery of viruses in the final concentration step

From the seeding experiments where the EOR of the five selected enteric viruses and process control (mengovirus) was determined it was evident that the final recovery efficiencies differed among viruses and the type of water (Figure 3.4).

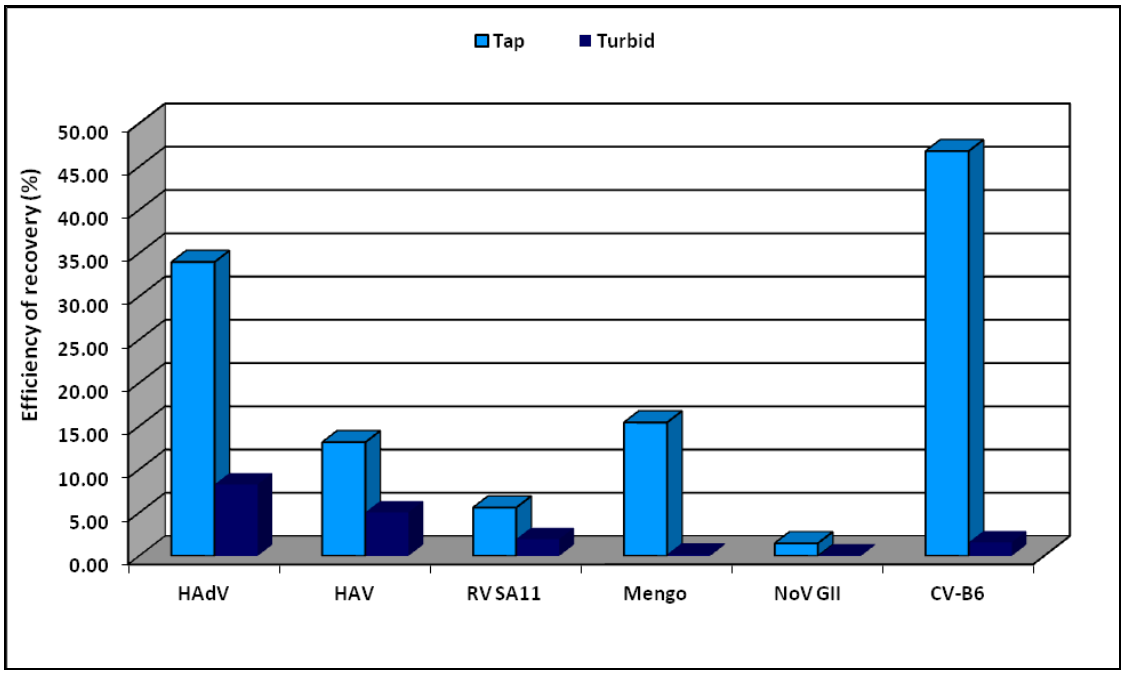

Figure 3.4: Percentage efficiency of recovery for the five enteric viruses and process control (mengovirus) from tap and turbid water.

From Figure 3.4 it is evident that for tap water, the EOR of the glass wool adsorption-elution method was the highest for CV-B6 (46.9% : range 17%-87%) followed by the EOR for HAdV (33.97% : range 10.37% - 86.03%). The EOR for NoV GII was the lowest (1.46%: range 0.54%-3.80%), with HAV (13.14%: range 0.96 – 25.00%) and RV SA11 (5.58%: range 3.92% - 6.84 %) at intermediate levels of EOR. The EOR for the mengovirus, the process control (15.42%: range 7.89% - 19.14%) was slightly higher than that for HAV but not as high as that determined for CV-B6 or HAdV.

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For turbid water, the EOR was highest for HAdV (8.26%: range 4.02% - 14.75%) followed by the EOR for HAV (5.04%: range 0.00% - 26.41%). For the other enteric viruses and process control the EOR was low, i.e. CV-B6 (1.57%: range 0.58% - 3.58%), NoV GII (0.04%: range 0.01% - 0.08 %), RV SA11 (1.95%: range 0.18% - 6.84%) and mengovirus (0.16%: range 0.11% - 0.20%).

The generalised linear statistical model applied to compare the EOR of each virus in each water type clearly indicated that the EOR varied for each virus (Figure 3.5). This was clearly noticeable for HAdV, HAV, mengovirus and CV-B6 when compared to RV SA11 and NoV GII. However the EOR calculated for tap water was higher than for turbid water for each virus (p = 0.00). The data clearly showed that there was no evidence of variation between the triplicate replicates for each sample (p = 0.39) confirming that the samples were similar sample. When the data for the three samples for each assay for each virus was analysed clear evidence of inter-assay variation was noted (p = 0.00).

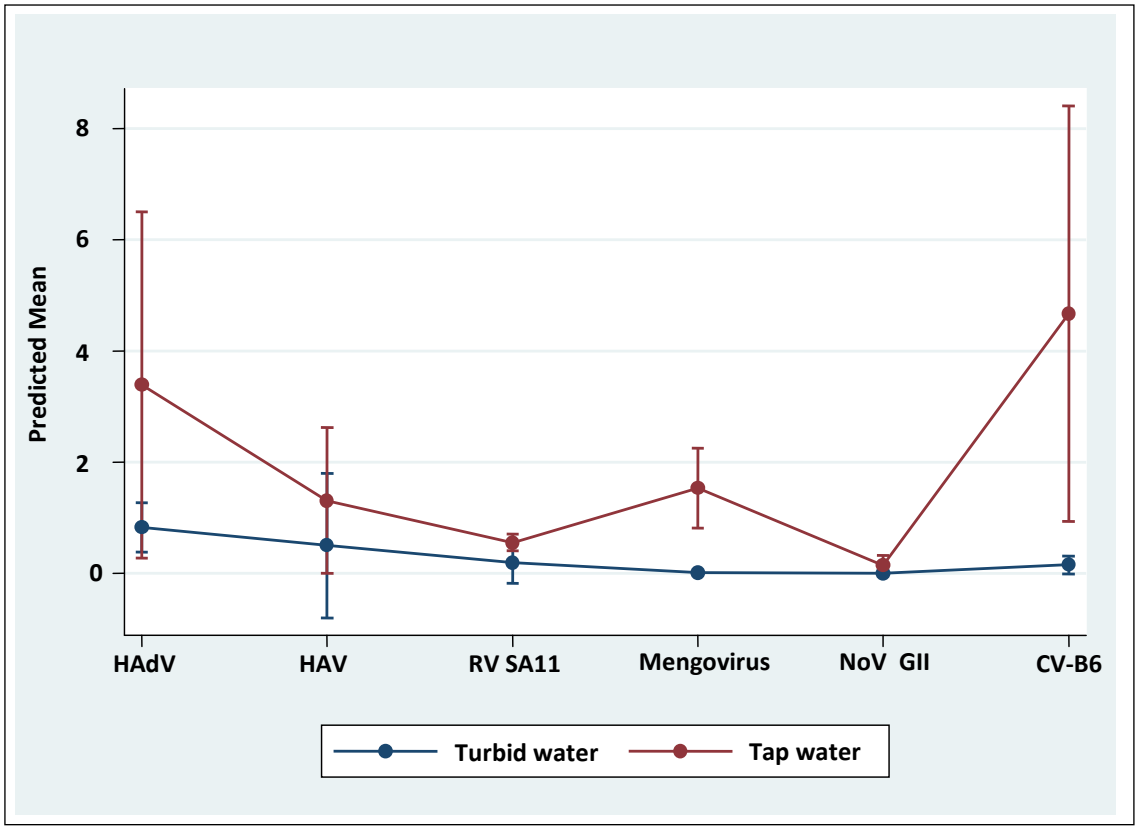

Figure 3.5: Predictive margins with 95% CIs of the efficiency of recovery between viruses in the same water types and between the different types of water.

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3.3.2 Assessment of virus loss at different stages of the adsorption-elution process

The comparison of the recoveries and loss of viruses at each stage of the adsorption-elution process between raw water and tap water is presented in Table 3.2.

Table 3.2: Percentage viral recovery and loss at different stages of the adsorption-elution process

	TAP WATER			TURBID WATER		
Virus	Final recovery %	Recovery in the eluate %	Loss in flow through %	Final recovery %	Recovery in the eluate %	Loss in flow through %
Human adenovirus	33.97	74.02	4.06	8.26	12.62	67.68
Hepatitis A virus	13.14	28.23	70.66	5.04	1.98	38.49
Rotavirus SA11	5.58	11.41	108.84	1.95	5.53	745.00
Mengovirus	15.42	54.73	60.32	0.16	3.62	82.87
Norovirus GII	1.46	7.57	110.28	0.05	1.86	31.30
Coxsackie virus B6	46.91	50.81	47.95	1.57	3.82	62.17

As described previously the virus recovery values differed from one virus to the other and was also affected by the water matrix from which they are recovered. The EOR at each stage of the glass wool adsorption-elution process for each virus in tap and turbid water are presented in Figures 3.6 (HAdV), 3.7 (HAV), 3.8 (RV SA11), 3.9 (mengovirus), 3.10 (NoV GII) and 3.11 (CV-B6). From Table 3.2 and Figures 3.6-3.11 it is clearly evident that the recoveries in the eluate and final concentrate are higher for the tap than for the turbid water. There was extensive loss of virus in the flow through and this was also higher for the turbid water than the tap water.

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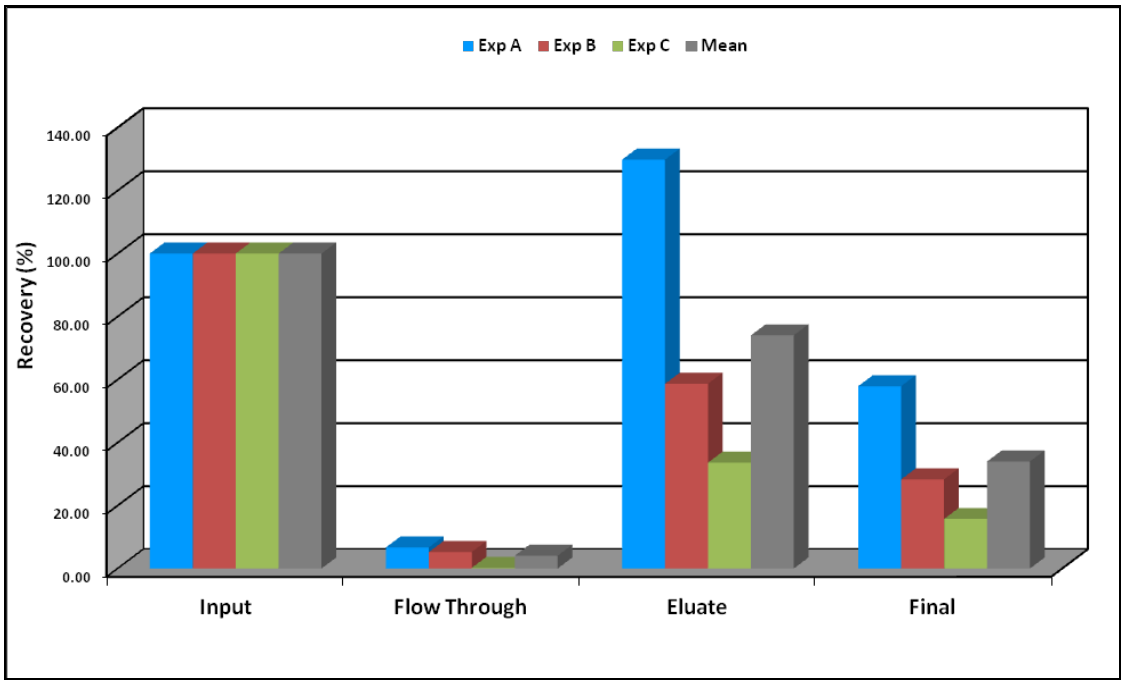

Figure 3.6a: Percentage recovery of human adenovirus from tap water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

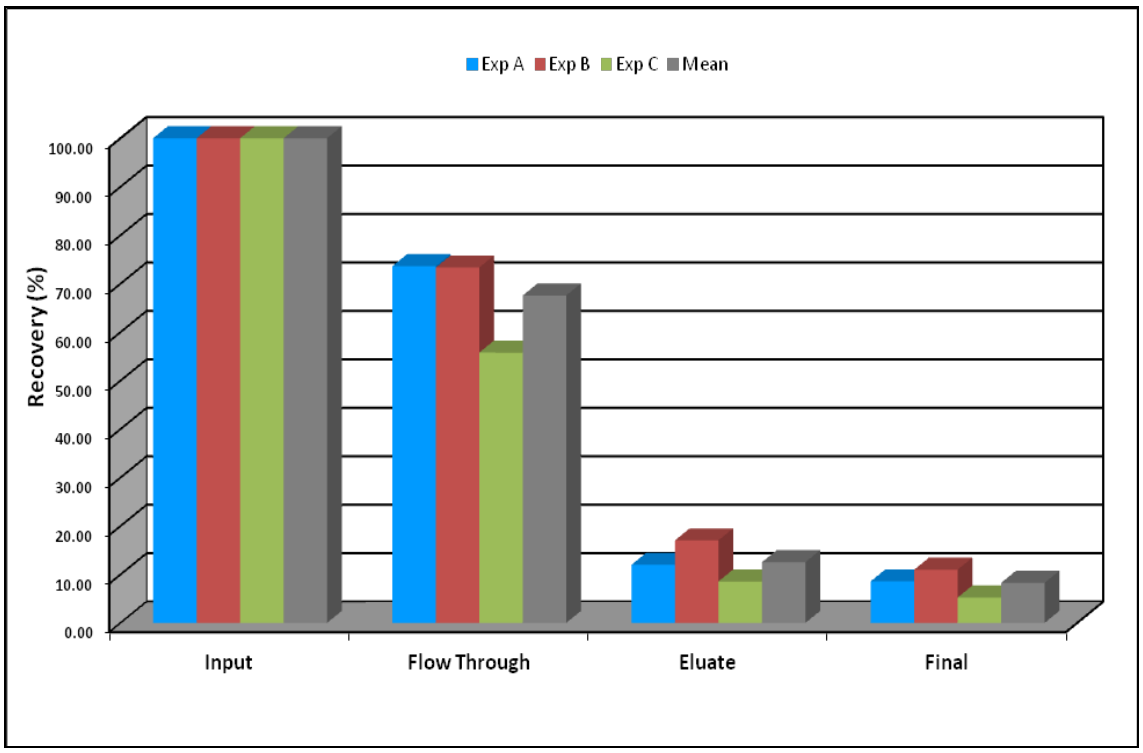

Figure 3.6b: Percentage recovery of human adenovirus from turbid water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

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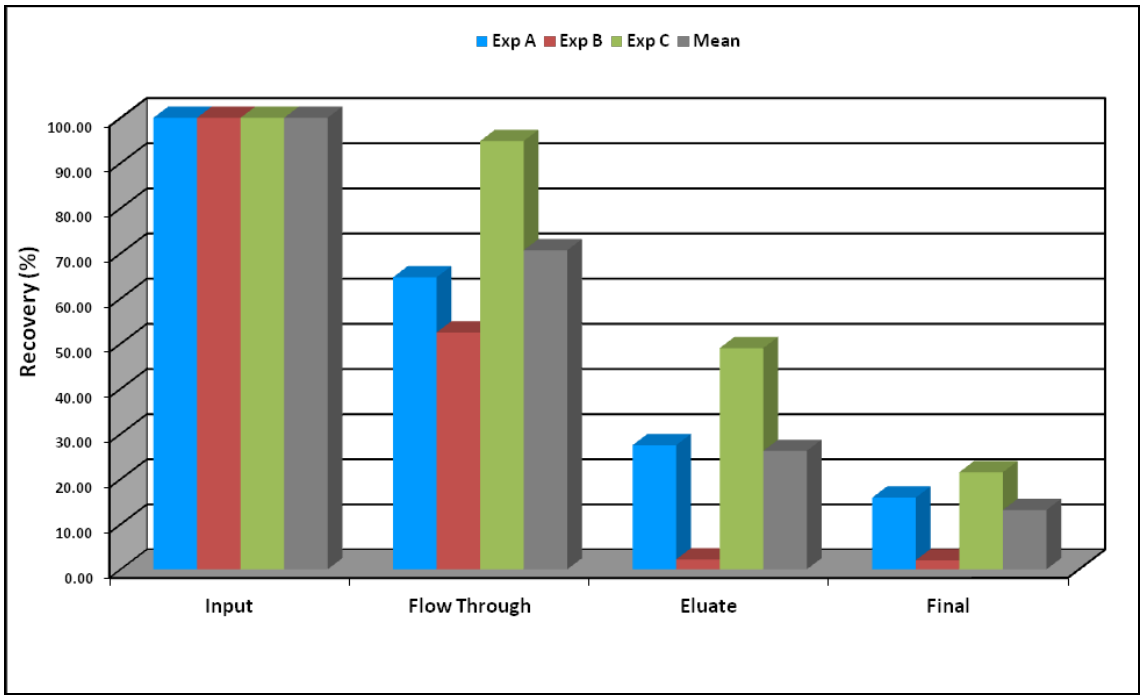

Figure 3.7a: Percentage recovery of hepatitis A virus from tap water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

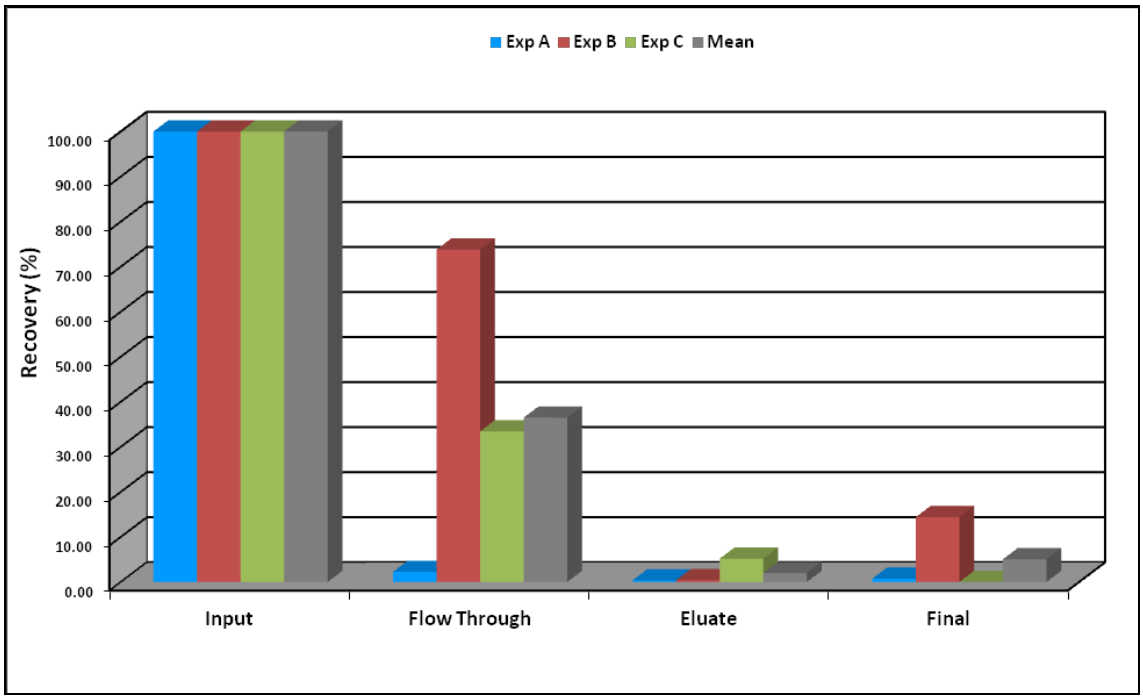

Figure 3.7b: Percentage recovery of hepatitis A virus from turbid water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

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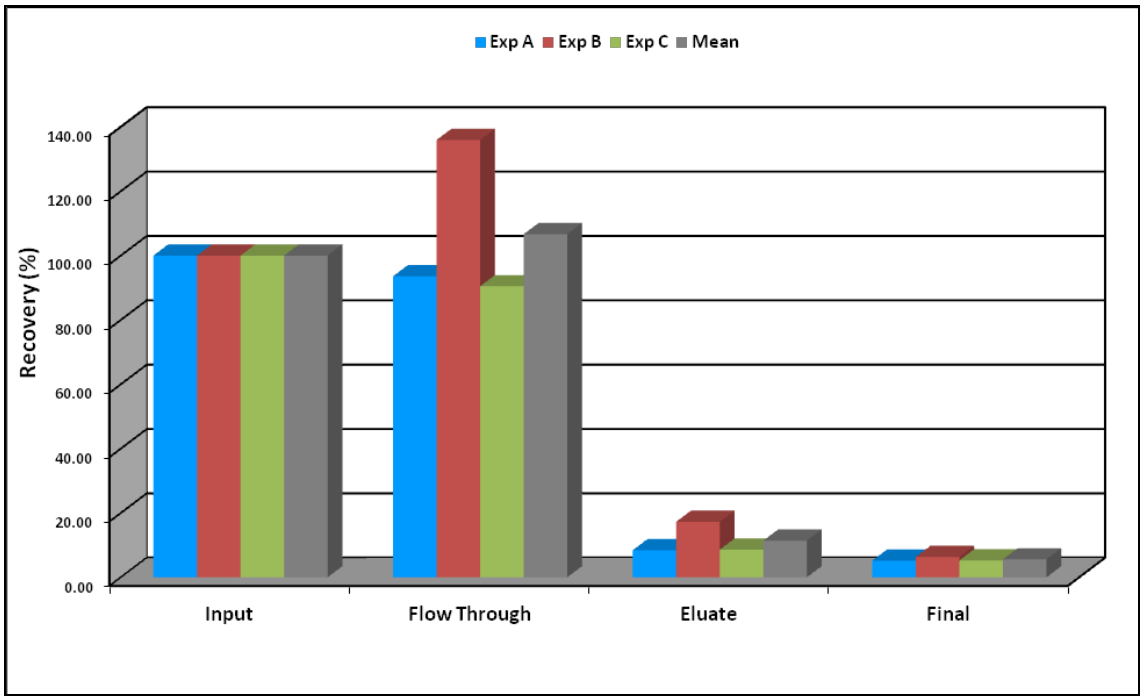

Figure 3.8a: Percentage recovery of rotavirus SA11 from tap water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

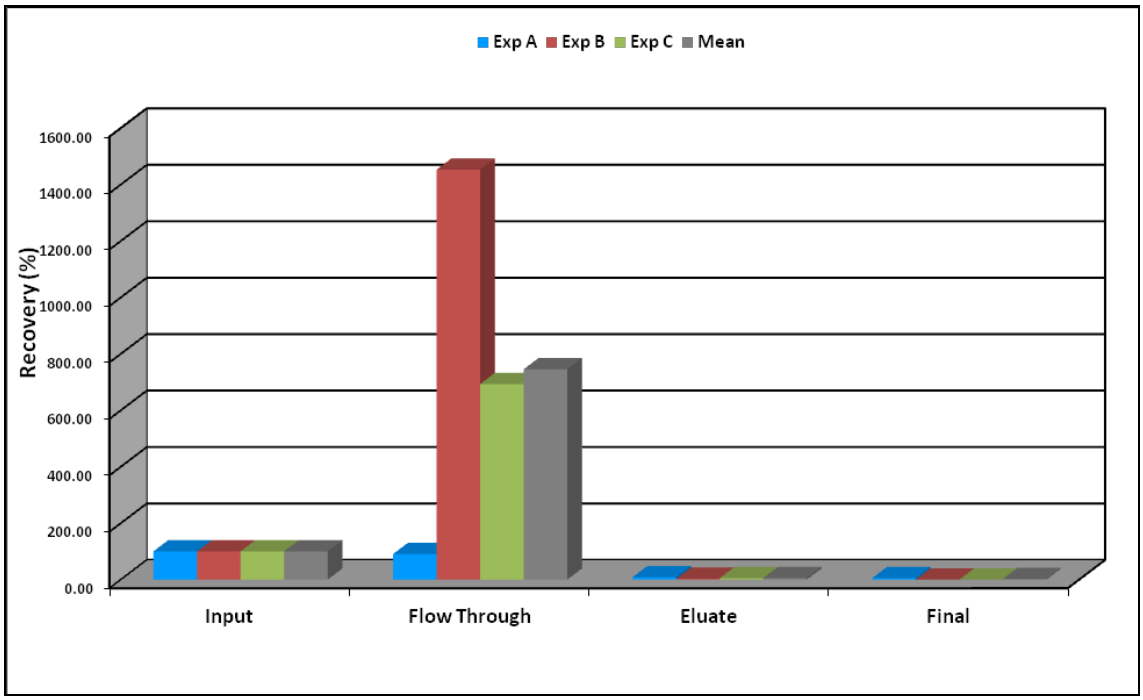

Figure 3.8b: Percentage recovery of rotavirus SA11 from turbid water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

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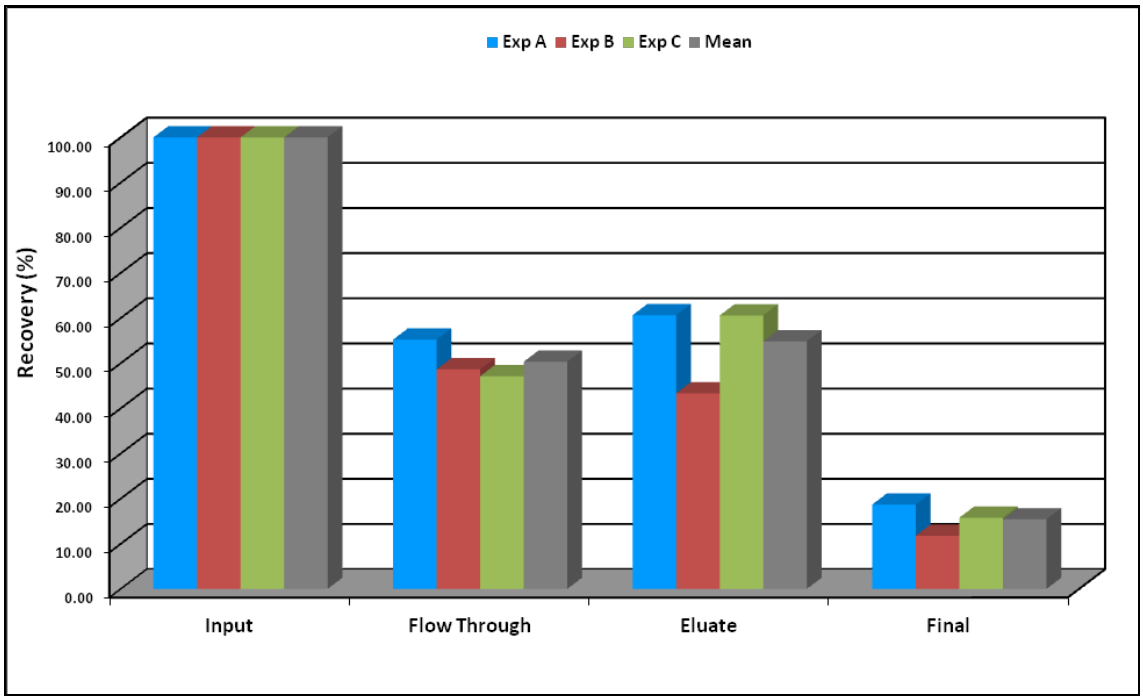

Figure 3.9a: Percentage recovery of mengovirus from tap water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

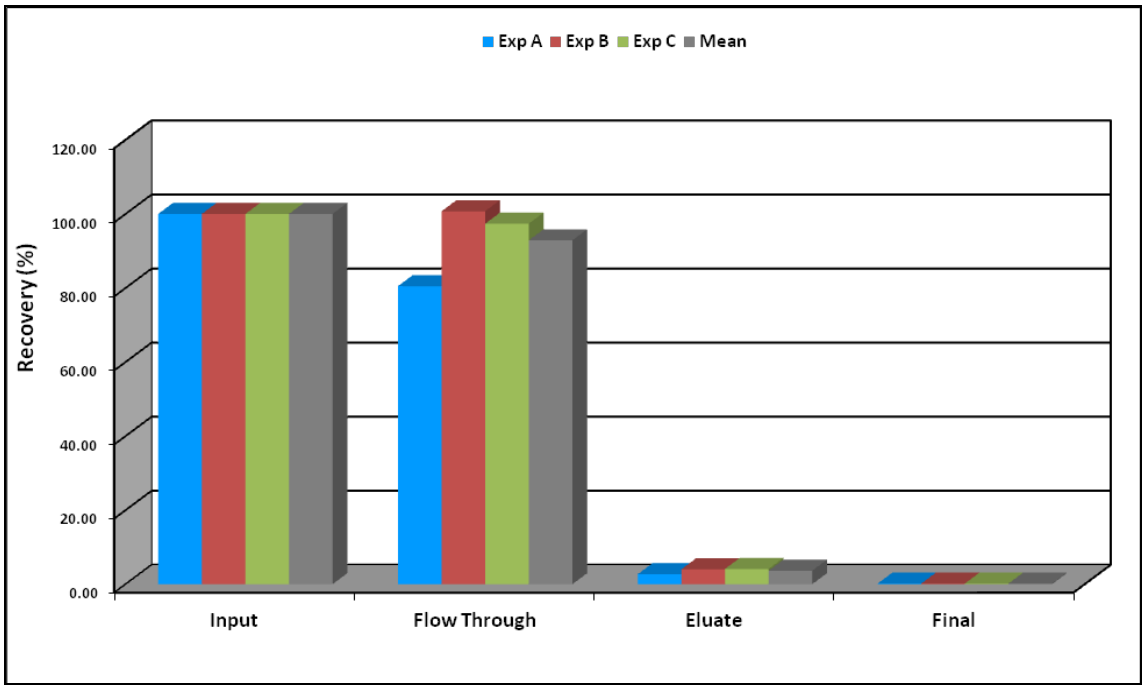

Figure 3.9b: Percentage recovery of mengovirus from turbid water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

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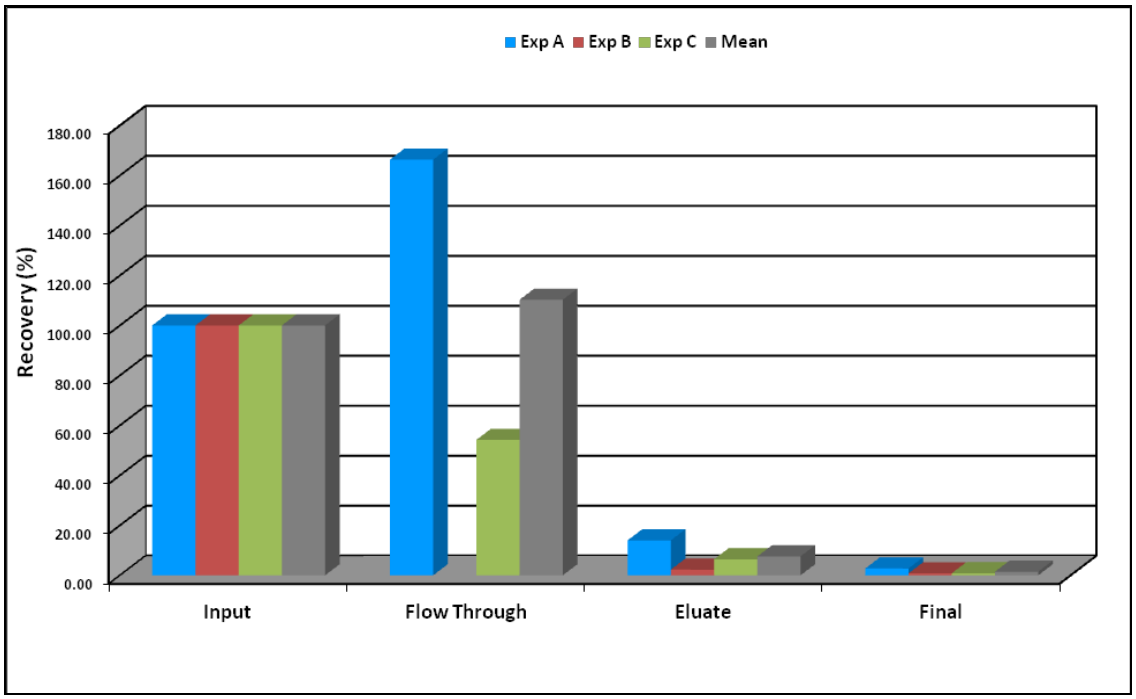

Figure 3.10a: Percentage recovery of norovirus GII from tap water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

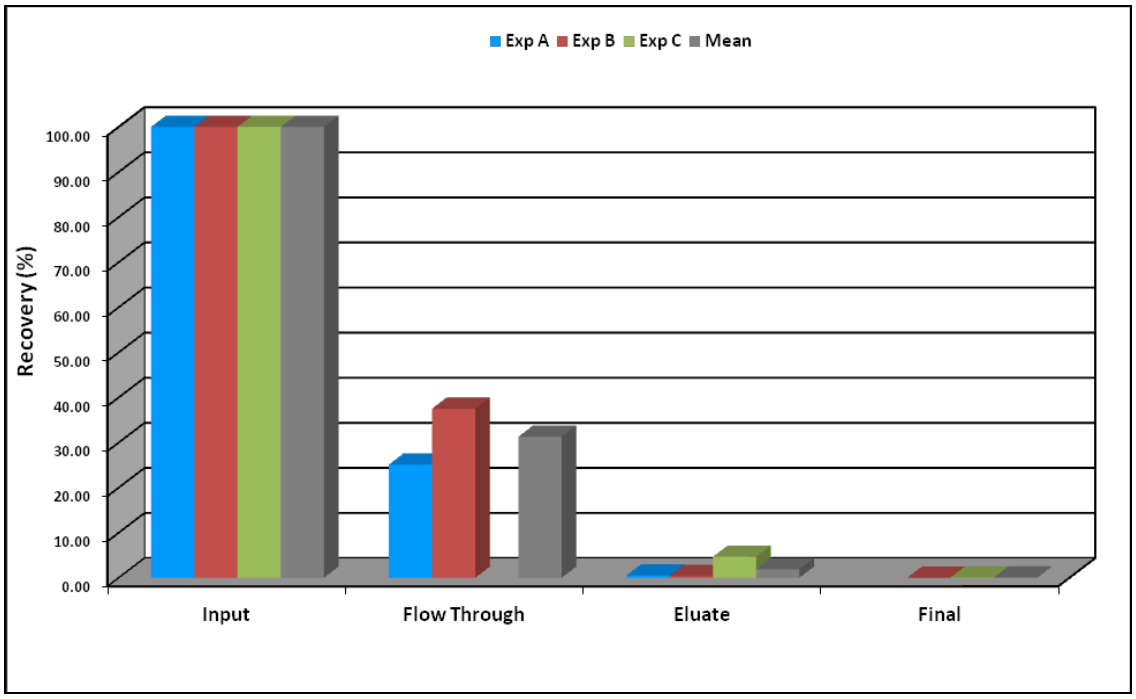

Figure 3.10b: Percentage recovery of norovirus GII from turbid water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

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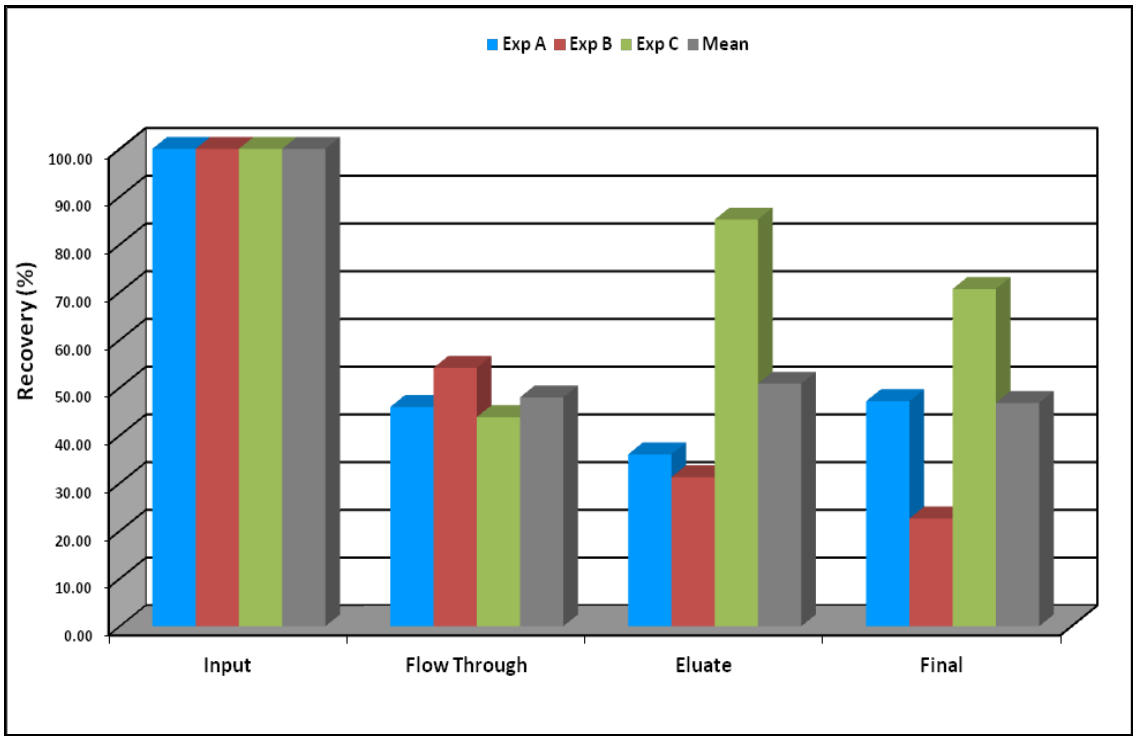

Figure 3.11a: Percentage recovery of coxsackievirus B6 from tap water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

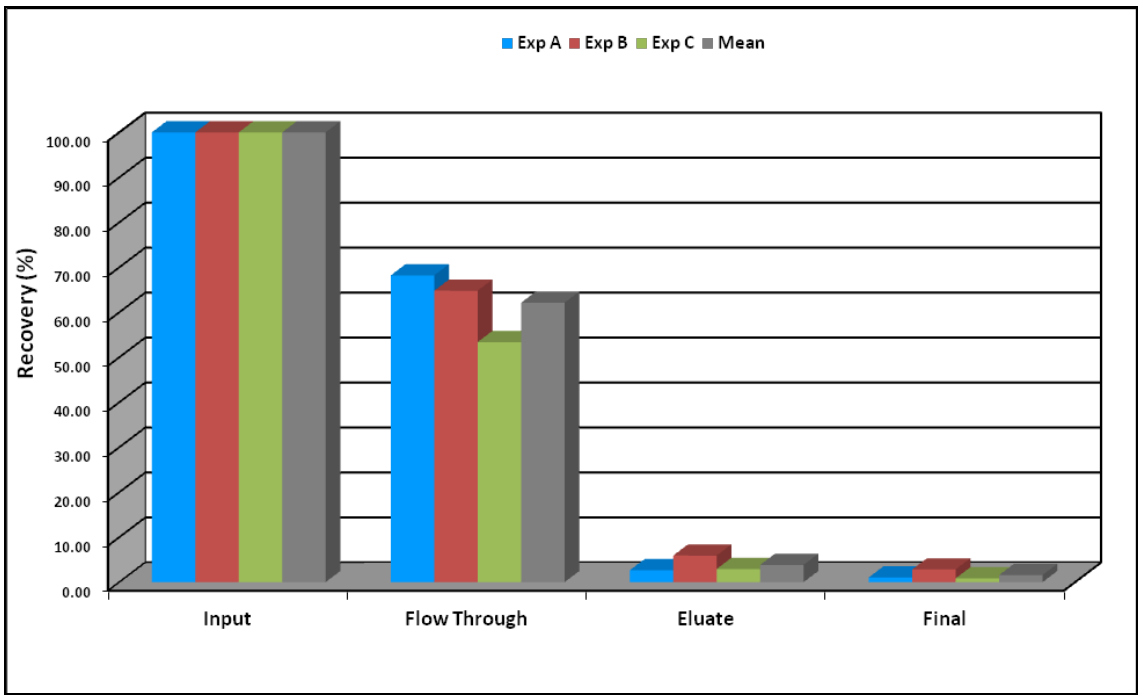

Figure 3.11b: Percentage recovery of coxsackievirus B6 from turbid water at each stage of the glass wool adsorption-elution process. Recovery was evaluated with three replicates per experiment.

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Statistical analysis using the generalised linear model clearly indicates that the predicted mean EOR in the eluate varied for each virus (Figure 3.12). A similar trend to that observed for the final recovery concentrate was noted, i.e. there were clear difference in efficiencies of recovery for all viruses between tap water and raw water.

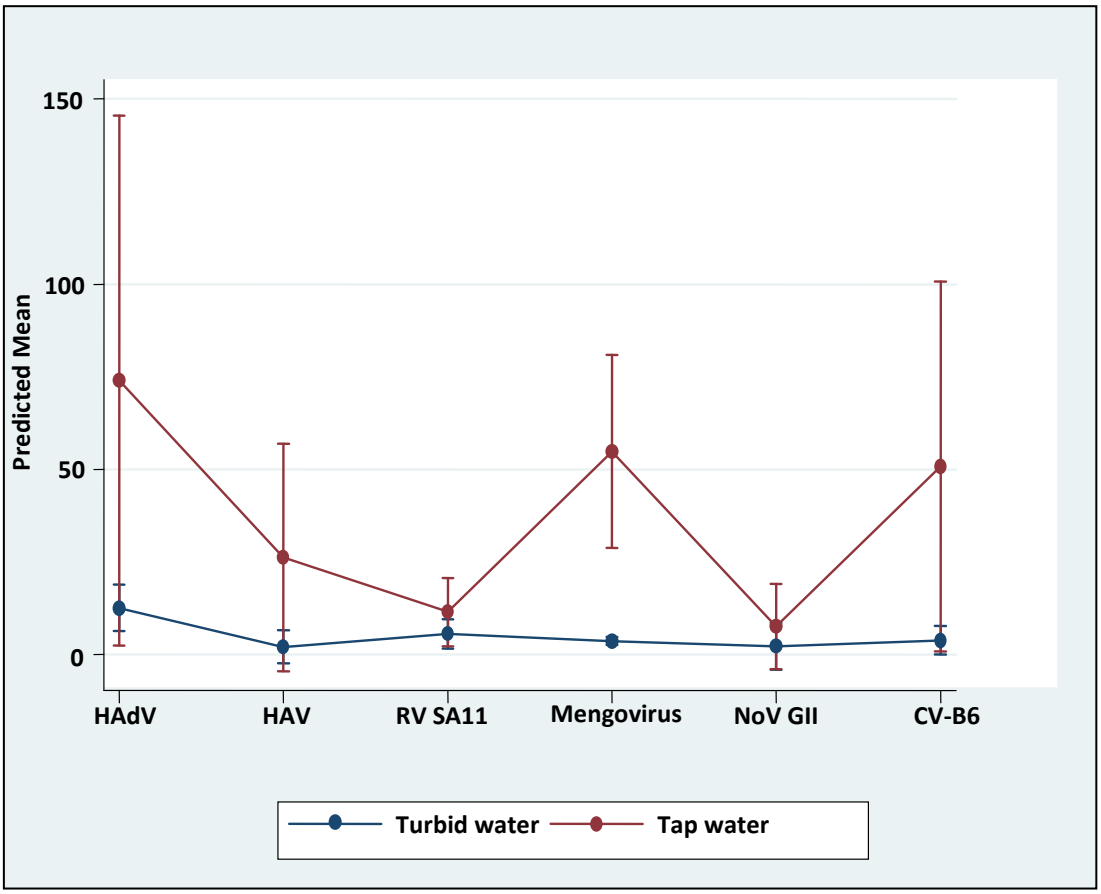

Figure 3.12: Predictive margins, with 95% CIs, of the relationship between the virus, water matrix and efficiency of recovery from the eluate.

Using the same analysis it was evident that the virus recovered the in the flow through, i.e. lost in the recovery process, was by the type of virus and source of water (Figure 3.13).

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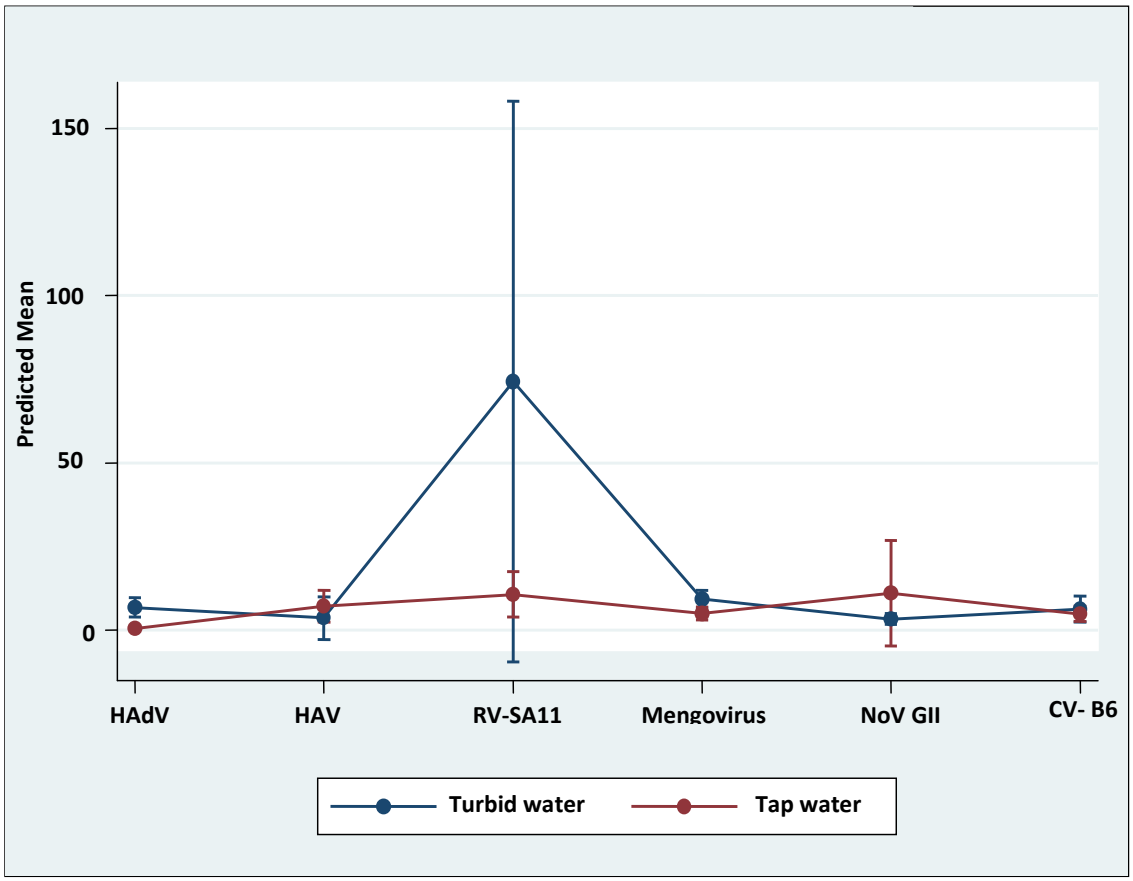

Figure 3.13: Predictive margins, with 95% CI, of the the relationship between the virus, the water matrix and efficiency of recovery from the flow through.

3.3.3 Assessment of the effect of modifications to the glass wool column on the efficiency of recovery using mengovirus as a model virus

The average percentage of recovered viruses from tap water for each stage of the adsorption elution process in the modified glass wool columns is presented in Table 3.3

Table 3.3 Average percentage of recovered virus for each glass wool column modification

Glass wool column modification	Final recovery %	SD	Recovery in eluate %	SD	Loss in flow through %	SD
15 g plus grid	15.42	5.09	54.78	17.92	50.31	13.00
15 g no grid	17.06	10.63	71.56	54.66	104.12	28.66
20 g no grid	5.57	3.52	58.61	8.81	86.07	36.67

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a) Standard column: 15 grams glass wool with wire gauze grid: When an average of 1.76 x 10^5 genome copies (SD = 3.19 x 10^4) was seeded, an average of 2.65 x 10^4 genome copies (SD = 8.9 x 10^3) was recovered in the final concentrate which translates to a final recovery of 15.42% (SD = 5.09%: range 7.89% - 24.47%)(Table 3.3). An average of 8.71 x 10^4 genome copies (SD = 1.93 x10^4), which represents 50.31% (SD = 13.00% : range 33.79% -71.3%) of the seeded viruses was lost in the flow through. In the elution stage of the recovery process 9.46 x 10^4 genome copies (SD = 3.18 x $10^{4:}$ range 6.5 x 10^4 – 1.67 x 10^5) giving an average recovery of 54.79% (SD = 17.79%: 26.78% - 88.83%) was recorded.

b) Modification: 15 grams glass wool without wire gauze grid: When an average of 1.38 x 10^5 genome copies (SD = 7.34 x 10^4: range 4.04 x 10^4 – 2.3 x 10^5 of mengovirus was seeded into tap water and run through columns prepared using 15 g glass wool with no interleaved grids an average of 2.91 x10^4 genome copies (SD = 2.43 x 10^4: range 5.38 x 10^1 – 6.06 x 10^4) were recovered in the final concentrate (Table 3.3). Therefore the average EOR was 17.06% (SD = 10.62%: range 0.86% - 29.42%). An average of 1.30 x 10^5 genome copies (SD =5.62 x 10^4: range 4.74 x 10^4 – 1.96 x 10^5) was lost in the flow through. This translates to a calculated average of 104.12% (SD = 28.67: range 76.45% -163.40%) loss of viruses in the flow through. In the eluate, an average of 2.41 x 10^5 genome copies (SD = 3.44 x 10^5: range 4.92 x 10^0 - 1.10 x 10^6) were recovered. Therefore 71.56% (SD = 54.67%: range 3.62 – 137.86%) of the viruses were recovered in the eluate.

c) Modification: 20 grams glass wool without wire gauze grid: Of the average of 2.96 x 10^5 genome copies (SD = 3.70 x 10^4: range 2.28 x 10^5 – 3.70 x 10^5) of mengovirus was passed through 20 g glass wool column without interleaved gauze grids 1.37 x10^4 genome copies (SD = 1.01 x 10^4: range 2.36 x 10^3 – 2.54 x 10^4) were recovered in the final concentrate. Therefore the average EOR was 5.57% (SD = 3.52%: range 1.13% - 10.35%). An average of 2.53 x 10^5 genome copies (SD = 1.09 x 10^5: range 1.58 x 10^5 – 4.38 x 10^5), which means an average of 86.07% (SD = 36.67: 58.08% -147.97%) of the viruses were lost in the flow through. In the eluate, an average of 1.72 x10^5 genome copies (SD = 2.67 X 10^4: 1.26 x 10^5 – 2.04 x 10^5) calculated to be 58.61% (SD = 8.81: range 46.32 – 70.83%) of the seeded viruses was recovered in the eluate.

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d) Statistical Analysis: Using the two-sample mean comparison t-test to assess the effect of glass wool column modifications on the recovery of mengovirus from tap water no significant difference in the final percentage recovery between standard column and modified column of 15 g without an interleaved grid (p = 0.3420) was noted. The modification of 15 g glass wool with no grid did not result in significant difference on the percentage recovery in the elution stage (p = 0.2013). However when the percentage recoveries in the flow through between the standard column and the modified column (15 g glass wool with no grid) were compared a significant difference between the percentage of viruses lost in the flow through (p = 0.0002) was evident. There was a significant difference in the EOR in the final concentrate between the 15 g glass wool column without grid and 20g glass wool column without grid (p = 0036) and no significant difference on the percentage recovery in the elution (*p = 0.2465*) as well as in the percentage of viruses lost in the flow through (p = 0.1308). Using the same test to the compare percentage of viruses recovered in the eluate and in the final concentrate revealed that the recovery in the eluate was significantly higher than in the final concentrate in the standard and modified the three glass wool columns, i.e. 15 g glass wool with grid (p = 0.0000), 15 g glass wool without grid (p = 0.00048), and 20 g glass wool without grid (p = 0.0000).

3.3.4 Comparison of the effect of liquid and solid PEG/NaCl precipitation on the efficiency of recovery using mengovirus as a model virus

When the effect of the two methods of PEG/NaCl precipitation, namely solid PEG_{6000}/NaCl and liquid PEG_{8000}/NaCl precipitation were compared results showed that with the solid PEG_{6000}/NaCl precipitation method the EOR was 12.96% (SD = 12.41 : range 3.10% - 45.00%) while with the liquid PEG_{8000}/NaCl precipitation the average EOR was 9.67% (SD = 2.83 : range 4.78% – 12.97%). Application of the two sample mean comparison t-test showed that the observed difference in the EOR was not significant (p = 0.7753).

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3.3.5 Effect of water pH on the efficiency of recovery in turbid water using hepatitis A virus as a model virus

The EOR at three pH values, namely. pH 7.0, pH 6.0 and pH 3.5 were assessed. The lowest EOR, i.e. 0.28% (SD = 0.42%: range 0.02% – 1.01%) was obtained at pH 3.5. The percentage of the viruses lost in the flow through averaged 108.37% (range 0 – 525.10%). A water pH 7.0 resulted in the second lowest EOR, with an average of 5.04% (SD = 8.94%: range 0 – 26.41%). At the latter pH the percentage recovery in the eluate was lower than in the final recovery (1.98%: SD = 3.08: range 0.03% -9.49%). An average of 54.09% (SD = 57.99%: range 1.51% - 161.00%) of HAV was lost in the flow through. The highest final recovery of 13.65% was obtained at pH 6.0 with the recovery in the eluate being 0.16% and a virus loss in the flow through of 75.95%.

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CHAPTER 4

DISCUSSION

One of the major challenges in the detection of viral pathogens from water is the low concentration in water sources (Soule et al.,, 2000). Therefore, large volumes (10 – 1000 ℓ) of water need to be analysed for the detection of these viruses (Köster et al., 2003; Gerba, 2007). These volumes however are too large to be used in the available analyses for detection of viruses such as cell culture, plaque assays or for molecular detection. It is therefore necessary to recover the viruses from large volumes of water and concentrate them to volumes required for the available detection methods (Wyn-Jones, 2007). Of the described viral recovery methods the glass wool adsorption-elution method for recovery of viruses and subsequent concentration of viruses from water is a cost-effective and feasible method to apply in resource-limited settings. In order to ensure the efficacy of this method for the recovery of different enteric viruses from both treated tap and turbid surface water a comprehensive assessment of the method's performance was necessary as previous studies used treated or laboratory water (Menut et al., 1993; Gassilloud et al., 2003) and cell culture-based quantification methods (Menut et al., 1993; Vilaginés et al., 1993, 1997). This was achieved by determining of the total amount of viruses seeded in the water samples which were passed through the glass wool column, the determination of the percentage of viruses adsorbed and eluted by the buffer, the percentage of viruses lost in the flow through and calculation of the recovery in the final concentrate. This required accurate quantification of the total viral load (infectious and non-infectious particles) in samples (Martinez-Martinez et al., 2011). This information is required for further optimisation of the recovery method for the assessment of risk posed by enteric viruses in the environment and drinking water. Accurate quantification of viruses is also very important to determine the level of contamination of source waters, to determine the efficiency of disinfection treatment processes in the reduction of virus contamination and also to determine the possible linkages between virus levels and risk of infection or disease outbreaks (Rodriguez et al., 2012).

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In this study *rt* qRT-PCR/PCR was used to determine the absolute copy number of viruses present at each stage of the glass wool adsorption-elution process by comparing the unknown samples to standard curves prepared with known concentrations of each virus. The accuracy of quantification and subsequent determination of the correct EOR for the glass wool adsorption-elution is dependent on the accuracy of the constructed standard curves (Forlenza et al., 2012). The standard curves constructed for RV SA11, HAV and HAdV (Figures 3.1, 3.2 and 3.3) in this investigation met important criteria such as high efficiencies and small errors. Under optimal conditions every cycle in the PCR should result in doubling of amplification product, meaning that the amplification efficiency should be 2, indicated by two-fold increase in fluorescence (Forlenza et al., 2012). The efficiencies of the standard curves for RV SA11, HAV and HAdV (Figures 3.1, 3.2 and 3.3, respectively) were between 1.85 and 2.1 which was within the acceptable limits. In this study amplification of RV SA11 (dsRNA virus) was more efficient than HAdV (dsDNA virus) which was contrary to reports in literature (Martinez-Martinez et al., 2011) which indicated that amplification of DNA viruses is more efficient than RNA viruses. Positive sense ssRNA virus, HAV, had the lowest amplification efficiency (1.817). The detection limit of fluorescencent *rt* qRT-PCR/PCR is determined by the highest dilution of virus producing a CP value (Guo et al., 2009). However HAdV had the lowest limit of detection (1 genome copy) followed by RV SA11 (4 genome copies) and HAV had the highest limit of detection (50 genome copies). The low limits of detection shown by these standard curves indicated the high sensitivity of the *rt* qRT-PCR/PCR assays used to quantify the viruses under investigation (Saunders, 2008; Gibson et al., 2012) which subsequently allowed for the accurate calculation of the EOR. For the accurate determination of the detection limit, the viral nucleic acid should be consistently detected in all replicates of the highest dilution qRT-PCR/PCR assay (Watzinger et al., 2004). This was observed in the standard curves RV SA11, HAV and HAdV (Figures 3.1, 3.2 and 3.3, respectively). Another important feature which needs to be considered in the construction of standard curves for accurate quantification of viruses is the dynamic ranges of the standards, i.e., the lowest and highest concentration and associated CP values as well as the number of replicates performed (Kristen and Borchardt, 2013). The standard curves constructed in this investigation had a wider

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range of at least five dilutions (standards) with three replicates each, indicating that the standard curves offered accurate quantification over a wide range of concentrations (Saunders, 2008).

Sample quality plays a critical role on the accuracy of *rt* qRT-PCR/PCR as the absence or presence of inhibitory substances can affect the sensitivity of the reaction (Gibson et al., 2012). The relative copy numbers between the samples of different quality can be estimated by the differences in their CP values (Bernard and Wittwer, 2002). By comparing the CP values and fluorescence intensities for the number of virus genome copies detected in tap and turbid water which were seeded with the same amount of viruses, the effect of sample quality can be evaluated (Rodriguez et al., 2012). The presence of inhibitors in a water sample can decrease the sensitivity by causing shift or increase in the CP values (Gibson et al., 2012). In this evaluation the sensitivity of *rt* qRT-PCR/PCR was shown to be greatly affected by sample quality as indicated by higher CP values in turbid water than tap water which had been seeded with the same concentration of virus, e.g. 31.48 in turbid water and 25.57 in tap water for HAV and 36.33 in turbid water and 33.80 in tap water for NoV. Inhibitors in the samples also reduced the efficiency of PCR amplification as shown by the reduced steepness of the amplification curves in the turbid waters as compared to tap waters (Appendix A). The effect of sample quality on efficiency of amplification was more pronounced for NoV than the rest of the viruses assessed in this evaluation. It is therefore very important to account for inhibition when reporting *rt* qRT-PCR/PCR results for viral pathogens in environmental waters and drinking waters since inhibition can result in underestimating pathogen concentrations (Gibson et al., 2012). In this evaluation the effect of inhibitors in water samples appeared to have had a strong bearing on the final calculation of the EOR of glass wool adsorption-elution process.

In this investigation the six selected enteric viruses (HAdV, HAV, RV SA11, mengovirus, NoV GII.4 and CV-B6) could all be recovered from treated tap water and turbid water using the glass wool adsorption-elution technique. However, the final EOR differed between virus types and water quality (Table 3.2). In the treated tap water the highest EOR of 46.91% was recorded for CV-B6 (representing the enteroviruses) followed by

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HAdV (representing the proposed indicators for virus contamination of water) with an EOR of 33.7%. The EOR for mengovirus (process control) was 15.42% which was slightly more efficient than that for HAV (EOR of 13.14%). Gastroenteritis viruses NoV, the leading cause of non-bacterial gastroenteritis, and RV, the leading cause of diarrhoea in children, had the lowest EORs of 1.46% and 5.58%, respectively in tap water. In the turbid water, NoV had the lowest EOR of 0.05% followed by CV-B6 (1.57%). These results are in agreement with other studies which reported that the EOR of glass wool adsorption-elution for the recovery of viruses from water was dependent the type of viruses (Vilaginés et al., 1993; Gassilloud et al., 2003; Lambertini et al., 2008). In the glass wool recovery experiments of Lambertini et al. (2008), EORs of 98% for PV in tap water and 56% in well water, 28% for HAdV 41 in tap water and 22% in well water were reported. This evaluation also showed that there is variation in recovery efficiencies for a single virus type as shown by the standard deviations and recovery efficiency ranges for replicate assays of the same virus type (Table 3.3 ; Figure 3.12; Appendix A) which was also demonstrated in previous studies (Lambertini et al., 2008; Calgua et al., 2013). This study has therefore shown quantitatively that the EOR for glass wool for the selected enteric viruses are higher in tap water than the turbid waters. There are three possible explanations for this observation: i) the glass wool filters clogged when recovering viruses from raw surface water. Most of the seeded viruses could have been trapped in the debris and particulate matter such as silt and other salt particles such that the buffer could not elute all the viruses for the downstream precipitation and molecular detection steps, ii) the presence of inhibitory substances in the turbid/raw water like humic and fulvic acids which interfere with molecular-based assays such as *rt* RT-PCR/PCR (Bosch et al., 2011; Ikner et al., 2012). There is need for clarification of the water matrix to remove sediments and inhibitors associated with turbid surface water, but this should be validated to ensure that the viruses are not lost prior to the adsorption step (Mattison and Bidawid; 2009), and iii) the suspended solids and ionic organic compounds naturally present in turbid waters preferentially adsorb to the glass wool reducing the effectiveness of viruses to adsorb to glass wool (Bosch et al., 2011; Ikner et al., 2012). Pre-treatment of turbid surface may be required to facilitate for effective adsorption of viruses to the glass wool.

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In this study very low EORs were recorded for NoV from tap water (1.46%) and turbid water (0.05%). In their investigations using FCV, which was used as a surrogate for NoV, Gassilloud et al. (2003) also reported low EORs from tap water with an average recovery of 0.5% (0.3-0.8%). They concluded that glass wool adsorption-elution technique was not suitable for FCVs, and therefore NoVs. However Lambertini and colleagues, who were the first to recover NoV from water using glass wool adsorption-elution, obtained an average EOR of 29% (Lambertini et al., 2008). This value was an average for NoV GII and NoV GI recovered from three water matrices, namely tap water and water from two different wells whereas in this study NoV GII.4 was used to determine the EOR from dechlorinated tap water and turbid surface water. The physiochemical parameters of the water matrices like pH, conductivity, turbidity, hardness and presence of organic acids therefore appear to affect the isoelectric points of viruses which in turn influence adsorption and the final recovery (Sobsey and Glass, 1984; Bosch et al., 2011).

This study is one of the first investigations to systematically compare the elution efficiency and the final recovery efficiency of glass wool adsorption-elution technique using *rt* qRT-PCR/PCR. In the assessment of the different stages of the adsorption-elution process it was found that higher recovery efficiencies were obtained in the eluate than in the final recovered virus suspension for all five selected enteric viruses and the process control (mengovirus). The secondary concentration step, i.e. PEG/NaCl precipitation, resulted in a loss of viruses (Ikner et al., 2012). There are few studies which have been done on the elution efficiency of the GBEB (Vilaginés et al., 1993) where the eluted virus was titrated by plaque assay and recovery efficiency expressed as a percentage of the virus before glass wool filtration. In these early experiments, elution efficiency was described as concentration efficiency with results (72% and 62% in 400 ℓ and 1000 ℓ, respectively) comparable to the values obtained in this evaluation. However using PV, glycine buffer was evaluated on positively charged filters, i.e. cellulose filters and resin filters (Zeta plus 50S, 0.75 uM pore size) and produced elution efficiencies of 67% and 60%, respectively (Sobsey and Jones, 1979). Beef extract (3%) buffer was evaluated on Millipore HA filters for the elution of poliovirus and produced elution efficiency of 53-123% (Sobsey and Glass, 1984). In this

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study the elution efficiency of GBEB pH 9.5 (74.02% for HAdV; 54.73% for mengovirus; 50.81% for CV-B6) was comparable to that reported for glycine buffer (pH 9.0) and beef extract buffer (pH 6-8) from positive filters (Sobsey and Jones, 1979). This investigation also showed that large quantities of viruses, not adsorbed to the glass wool, were lost in the flow through (Table 3.2).

In treated tap water mengovirus, a proposed sample process control (da Silva et al., 2007; Comelli et al., 2008; Pintó et al., 2009) for the recovery and concentration of viruses from food and environmental matrices, had an EOR of 15.42% (range 5.10% - 24.47%) which compared well with the EOR for the other five selected enteric viruses used in this study. As observed for the other enteric viruses the percentage mengovirus recovered in the eluate (54.73%) was higher than in the final recovered virus suspension (15.42%) but was within the range of recoveries recorded for the other five viruses (7.57% for NoV GII.4 to 74.02% for HAdV). The quantity of viruses lost in the flow through (50.32%) was within the range of other five enteric viruses (4.06% for HAdVs to 110.28% for NoV GII.4). A similar pattern of virus recovery and virus loss for mengovirus was also observed for turbid water. Therefore this study supports the application of mengovirus (strain MC_0) as a sample process control because: i) its behavior was similar to the other selected enteric viruses, ii) it is a murine virus which is not likely to be found associated with water (Mattison and Bidawid; 2009), and iii) it is not infectious to humans and other animal species (Pintó et al., 2009). It is therefore possible that mengovirus can be co-adsorbed on the glass wool, co-eluted in the buffer, co-concentrated by PEG/NaCl precipitation with either PEG_{6000} or PEG_{8000} and its nucleic acid co-extracted with the target enteric viruses for detection by molecular-based methods. The advantage of using a process control is that it can be traced at all the stages of the analytical process unlike other control systems, such internal or external amplification controls, which are introduced at the final detection stage and evaluate potential inhibition of the amplification process (Casas et al., 2007; Bosch et al., 2011). Using mengovirus as a process control it was possible to define the adsorption efficiency of glass wool filters, the elution efficiency of the elution buffer (GBEB), the precipitation efficiency using PEG/NaCl, the nucleic acid extraction efficiency and to control for RT-PCR/PCR inhibition for each sample.

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When compared with FCV, another proposed process control (Mattison et al., 2009), the EOR for mengovirus from the glass wool filters was higher than that reported for FCV. Mengovirus would be a more appropriate process control virus. The average EOR of 33.97% (10.38% - 86.02%) for HAdVs, a proposed indicator for viral pollution, from tap water compared well with findings of Lambertini and co-workers who reported an average EOR of 21% (4% - 58%) (Lambertini et al., 2008). Adenoviruses also showed the highest recovery efficiencies in the eluate for both tap water and raw surface water. It was also demonstrated in this study that the virus adsorbs well to the glass wool columns as shown by the least amount of viruses lost in the flow through (Table 3.2).

In the evaluation of the effect of modifications in the glass wool column preparation on the EOR of mengovirus there was no statistically significant difference in the EOR between standard glass wool column and modified column (15 g without grid). There was however a larger variation of final EOR in the modified column (SD = 10.62%) than the standard column (SD = 5.09%). This therefore means that the final EORs in the standard glass wool column are more reproducible than for the modified column. In addition more viruses were also lost in the flow through of the modified column than the standard column, indicating that the standard column retains more viruses (higher adsorption efficiency) than the modified column. When more glass wool was added in the modified column to 20 g there was a statistically significant decrease in the final EOR. Therefore, an increase in the mass of glass wool column resulted in the decrease in adsorption capacity. However, more data is required to fully describe the relationship between adsorption efficiency and the mass of glass wool in the column or the relationship between mass of glass wool and EOR.

Using mengovirus as a model, a comparison of different precipitation methods showed that the PEG_{8000}/NaCl precipitation protocol was less efficient than the PEG_{6000}/NaCl precipitation protocol for secondary concentration although the difference was not statistically different. However, the PEG_{6000}/NaCl protocol has a disadvantage of an overnight precipitation step. Therefore, the choice of the precipitation method is dependent on the turn-around-time required for the reporting of results. Since

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mengovirus has been shown to behave like the other selected enteric viruses, these findings may be representative of the precipitation efficiency of the two methods for the secondary concentration of enteric viruses from environmental samples.

The findings of this study are in agreement with other studies which showed that the EOR of viruses using glass wool adsorption-elution is affected by the pH of the sample. The USEPA guidelines recommend a pH of 7.0 for the water sample and that the pH be adjusted downwards if it is above pH 8.0 (USEPA, 2006; Lambertini et al., 2008). For HAV the optimal recovery was obtained from turbid water at pH 6.0 (EOR = 13.65%) and was very poor (EOR = 0.28%) at very acidic (pH 3.5) and neutral pH (EOR = 5.04%). The optimal recovery for HAV at pH 6 comparable to that reported for HAdV 41 (15.8%) and differed widely from the optimal recovery for PV of 85%, obtained at pH 6.5 (Lambertini et al., 2008). Therefore, it is recommended to adjust the pH of the water matrix to the ranges optimal for recovery of specific viruses. Experiments with HAV on sample pH variation have shown unusual outcome where the recovery in the eluate was lower than in the final PEG/NaCl precipitation showing that pH influences virus elution (Lambertini et al., 2008).

From the discussion it is evident the hypothesis of this study was correct. That is, the glass wool adsorption-elution technique was more efficient in the recovery of enteric viruses from treated tap water than from turbid surface water. It was also demonstrated that mengovirus behaved similarly to the enteric viruses and could therefore be used as a process control when using the glass wool adsorption-elution technique for the recovery of enteric viruses from environmental samples. Although the EORs were affected by virus type and water quality, this study has shown that the glass wool adsorption-elution technique is a practical cost-effective viral recovery method for use in resource-poor settings. Future research should however investigate methods to improve the EOR of enteric viruses from turbid water to facilitate more accurate prevalence and quantification data for disinfection studies.

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CHAPTER 5

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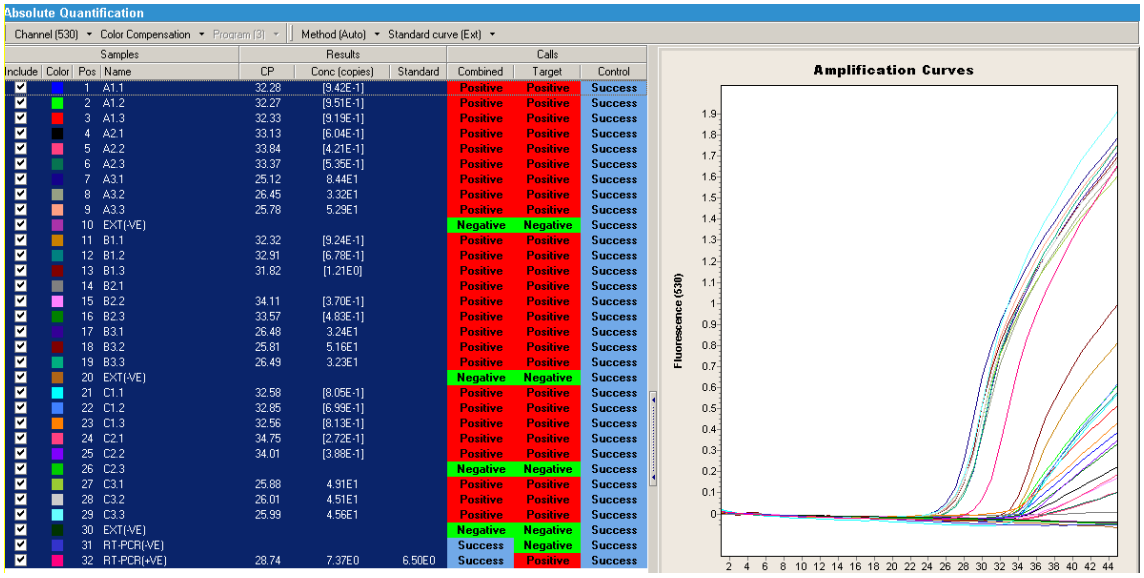
APPENDIX A

AMPLIFICATION CURVES AND WORKSHEETS

A1: Mengovirus

Mengovirus in treated tap water



Include	Color	Pos	Name	Samples CP	Results Conc (copies)	Results Standard	Calls Combined	Calls Target	Calls Control
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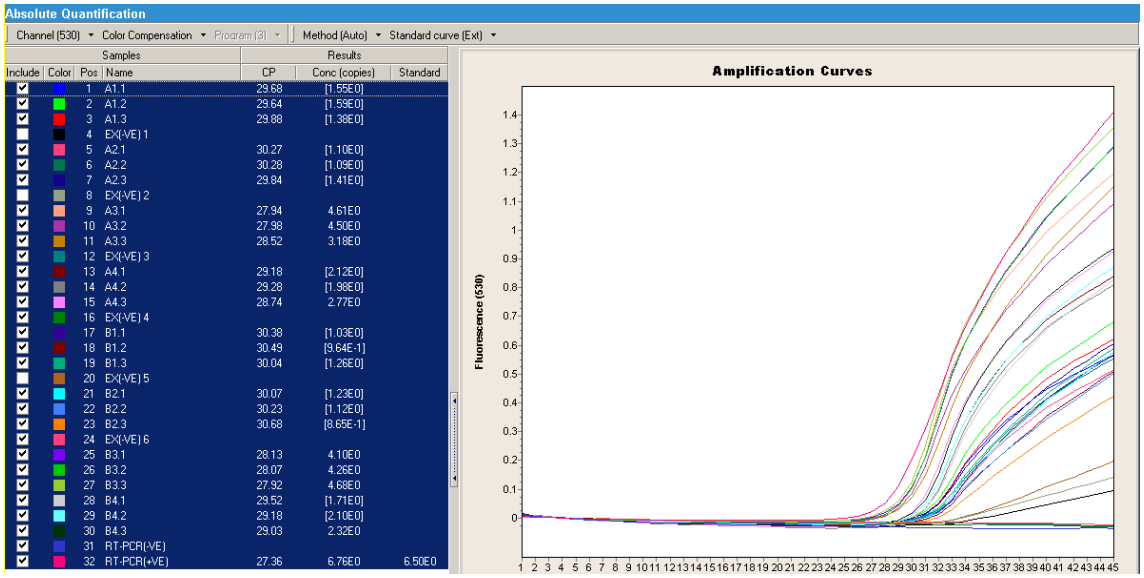
☑		2	A1.2	32.27	[9.51E-1]		Positive	Positive	Success
☑		3	A1.3	32.33	[9.19E-1]		Positive	Positive	Success
☑		4	A2.1	33.13	[6.04E-1]		Positive	Positive	Success
☑		5	A2.2	33.84	[4.21E-1]		Positive	Positive	Success
☑		6	A2.3	33.37	[5.35E-1]		Positive	Positive	Success
☑		7	A3.1	25.12	8.44E1		Positive	Positive	Success
☑		8	A3.2	26.45	3.32E1		Positive	Positive	Success
☑		9	A3.3	25.78	5.29E1		Positive	Positive	Success
☑		10	EXT(-VE)				Negative	Negative	Success
☑		11	B1.1	32.32	[9.24E-1]		Positive	Positive	Success
☑		12	B1.2	32.91	[6.78E-1]		Positive	Positive	Success
☑		13	B1.3	31.82	[1.21E0]		Positive	Positive	Success
☑		14	B2.1				Positive	Positive	Success
☑		15	B2.2	34.11	[3.70E-1]		Positive	Positive	Success
☑		16	B2.3	33.57	[4.83E-1]		Positive	Positive	Success
☑		17	B3.1	26.48	3.24E1		Positive	Positive	Success
☑		18	B3.2	25.81	5.16E1		Positive	Positive	Success
☑		19	B3.3	26.49	3.23E1		Positive	Positive	Success
☑		20	EXT(-VE)				Negative	Negative	Success
☑		21	C1.1	32.58	[8.05E-1]		Positive	Positive	Success
☑		22	C1.2	32.85	[6.99E-1]		Positive	Positive	Success
☑		23	C1.3	32.56	[8.13E-1]		Positive	Positive	Success
☑		24	C2.1	34.75	[2.72E-1]		Positive	Positive	Success
☑		25	C2.2	34.01	[3.88E-1]		Positive	Positive	Success
☑		26	C2.3				Negative	Negative	Success
☑		27	C3.1	25.88	4.91E1		Positive	Positive	Success
☑		28	C3.2	26.01	4.51E1		Positive	Positive	Success
☑		29	C3.3	25.99	4.56E1		Positive	Positive	Success
☑		30	EXT(-VE)				Negative	Negative	Success
☑		31	RT-PCR(-VE)				Success	Negative	Success
☑		32	RT-PCR(+VE)	28.74	7.37E0	6.50E0	Success	Positive	Success

Mengovirus in turbid surface water



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☑		3	A1.3	29.88	[1.38E0]	
☐		4	EX(-VE) 1			
☑		5	A2.1	30.27	[1.10E0]	
☑		6	A2.2	30.28	[1.09E0]	
☑		7	A2.3	29.84	[1.41E0]	
☐		8	EX(-VE) 2			
☑		9	A3.1	27.94	4.61E0	
☑		10	A3.2	27.98	4.50E0	
☑		11	A3.3	28.52	3.18E0	
☑		12	EX(-VE) 3			
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☑		14	A4.2	29.28	[1.98E0]	
☑		15	A4.3	28.74	2.77E0	
☑		16	EX(-VE) 4			
☑		17	B1.1	30.38	[1.03E0]	
☑		18	B1.2	30.49	[9.64E-1]	
☑		19	B1.3	30.04	[1.26E0]	
☐		20	EX(-VE) 5			
☑		21	B2.1	30.07	[1.23E0]	
☑		22	B2.2	30.23	[1.12E0]	
☑		23	B2.3	30.68	[8.65E-1]	
☑		24	EX(-VE) 6			
☑		25	B3.1	28.13	4.10E0	
☑		26	B3.2	28.07	4.26E0	
☑		27	B3.3	27.92	4.68E0	
☑		28	B4.1	29.52	[1.71E0]	
☑		29	B4.2	29.18	[2.10E0]	
☑		30	B4.3	29.03	2.32E0	
☑		31	RT-PCR(-VE)			
☑		32	RT-PCR(+VE)	27.36	6.76E0	6.50E0

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Mengovirus: Calculation of virus titres and recovery efficiencies

MSc study Vurayai Ruhanya: Seeding experiment										
Date:06/07/10		Water type:Tap			Virus:Mengovirus			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$1.85X10^5$					
1.2	Seeded water	10ℓ	1mℓ		$1.36X10^5$					
1.3	Seeded water	10ℓ	1mℓ		$2.42X10^5$					
2.1	Flow Through	10ℓ	1mℓ				$7.40X10^4$	0	40.00%	
2.2	Flow Through	10ℓ	1mℓ				$9.66X10^4$	1	71.03%	
2.3	Flow Through	10ℓ	1mℓ				$8.44X10^4$	0	34.88%	48.64%
3.1	Eluate	100 mℓ	1mℓ				$1.03X10^5$	0	55.68%	
3.2	Eluate	100 mℓ	1mℓ				$6.46X10^4$	0	47.50%	
3.3	Eluate	100 mℓ	1mℓ				$6.48X10^4$	1	26.78%	43.32%
4.1	Final concentrate	10 mℓ	1mℓ				$1.46X10^4$	1	7.89%	
4.2	Final concentrate	10 mℓ	1mℓ				$2.38X10^4$	1	17.50%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.26X10^4$	1	10.00%	11.80%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment										
Date:06/08/10		Water type: Raw			Virus:Mengovirus			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$3.10X10^5$					
1.2	Seeded water	10ℓ	1mℓ		$3.18X10^5$					
1.3	Seeded water	10ℓ	1mℓ		$2.76X10^5$					
2.1	Flow Through	10ℓ	1mℓ				$2.20X10^5$	0	70.77%	
2.2	Flow Through	10ℓ	1mℓ				$2.18X10^5$	0	68.55%	
2.3	Flow Through	10ℓ	1mℓ				$2.82X10^5$	0	102.17%	80.50%
3.1	Eluate	100 mℓ	1mℓ				$9.22X10^3$	2	2.97%	
3.2	Eluate	100 mℓ	1mℓ				$9.00X10^3$	2	2.83%	
3.3	Eluate	100 mℓ	1mℓ				$6.36X10^3$	2	2.30%	2.70%
4.1	Final concentrate	10 mℓ	1mℓ				$4.24X10^2$	3	0.14%	
4.2	Final concentrate	10 mℓ	1mℓ				$3.96X10^2$	3	0.12%	
4.3	Final concentrate	10 mℓ	1mℓ				$5.54X10^2$	3	0.20%	0.15%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment											
Date:06/07/10			Water type:Raw			Virus:Mengovirus			Replicate No:B		
No	Sample type		Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water		10ℓ	1mℓ		$2.06X10^5$					
1.2	Seeded water		10ℓ	1mℓ		$1.93X10^5$					
1.3	Seeded water		10ℓ	1mℓ		$2.52X10^5$					
2.1	Flow Through		10ℓ	1mℓ				$2.46X10^5$	0	117.48%	
2.2	Flow Through		10ℓ	1mℓ				$2.24X10^5$	0	116.06%	
2.3	Flow Through		10ℓ	1mℓ				$1.73X10^5$	0	68.65%	100.73%
3.1	Eluate		100 mℓ	1mℓ				$8.20X10^3$	2	3.98%	
3.2	Eluate		100 mℓ	1mℓ				$8.52X10^3$	2	4.41%	
3.3	Eluate		100 mℓ	1mℓ				$9.36X10^3$	2	3.71%	4.03%
4.1	Final concentrate		10 mℓ	1mℓ				$2.34X10^2$	3	0.11%	
4.2	Final concentrate		10 mℓ	1mℓ				$4.20X10^2$	3	0.17%	
4.3	Final concentrate		10 mℓ	1mℓ				$4.64X10^2$	3	0.18%	0.15%
	Key words										
	Stock virus titre:										
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction										

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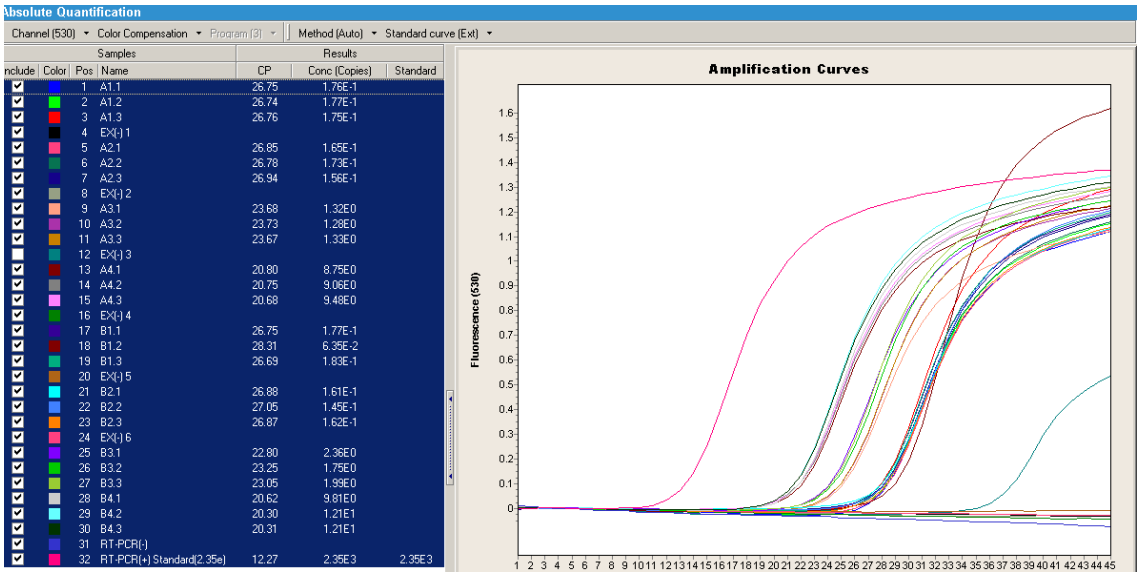
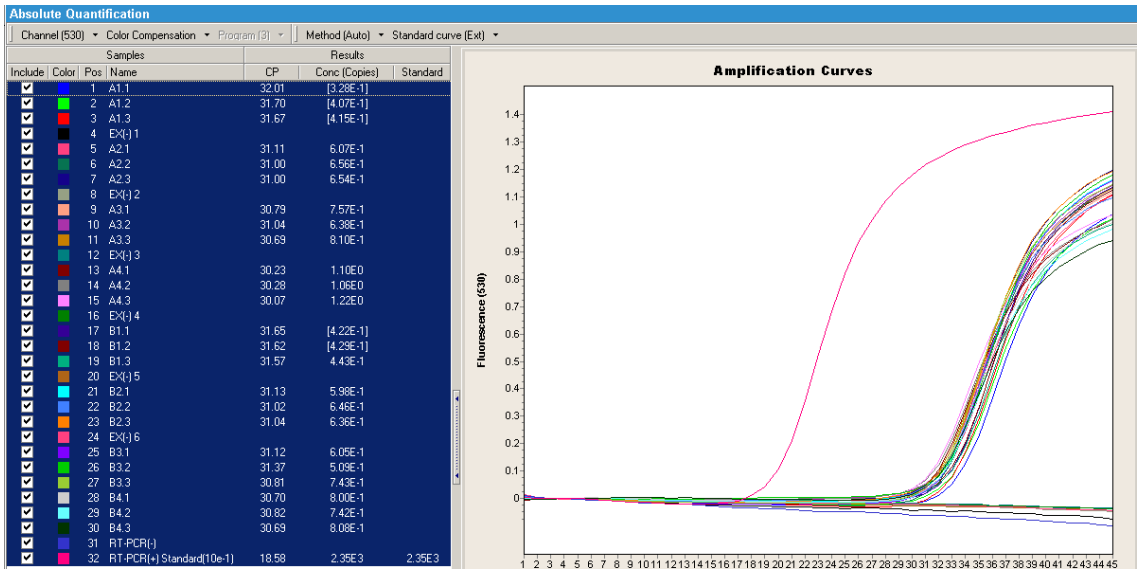
A2: Rotavirus-SA11

RV-SA11 in treated tap water



RV-SA11 in turbid surface water



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Rotavirus SA-11: Calculation of virus titres and recovery efficiencies

MSc study Vurayai Ruhanya: Seeding experiment										
Date:16/02/11		Water type: Tap			Virus:RV			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$3.54X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$1.27X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$3.66X10^4$					
2.1	Flow Through	10ℓ	1mℓ				$3.22X10^4$	0	90.96%	
2.2	Flow Through	10ℓ	1mℓ				$2.90X10^4$	0	228.30%	
2.3	Flow Through	10ℓ	1mℓ				$3.24X10^4$	0	88.52%	135.92%
3.1	Eluate	100 mℓ	1mℓ				$4.72X10^3$	1	13.33%	
3.2	Eluate	100 mℓ	1mℓ				$3.50X10^3$	1	27.55%	
3.3	Eluate	100 mℓ	1mℓ				$3.98X10^3$	1	10.87%	17.25%
4.1	Final concentrate	10 mℓ	1mℓ				$2.42X10^3$	1	6.84%	
4.2	Final concentrate	10 mℓ	1mℓ				$2.42X10^3$	1	6.84%	
4.3	Final concentrate	10 mℓ	1mℓ				$1.96X10^3$	1	5.36%	6.35%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment										
Date:16/02/11		Water type: RAW			Virus:RV-SA11			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$4.15X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$4.07X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$3.28X10^4$					
2.1	Flow Through	10ℓ	1mℓ				$6.07X10^4$	0	65.27%	
2.2	Flow Through	10ℓ	1mℓ				$4.32X10^4$	0	101.88%	
2.3	Flow Through	10ℓ	1mℓ				$4.42X10^4$	0	104.24%	90.46%
3.1	Eluate	100 mℓ	1mℓ				$4.24X10^3$	1	6.82%	
3.2	Eluate	100 mℓ	1mℓ				$4.0X10^3$	1	9.43%	
3.3	Eluate	100 mℓ	1mℓ				$4.02X10^3$	1	9.48%	8.58%
4.1	Final concentrate	10 mℓ	1mℓ				$2.44X10^3$	1	3.92%	
4.2	Final concentrate	10 mℓ	1mℓ				$2.10X10^3$	1	4.95%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.90X10^3$	1	6.84%	5.24%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment										
Date:16/02/11		Water type: Tap			Virus:RV			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$3.54X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$3.52X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$3.50X10^4$					
2.1	Flow Through	10ℓ	1mℓ				$3.3X10^4$	0	93.22%	
2.2	Flow Through	10ℓ	1mℓ				$3.46X10^4$	0	98.29%	
2.3	Flow Through	10ℓ	1mℓ				$3.12X10^4$	0	89.14%	89.14%
3.1	Eluate	100 mℓ	1mℓ				$2.64X10^3$	1	7.45%	
3.2	Eluate	100 mℓ	1mℓ				$2.56X10^3$	1	10.11%	
3.3	Eluate	100 mℓ	1mℓ				$2.66X10^3$	1	7.60%	8.39%
4.1	Final concentrate	10 mℓ	1mℓ				$1.75X10^3$	1	4.94%	
4.2	Final concentrate	10 mℓ	1mℓ				$1.81X10^3$	1	5.14%	
4.3	Final concentrate	10 mℓ	1mℓ				$1.90X10^3$	1	5.43%	5.17%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment										
Date:16/02/11		Water type: RAW			Virus:RV-SA11			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$4.15X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$4.07X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$3.28X10^4$					
2.1	Flow Through	10ℓ	1mℓ				$6.07X10^4$	0	65.27%	
2.2	Flow Through	10ℓ	1mℓ				$4.32X10^4$	0	101.88%	
2.3	Flow Through	10ℓ	1mℓ				$4.42X10^4$	0	104.24%	90.46%
3.1	Eluate	100 mℓ	1mℓ				$4.24X10^3$	1	6.82%	
3.2	Eluate	100 mℓ	1mℓ				$4.0X10^3$	1	9.43%	
3.3	Eluate	100 mℓ	1mℓ				$4.02X10^3$	1	9.48%	8.58%
4.1	Final concentrate	10 mℓ	1mℓ				$2.44X10^3$	1	3.92%	
4.2	Final concentrate	10 mℓ	1mℓ				$2.10X10^3$	1	4.95%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.90X10^3$	1	6.84%	5.24%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment										
Date:16/02/11		Water type: RAW			Virus:RV-SA11			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$4.22X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$4.29X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$4.43X10^4$					
2.1	Flow Through	10ℓ	1mℓ				$5.98X10^5$	-1	1417.00%	
2.2	Flow Through	10ℓ	1mℓ				$6.46X10^5$	-1	1505.80%	
2.3	Flow Through	10ℓ	1mℓ				$6.36X10^5$	-1	1435.66%	1452.82%
3.1	Eluate	100 mℓ	1mℓ				$1.21X10^3$	1	2.87%	
3.2	Eluate	100 mℓ	1mℓ				$1.02X10^3$	1	2.38%	
3.3	Eluate	100 mℓ	1mℓ				$1.49X10^3$	1	3.36%	2.87%
4.1	Final concentrate	10 mℓ	1mℓ				$1.60X10^2$	1	0.38%	
4.2	Final concentrate	10 mℓ	1mℓ				$1.48X10^2$	1	0.34%	
4.3	Final concentrate	10 mℓ	1mℓ				$1.62X10^2$	1	0.37%	0.36%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment										
Date:16/02/11		Water type: RAW			Virus:RV-SA11			Replicate No:C		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$1.16X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$6.12X10^3$					
1.3	Seeded water	10ℓ	1mℓ		$8.04X10^3$					
2.1	Flow Through	10ℓ	1mℓ				$6.42X10^4$	-1	553.00%	
2.2	Flow Through	10ℓ	1mℓ				$5.62X10^4$	-1	918.30%	
2.3	Flow Through	10ℓ	1mℓ				$4.88X10^4$	-1	606.96%	692.75%
3.1	Eluate	100 mℓ	1mℓ				$3.42X10^2$	2	2.94%	
3.2	Eluate	100 mℓ	1mℓ				$4.34X10^2$	1	7.09%	
3.3	Eluate	100 mℓ	1mℓ				$4.36X10^2$	1	5.42%	5.15%
4.1	Final concentrate	10 mℓ	1mℓ				$2.06X10^1$	3	0.18%	
4.2	Final concentrate	10 mℓ	1mℓ				$1.91X10^1$	2	0.31%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.0X10^1$	2	0.25%	0.25%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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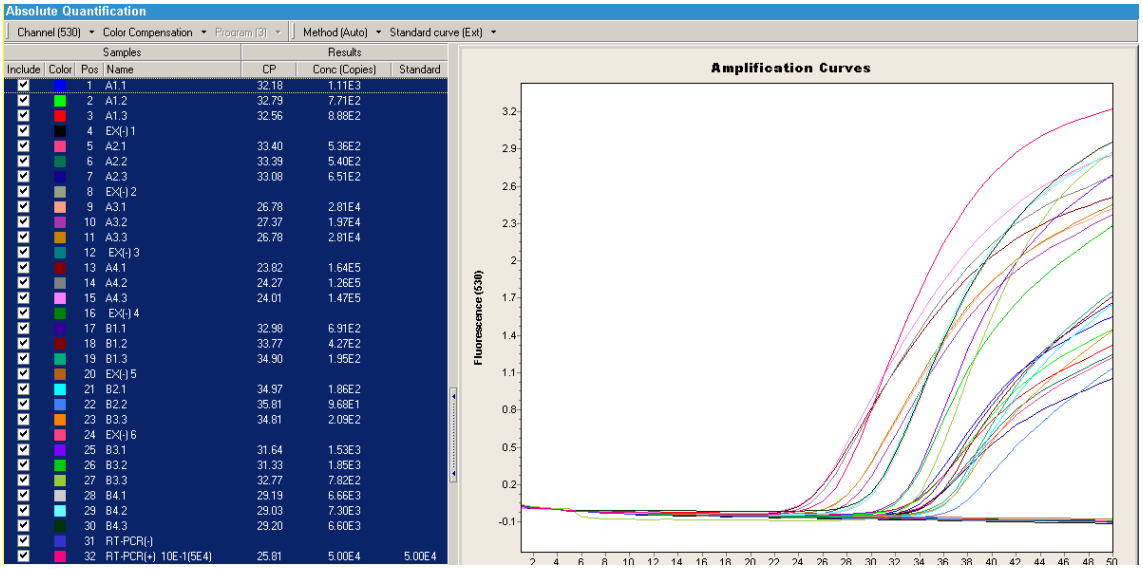
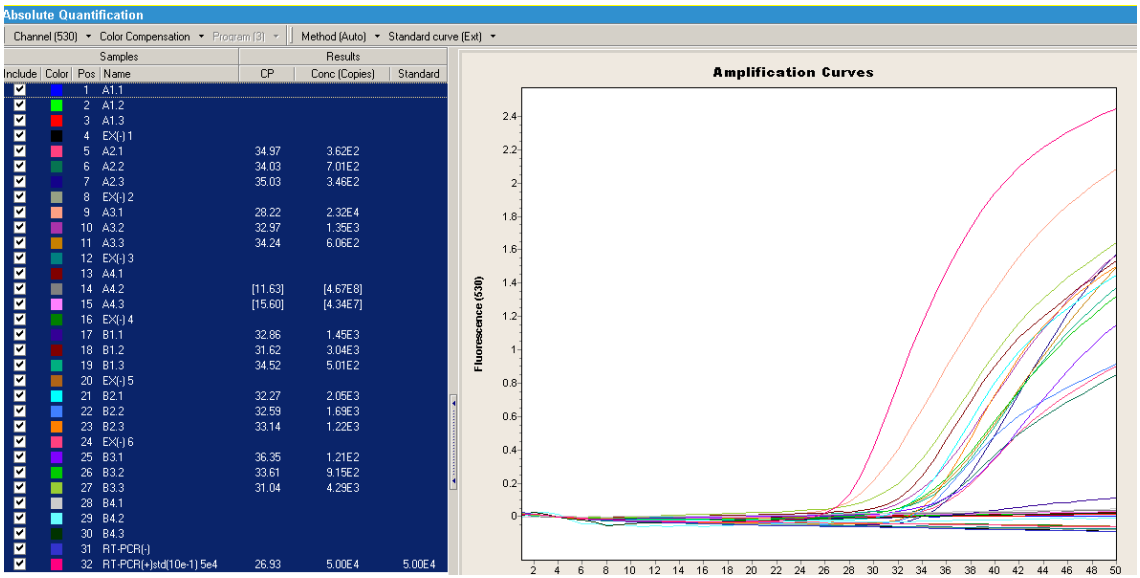
A3: Hepatitis A Virus

HAV in treated tap water



HAV in turbid surface water



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Hepatitis A virus: Calculation of virus titres and recovery efficiencies

MSc study Vurayai Ruhanya: Seeding experiment											
Date:04/10/10		Water type: Tap			Virus:HAV			Replicate No:A			
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery	
1.1	Seeded water	10ℓ	1mℓ		$2.22X10^8$						
1.2	Seeded water	10ℓ	1mℓ		$1.54X10^8$						
1.3	Seeded water	10ℓ	1mℓ		$1.78X10^8$						
2.1	Flow Through	10ℓ	1mℓ				$1.10X10^8$	0	49.55%		
2.2	Flow Through	10ℓ	1mℓ				$1.10X10^8$	0	71.43%		
2.3	Flow Through	10ℓ	1mℓ				$1.30X10^8$	0	73.03%	64.67%	
3.1	Eluate	100 mℓ	1mℓ				$5.62X10^7$	1	25.33%		
3.2	Eluate	100 mℓ	1mℓ				$3.94X10^7$	1	25.58%		
3.3	Eluate	100 mℓ	1mℓ				$5.62X10^7$	1	31.57%	27.49%	
4.1	Final concentrate	10 mℓ	1mℓ				$3.28X10^7$	1	14.77%		
4.2	Final concentrate	10 mℓ	1mℓ				$2.52X10^7$	1	16.36%		
4.3	Final concentrate	10 mℓ	1mℓ				$2.94X10^7$	1	16.52%	15.88%	
	Key words										
	Stock virus titre:										
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction										

MSc study Vurayai Ruhanya: Seeding experiment										
Date:04/10/10		Water type: Tap			Virus:HAV			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$1.38X10^8$					
1.2	Seeded water	10ℓ	1mℓ		$8.54X10^7$					
1.3	Seeded water	10ℓ	1mℓ		$3.90X10^7$					
2.1	Flow Through	10ℓ	1mℓ				$3.72X10^7$	1	26.95%	
2.2	Flow Through	10ℓ	1mℓ				$1.94X10^7$	0	22.72%	
2.3	Flow Through	10ℓ	1mℓ				$4.20X10^7$	0	107.70%	52.45%
3.1	Eluate	100 mℓ	1mℓ				$3.06X10^6$	1	2.22%	
3.2	Eluate	100 mℓ	1mℓ				$3.70X10^6$	1	0.43%	
3.3	Eluate	100 mℓ	1mℓ				$1.54X10^6$	1	3.95%	2.20%
4.1	Final concentrate	10 mℓ	1mℓ				$1.33X10^6$	1	0.96%	
4.2	Final concentrate	10 mℓ	1mℓ				$1.46X10^6$	1	1.71%	
4.3	Final concentrate	10 mℓ	1mℓ				$1.32X10^6$	1	3.40%	2.02%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10 | **Water type: Tap** | **Virus:HAV** | **Replicate No:C**

No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$2.88X10^{8}$					
1.2	Seeded water	10ℓ	1mℓ		$2.08X10^{8}$					
1.3	Seeded water	10ℓ	1mℓ		$2.90X10^{8}$					
2.1	Flow Through	10ℓ	1mℓ				$2.80X10^{8}$	0	97.22%	
2.2	Flow Through	10ℓ	1mℓ				$2.44X10^{8}$	0	117.40%	
2.3	Flow Through	10ℓ	1mℓ				$2.02X10^{8}$	0	69.97%	92.86%
3.1	Eluate	100 mℓ	1mℓ				$1.21X10^{8}$	0	42.01%	
3.2	Eluate	100 mℓ	1mℓ				$1.23X10^{8}$	0	59.13%	
3.3	Eluate	100 mℓ	1mℓ				$1.33X10^{8}$	0	45.86%	49.00%
4.1	Final concentrate	10 mℓ	1mℓ				$5.14X10^{7}$	1	17.84%	
4.2	Final concentrate	10 mℓ	1mℓ				$5.20X10^{7}$	1	25.00%	
4.3	Final concentrate	10 mℓ	1mℓ				$6.30X10^{7}$	1	21.70%	21.51%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10 | **Water type: Raw** | **Virus:HAV** | **Replicate No:B**

No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$2.90X10^{8}$					
1.2	Seeded water	10ℓ	1mℓ		$6.08X10^{8}$					
1.3	Seeded water	10ℓ	1mℓ		$1.00X10^{9}$					
2.1	Flow Through	10ℓ	1mℓ				$4.10X10^{8}$	0	141.40%	
2.2	Flow Through	10ℓ	1mℓ				$3.38X10^{8}$	0	55.59%	
2.3	Flow Through	10ℓ	1mℓ				$2.44X10^{8}$	1	24.40%	73.79%
3.1	Eluate	100 mℓ	1mℓ				$2.42X10^{5}$	3	0.08%	
3.2	Eluate	100 mℓ	1mℓ				$1.83X10^{6}$	2	0.30%	
3.3	Eluate	100 mℓ	1mℓ				$8.50X10^{6}$	3	0.85%	0.41%
4.1	Final concentrate	10 mℓ	1mℓ				$7.66X10^{7}$	1	26.41%	
4.2	Final concentrate	10 mℓ	1mℓ				$7.38X10^{7}$	1	12.14%	
4.3	Final concentrate	10 mℓ	1mℓ				$4.64X10^{7}$	2	4.64%	14.40%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10 | **Water type: Raw** | **Virus:HAV** | **Replicate No:A**

No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$4.78X10^{9}$					
1.2	Seeded water	10ℓ	1mℓ		$3.84X10^{9}$					
1.3	Seeded water	10ℓ	1mℓ		$4.36X10^{9}$					
2.1	Flow Through	10ℓ	1mℓ				$7.24X10^{7}$	0	1.51%	
2.2	Flow Through	10ℓ	1mℓ				$1.40X10^{8}$	0	3.60%	
2.3	Flow Through	10ℓ	1mℓ				$6.92X10^{7}$	0	1.60%	2.24%
3.1	Eluate	100 mℓ	1mℓ				$4.64X10^{7}$	1	0.97%	
3.2	Eluate	100 mℓ	1mℓ				$2.70X10^{6}$	1	0.07%	
3.3	Eluate	100 mℓ	1mℓ				$1.20X10^{6}$	1	0.03%	0.36%
4.1	Final concentrate	10 mℓ	1mℓ				$3.70X10^{7}$	1	0.77%	
4.2	Final concentrate	10 mℓ	1mℓ				$4.22X10^{7}$	1	1.09%	
4.3	Final concentrate	10 mℓ	1mℓ				$1.19X10^{7}$	1	0.27%	0.71%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10 | **Water type: Raw** | **Virus:HAV** | **Replicate No:C**

No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$1.82X10^{9}$					
1.2	Seeded water	10ℓ	1mℓ		$6.24X10^{8}$					
1.3	Seeded water	10ℓ	1mℓ		$2.54X10^{9}$					
2.1	Flow Through	10ℓ	1mℓ				$5.36X10^{8}$	1	29.45%	
2.2	Flow Through	10ℓ	1mℓ				$3.26X10^{8}$	0	52.24%	
2.3	Flow Through	10ℓ	1mℓ				$4.72X10^{8}$	1	18.58%	33.42%
3.1	Eluate	100 mℓ	1mℓ				$6.90X10^{7}$	2	3.79%	
3.2	Eluate	100 mℓ	1mℓ				$5.92X10^{7}$	1	9.49%	
3.3	Eluate	100 mℓ	1mℓ				$5.66X10^{7}$	2	2.23%	5.17%
4.1	Final concentrate	10 mℓ	1mℓ				$1.14X10^{1}$	8	0.00%	
4.2	Final concentrate	10 mℓ	1mℓ				$1.26X10^{3}$	5	0.00%	
4.3	Final concentrate	10 mℓ	1mℓ				$1.14X10^{3}$	6	0.00%	0.00%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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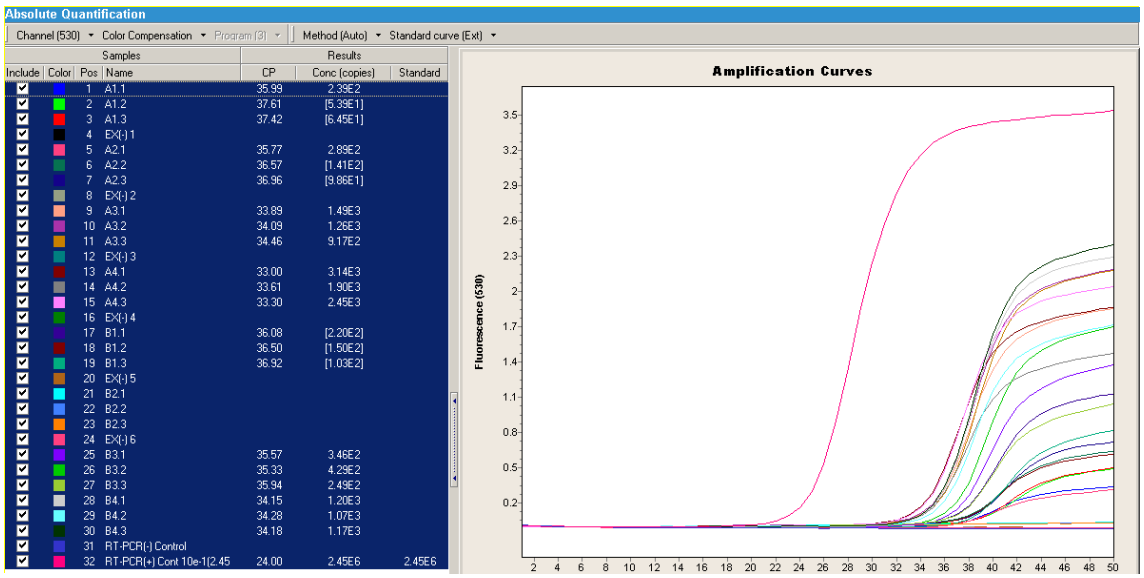
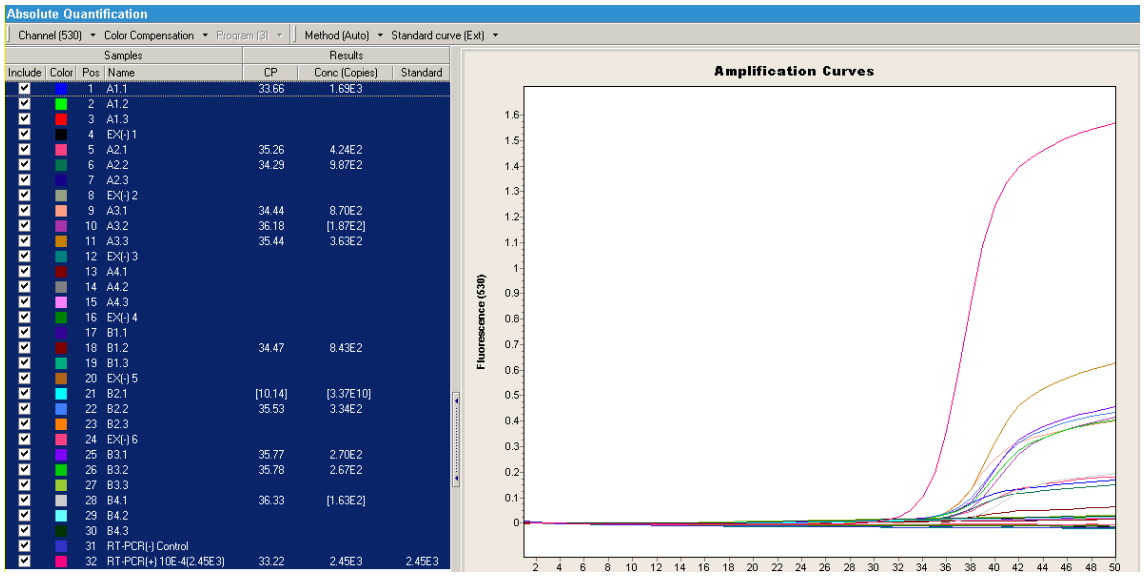
A4: Norovirus GII.4

NoV in treated tap water



NoV in turbid surface water



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Norovirus GII.4: Calculation of virus titres and recovery efficiencies

MSc study Vurayai Ruhanya: Seeding experiment										
Date:04/10/10		Water type: Tap			Virus:NoV GII			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$6.78X10^7$					
1.2	Seeded water	10ℓ	1mℓ		$1.08X10^7$					
1.3	Seeded water	10ℓ	1mℓ		$1.29X10^7$					
2.1	Flow Through	10ℓ	1mℓ				$5.78X10^7$	0	85.25%	
2.2	Flow Through	10ℓ	1mℓ				$2.82X10^7$	0	261.11%	
2.3	Flow Through	10ℓ	1mℓ				$1.97X10^7$	0	152.70%	166.35%
3.1	Eluate	100 mℓ	1mℓ				$2.98X10^6$	1	4.40%	
3.2	Eluate	100 mℓ	1mℓ				$2.52X10^6$	1	23.33%	
3.3	Eluate	100 mℓ	1mℓ				$1.84X10^6$	1	14.26%	22.58%
4.1	Final concentrate	10 mℓ	1mℓ				$6.28X10^5$	2	0.93%	
4.2	Final concentrate	10 mℓ	1mℓ				$3.80X10^5$	2	3.52%	
4.3	Final concentrate	10 mℓ	1mℓ				$4.90X10^5$	2	3.80%	2.75%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment										
Date:04/10/10		Water type: Tap			Virus:NoV GII			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$4.40X10^7$					
1.2	Seeded water	10ℓ	1mℓ		$3.00X10^7$					
1.3	Seeded water	10ℓ	1mℓ		$2.06X10^7$					
2.1	Flow Through	10ℓ	1mℓ							
2.2	Flow Through	10ℓ	1mℓ							
2.3	Flow Through	10ℓ	1mℓ							
3.1	Eluate	100 mℓ	1mℓ				$6.92X10^5$	2	1.57%	
3.2	Eluate	100 mℓ	1mℓ				$8.58X10^5$	2	2.86%	
3.3	Eluate	100 mℓ	1mℓ				$4.98X10^5$	2	2.42%	2.28%
4.1	Final concentrate	10 mℓ	1mℓ				$2.40X10^5$	2	0.55%	
4.2	Final concentrate	10 mℓ	1mℓ				$2.14X10^5$	2	0.71%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.30X10^5$	2	1.12%	0.79%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10		Water type: Tap			Virus:NoV GII			Replicate No:C		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$4.40X10^{7}$					
1.2	Seeded water	10ℓ	1mℓ		$3.98X10^{7}$					
1.3	Seeded water	10ℓ	1mℓ		$2.03X10^{7}$					
2.1	Flow Through	10ℓ	1mℓ				$3.80X10^{6}$	1	8.64%	
2.2	Flow Through	10ℓ	1mℓ				$2.52X10^{7}$	1	63.32%	
2.3	Flow Through	10ℓ	1mℓ				$1.84X10^{7}$	0	90.64%	54.20%
3.1	Eluate	100 mℓ	1mℓ				$7.38X10^{5}$	2	1.68%	
3.2	Eluate	100 mℓ	1mℓ				$1.00X10^{6}$	2	2.51%	
3.3	Eluate	100 mℓ	1mℓ				$3.06X10^{6}$	2	15.07%	6.42%
4.1	Final concentrate	10 mℓ	1mℓ				$2.52X10^{5}$	2	0.57%	
4.2	Final concentrate	10 mℓ	1mℓ				$2.64X10^{5}$	2	0.66%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.60X10^{5}$	2	1.28%	0.84%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10		Water type: Raw			Virus:Nov			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$3.38X10^{8}$					
1.2	Seeded water	10ℓ	1mℓ							
1.3	Seeded water	10ℓ	1mℓ							
2.1	Flow Through	10ℓ	1mℓ				$8.48X10^{7}$	1	25.09%	
2.2	Flow Through	10ℓ	1mℓ				$1.97X10^{8}$			
2.3	Flow Through	10ℓ	1mℓ							
3.1	Eluate	100 mℓ	1mℓ				$1.74X10^{6}$	1	0.51%	
3.2	Eluate	100 mℓ	1mℓ				$3.74X10^{5}$			
3.3	Eluate	100 mℓ	1mℓ				$7.26X10^{5}$			
4.1	Final concentrate	10 mℓ	1mℓ							
4.2	Final concentrate	10 mℓ	1mℓ							
4.3	Final concentrate	10 mℓ	1mℓ							
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10		Water type: Raw			Virus:NoV GII			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$1.20X10^8$					
1.2	Seeded water	10ℓ	1mℓ		$1.69X10^8$					
1.3	Seeded water	10ℓ	1mℓ							
2.1	Flow Through	10ℓ	1mℓ				$4.26X10^7$	1	35.50%	
2.2	Flow Through	10ℓ	1mℓ				$6.68X10^7$	1	39.53%	
2.3	Flow Through	10ℓ	1mℓ							
3.1	Eluate	100 mℓ	1mℓ				$5.40X10^5$	3	0.45%	
3.2	Eluate	100 mℓ	1mℓ				$5.34X10^5$	3	0.32%	
3.3	Eluate	100 mℓ	1mℓ							
4.1	Final concentrate	10 mℓ	1mℓ				$3.26X10^4$	4	0.03%	
4.2	Final concentrate	10 mℓ	1mℓ				$2.44X10^4$	4	0.01%	
4.3	Final concentrate	10 mℓ	1mℓ							
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10		Water type: Raw			Virus:NoV GII			Replicate No:C		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$1.26X10^8$					
1.2	Seeded water	10ℓ	1mℓ		$1.43X10^7$					
1.3	Seeded water	10ℓ	1mℓ							
2.1	Flow Through	10ℓ	1mℓ							
2.2	Flow Through	10ℓ	1mℓ							
2.3	Flow Through	10ℓ	1mℓ							
3.1	Eluate	100 mℓ	1mℓ				$1.38X10^6$	2	1.10%	
3.2	Eluate	100 mℓ	1mℓ				$1.18X10^6$	2	8.25%	
3.3	Eluate	100 mℓ	1mℓ				$1.94X10^6$	2		
4.1	Final concentrate	10 mℓ	1mℓ				$1.03X10^5$	3	0.08%	
4.2	Final concentrate	10 mℓ	1mℓ							
4.3	Final concentrate	10 mℓ	1mℓ							
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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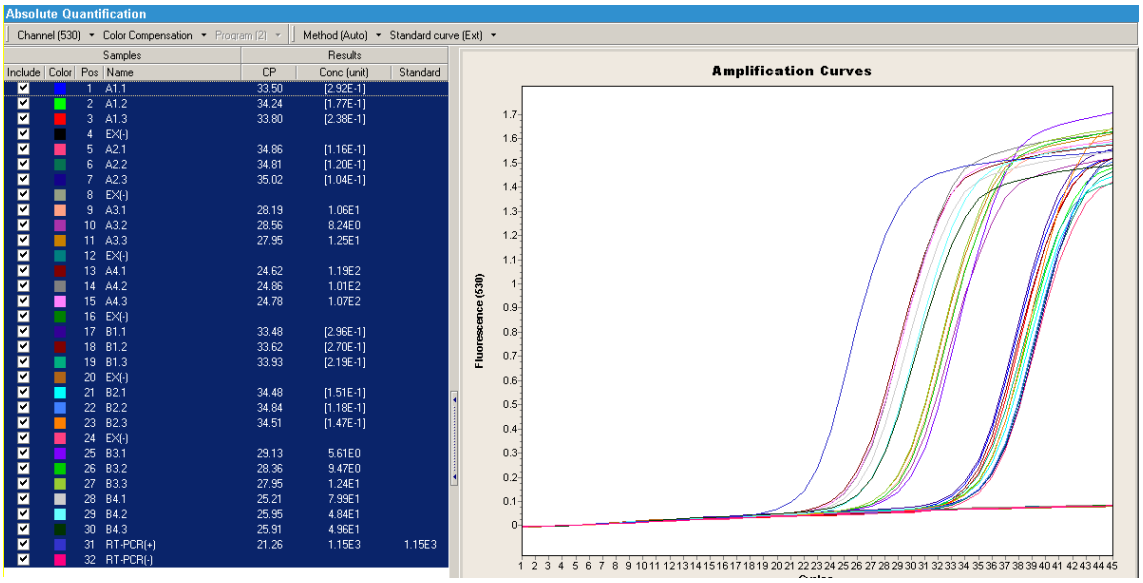
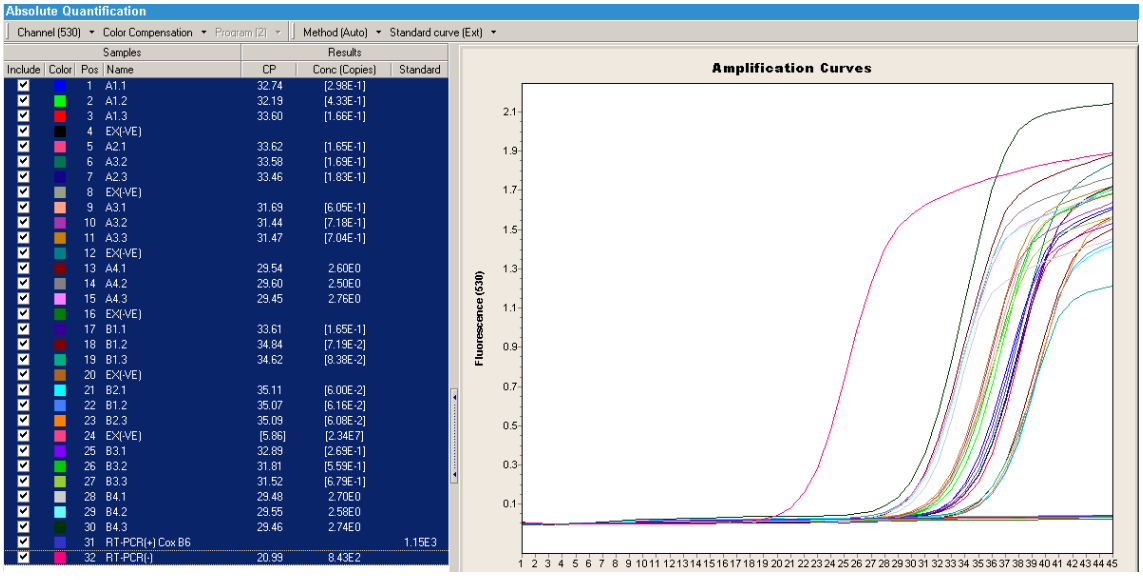
A5: Coxsackievirus-B6

CV-B6 in treated tap water



CV-B6 in turbid surface water



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Coxsackievirus B6: Calculation of virus titres and recovery efficiencies

MSc study Vurayai Ruhanya: Seeding experiment										
Date:04/10/10		Water type: Tap			Virus:Cox B6			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$2.34X10^5$					
1.2	Seeded water	10ℓ	1mℓ		$1.45X10^5$					
1.3	Seeded water	10ℓ	1mℓ		$1.90X10^5$					
2.1	Flow Through	10ℓ	1mℓ				$9.28X10^4$	1	27.78%	
2.2	Flow Through	10ℓ	1mℓ				$9.60X10^4$	1	66.21%	
2.3	Flow Through	10ℓ	1mℓ				$8.32X10^4$	1	43.79%	45.93%
3.1	Eluate	100 mℓ	1mℓ				$8.48X10^4$	1	36.24%	
3.2	Eluate	100 mℓ	1mℓ				$6.60X10^4$	1	45.52%	
3.3	Eluate	100 mℓ	1mℓ				$5.00X10^4$	1	26.32%	36.03%
4.1	Final concentrate	10 mℓ	1mℓ				$9.52X10^4$	1	40.68%	
4.2	Final concentrate	10 mℓ	1mℓ				$8.08X10^4$	1	55.72%	
4.3	Final concentrate	10 mℓ	1mℓ				$8.56X10^4$	1	45.05%	47.15%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment										
Date:04/10/10		Water type: Tap			Virus:Cox B6			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$2.36X10^5$					
1.2	Seeded water	10ℓ	1mℓ		$2.16X10^5$					
1.3	Seeded water	10ℓ	1mℓ		$1.75X10^5$					
2.1	Flow Through	10ℓ	1mℓ				$1.21X10^5$	0	51.27%	
2.2	Flow Through	10ℓ	1mℓ				$9.44X10^4$	1	43.70%	
2.3	Flow Through	10ℓ	1mℓ				$1.18X10^5$	0	67.43%	54.13%
3.1	Eluate	100 mℓ	1mℓ				$4.49X10^4$	1	1.90%	
3.2	Eluate	100 mℓ	1mℓ				$7.58X10^4$	1	35.09%	
3.3	Eluate	100 mℓ	1mℓ				$9.92X10^4$	1	56.69%	31.23%
4.1	Final concentrate	10 mℓ	1mℓ				$6.39X10^4$	1	27.08%	
4.2	Final concentrate	10 mℓ	1mℓ				$3.88X10^4$	1	17.96%	
4.3	Final concentrate	10 mℓ	1mℓ				$3.97X10^4$	1	22.69%	22.58%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10		Water type: Tap			Virus:Cox B6			Replicate No:C		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$1.18X10^5$					
1.2	Seeded water	10ℓ	1mℓ		$9.28X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$1.38X10^5$					
2.1	Flow Through	10ℓ	1mℓ				$2.88X10^4$	1	24.41%	
2.2	Flow Through	10ℓ	1mℓ				$5.79X10^4$	0	62.66%	
2.3	Flow Through	10ℓ	1mℓ				$6.12X10^4$	1	44.35%	43.81%
3.1	Eluate	100 mℓ	1mℓ				$8.96X10^4$	1	75.93%	
3.2	Eluate	100 mℓ	1mℓ				$1.07X10^5$	1	115.30%	
3.3	Eluate	100 mℓ	1mℓ				$8.88X10^4$	1	64.35%	85.19%
4.1	Final concentrate	10 mℓ	1mℓ				$7.08X10^4$	1	60.00%	
4.2	Final concentrate	10 mℓ	1mℓ				$8.08X10^4$	1	87.07%	
4.3	Final concentrate	10 mℓ	1mℓ				$8.96X10^4$	1	64.90%	
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment

Date:04/10/10		Water type: Raw			Virus:Cox B6			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$5.96X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$8.66X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$3.32X10^4$					
2.1	Flow Through	10ℓ	1mℓ				$3.30X10^4$	0	55.37%	
2.2	Flow Through	10ℓ	1mℓ				$3.38X10^4$	0	39.03%	
2.3	Flow Through	10ℓ	1mℓ				$3.66X10^4$	0	110.24%	68.21%
3.1	Eluate	100 mℓ	1mℓ				$1.21X10^3$	1	2.03%	
3.2	Eluate	100 mℓ	1mℓ				$1.44X10^3$	1	1.66%	
3.3	Eluate	100 mℓ	1mℓ				$1.40X10^3$	1	4.22%	2.64%
4.1	Final concentrate	10 mℓ	1mℓ				$5.20X10^2$	2	0.87%	
4.2	Final concentrate	10 mℓ	1mℓ				$5.0X10^2$	2	0.58%	
4.3	Final concentrate	10 mℓ	1mℓ				$5.52X10^2$	2	1.66%	1.04%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment										
Date:04/10/10		Water type: Raw			Virus:Cox B6			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$3.30X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$1.44X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$1.68X10^4$					
2.1	Flow Through	10ℓ	1mℓ				$1.20X10^4$	0	36.36%	
2.2	Flow Through	10ℓ	1mℓ				$1.23X10^4$	0	85.42%	
2.3	Flow Through	10ℓ	1mℓ				$1.22X10^4$	0	72.62%	64.80%
3.1	Eluate	100 mℓ	1mℓ				$5.38X10^2$	2	1.63%	
3.2	Eluate	100 mℓ	1mℓ				$1.36X10^3$	1	9.44%	
3.3	Eluate	100 mℓ	1mℓ				$1.12X10^3$	1	6.67%	5.91%
4.1	Final concentrate	10 mℓ	1mℓ				$5.35X10^2$	2	1.62%	
4.2	Final concentrate	10 mℓ	1mℓ				$5.16X10^2$	2	3.58%	
4.3	Final concentrate	10 mℓ	1mℓ				$5.48X10^2$	2	3.26%	2.82%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment										
Date:04/10/10		Water type: Raw			Virus:Cox B6			Replicate No:C		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$4.99X10^4$					
1.2	Seeded water	10ℓ	1mℓ		$7.56X10^4$					
1.3	Seeded water	10ℓ	1mℓ		$5.32X10^4$					
2.1	Flow Through	10ℓ	1mℓ				$2.48X10^4$	0	49.70%	
2.2	Flow Through	10ℓ	1mℓ				$2.38X10^4$	0	31.48%	
2.3	Flow Through	10ℓ	1mℓ				$4.20X10^4$	0	78.95%	53.22%
3.1	Eluate	100 mℓ	1mℓ				$1.90X10^3$	1	3.81%	
3.2	Eluate	100 mℓ	1mℓ				$1.48X10^3$	1	1.96%	
3.3	Eluate	100 mℓ	1mℓ				$1.60X10^3$	1	3.01%	2.93%
4.1	Final concentrate	10 mℓ	1mℓ				$3.90X10^2$	2	0.78%	
4.2	Final concentrate	10 mℓ	1mℓ				$4.50X10^2$	2	0.60%	
4.3	Final concentrate	10 mℓ	1mℓ				$5.80X10^2$	2	1.15%	0.84%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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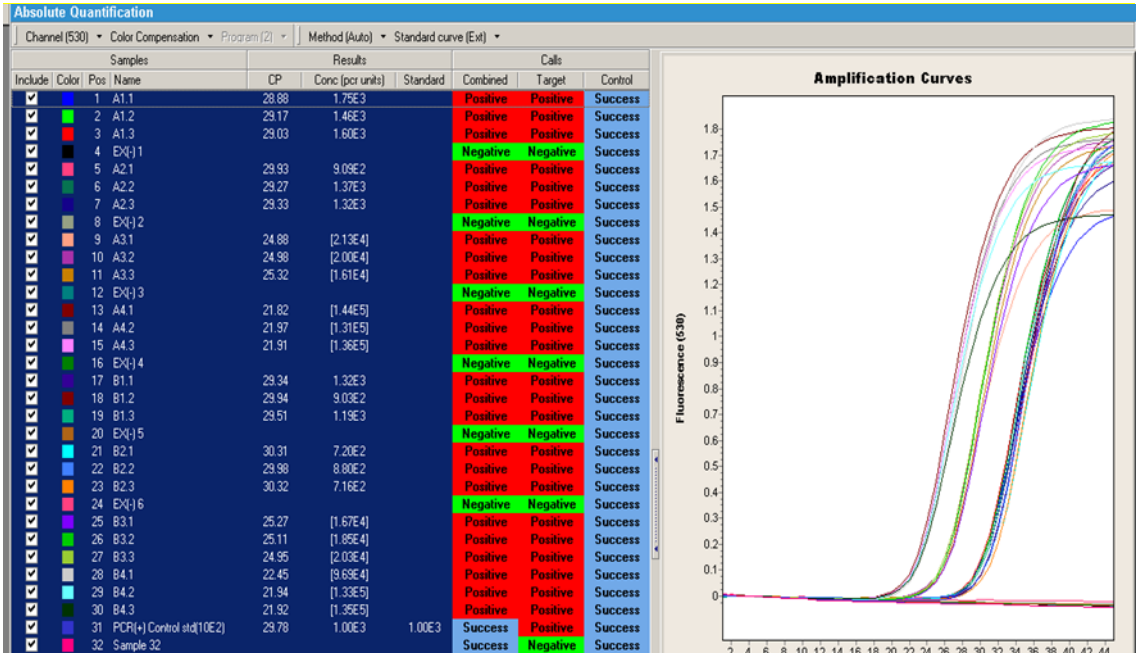
A6: Human adenovirus type 2

HAdV in treated tap water

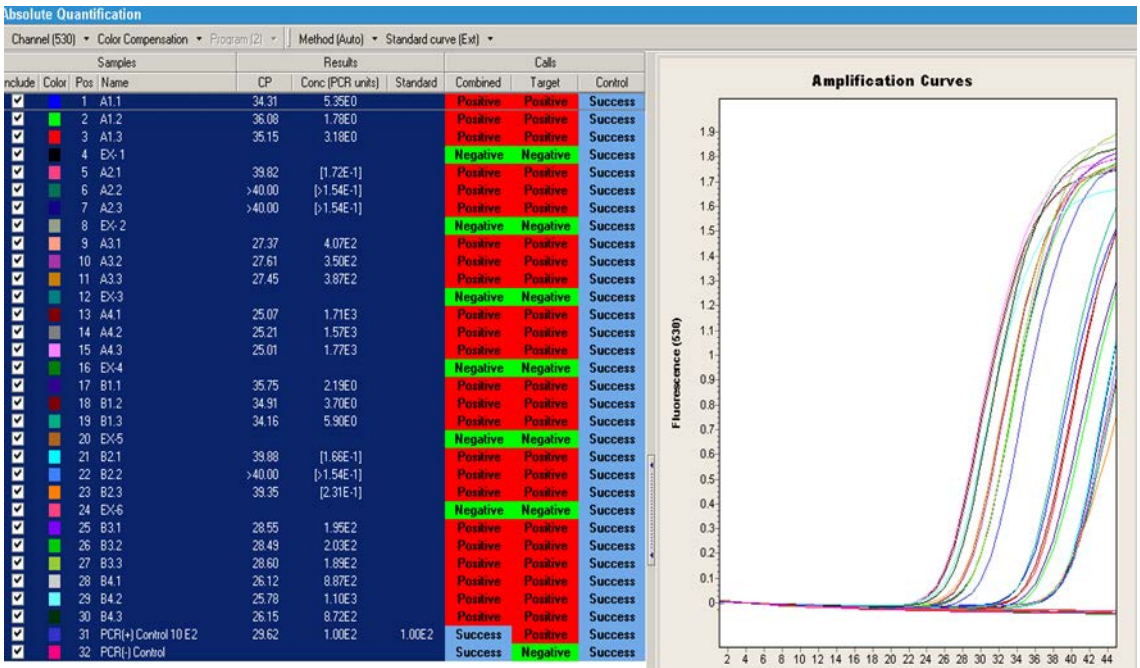


Absolute Quantification

Channel (530) ▾ Color Compensation ▾ Program (2) ▾ Method (Auto) ▾ Standard curve (Ext) ▾

Include	Color	Pos	Name	Samples CP	Results Conc (pcr units)	Results Standard	Calls Combined	Calls Target	Calls Control
☑		1	A1.1	28.88	1.75E3		Positive	Positive	Success
☑		2	A1.2	29.17	1.46E3		Positive	Positive	Success
☑		3	A1.3	29.03	1.60E3		Positive	Positive	Success
☑		4	EX(-)1				Negative	Negative	Success
☑		5	A2.1	29.93	9.09E2		Positive	Positive	Success
☑		6	A2.2	29.27	1.37E3		Positive	Positive	Success
☑		7	A2.3	29.33	1.32E3		Positive	Positive	Success
☑		8	EX(-)2				Negative	Negative	Success
☑		9	A3.1	24.88	[2.13E4]		Positive	Positive	Success
☑		10	A3.2	24.98	[2.00E4]		Positive	Positive	Success
☑		11	A3.3	25.32	[1.61E4]		Positive	Positive	Success
☑		12	EX(-)3				Negative	Negative	Success
☑		13	A4.1	21.82	[1.44E5]		Positive	Positive	Success
☑		14	A4.2	21.97	[1.31E5]		Positive	Positive	Success
☑		15	A4.3	21.91	[1.36E5]		Positive	Positive	Success
☑		16	EX(-)4				Negative	Negative	Success
☑		17	B1.1	29.34	1.32E3		Positive	Positive	Success
☑		18	B1.2	29.94	9.03E2		Positive	Positive	Success
☑		19	B1.3	29.51	1.19E3		Positive	Positive	Success
☑		20	EX(-)5				Negative	Negative	Success
☑		21	B2.1	30.31	7.20E2		Positive	Positive	Success
☑		22	B2.2	29.98	8.80E2		Positive	Positive	Success
☑		23	B2.3	30.32	7.16E2		Positive	Positive	Success
☑		24	EX(-)6				Negative	Negative	Success
☑		25	B3.1	25.27	[1.67E4]		Positive	Positive	Success
☑		26	B3.2	25.11	[1.85E4]		Positive	Positive	Success
☑		27	B3.3	24.95	[2.03E4]		Positive	Positive	Success
☑		28	B4.1	22.45	[9.69E4]		Positive	Positive	Success
☑		29	B4.2	21.94	[1.33E5]		Positive	Positive	Success
☑		30	B4.3	21.92	[1.35E5]		Positive	Positive	Success
☑		31	PCR(+) Control std(10E2)	29.78	1.00E3	1.00E3	Success	Positive	Success
☑		32	Sample 32				Success	Negative	Success

HAdV in turbid surface water



Absolute Quantification

Channel (530) ▾ Color Compensation ▾ Program (2) ▾ Method (Auto) ▾ Standard curve (Ext) ▾

Include	Color	Pos	Name	Samples CP	Results Conc (PCR units)	Results Standard	Calls Combined	Calls Target	Calls Control
☑		1	A1.1	34.31	5.35E0		Positive	Positive	Success
☑		2	A1.2	36.08	1.78E0		Positive	Positive	Success
☑		3	A1.3	35.15	3.18E0		Positive	Positive	Success
☑		4	EX-1				Negative	Negative	Success
☑		5	A2.1	39.82	[1.72E-1]		Positive	Positive	Success
☑		6	A2.2	>40.00	[>1.54E-1]		Positive	Positive	Success
☑		7	A2.3	>40.00	[>1.54E-1]		Positive	Positive	Success
☑		8	EX-2				Negative	Negative	Success
☑		9	A3.1	27.37	4.07E2		Positive	Positive	Success
☑		10	A3.2	27.61	3.50E2		Positive	Positive	Success
☑		11	A3.3	27.45	3.87E2		Positive	Positive	Success
☑		12	EX-3				Negative	Negative	Success
☑		13	A4.1	25.07	1.71E3		Positive	Positive	Success
☑		14	A4.2	25.21	1.57E3		Positive	Positive	Success
☑		15	A4.3	25.01	1.77E3		Positive	Positive	Success
☑		16	EX-4				Negative	Negative	Success
☑		17	B1.1	35.75	2.19E0		Positive	Positive	Success
☑		18	B1.2	34.91	3.70E0		Positive	Positive	Success
☑		19	B1.3	34.16	5.90E0		Positive	Positive	Success
☑		20	EX-5				Negative	Negative	Success
☑		21	B2.1	39.88	[1.66E-1]		Positive	Positive	Success
☑		22	B2.2	>40.00	[>1.54E-1]		Positive	Positive	Success
☑		23	B2.3	39.35	[2.31E-1]		Positive	Positive	Success
☑		24	EX-6				Negative	Negative	Success
☑		25	B3.1	28.55	1.95E2		Positive	Positive	Success
☑		26	B3.2	28.49	2.03E2		Positive	Positive	Success
☑		27	B3.3	28.60	1.89E2		Positive	Positive	Success
☑		28	B4.1	26.12	8.87E2		Positive	Positive	Success
☑		29	B4.2	25.78	1.10E3		Positive	Positive	Success
☑		30	B4.3	26.15	8.72E2		Positive	Positive	Success
☑		31	PCR(+) Control 10 E2	29.62	1.00E2	1.00E2	Success	Positive	Success
☑		32	PCR(-) Control				Success	Negative	Success

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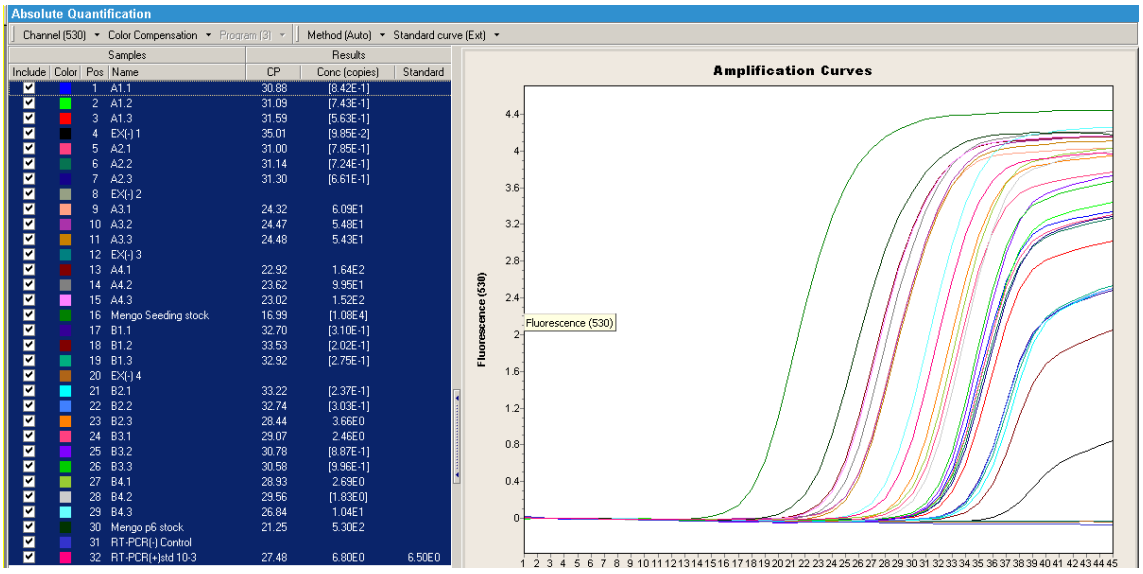
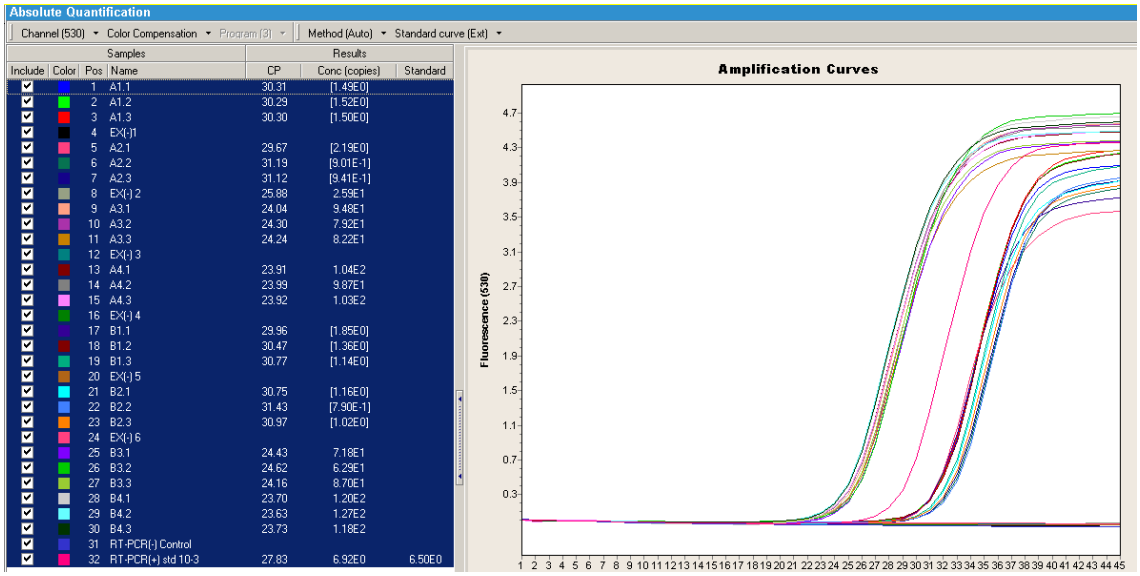
A7: Mengovirus: Glass wool modification

Glass wool modification: 15 g without grid using mengovirus



Glass wool modification: 20 g without grid using mengovirus



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MSc study Vurayai Ruhanya: Seeding experiment :Glasswool modification,15g no grid										
Date:25/05/11	Water type:tap		Virus: Mengovirus				Replicate no:B			
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ	$6.20x10^4$						
1.2	Seeded water	10ℓ	1mℓ	$4.04x10^4$						
1.3	Seeded water	10ℓ	1mℓ	$5.50x10^4$						
2.1	Flow Through	10ℓ	1mℓ				$4.74x10^4$	0	76.45%	
2.2	Flow Through	10ℓ	1mℓ				$6.06x10^4$	0	163.40%	
2.3	Flow Through	10ℓ	1mℓ				7.32x104	0	133.10%	124.32%
3.1	Eluate	100 mℓ	1mℓ				$4.92x10^3$	0	7.94%	
3.2	Eluate	100 mℓ	1mℓ				$1.77x10^3$	1	4.38%	
3.3	Eluate	100 mℓ	1mℓ				$1.99x10^3$	1	3.62%	5.31%
4.1	Final concentrate	10 mℓ	1mℓ				$5.38x10^2$	2	0.86%	
4.2	Final concentrate	10 mℓ	1mℓ				$3.66x10^3$	1	9.05%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.23x10^3$	1	4.05%	4.65%

MSc study Vurayai Ruhanya: Seeding experiment :Glasswool modification,15g no grid										
Date:25/05/11	Water type:tap		Virus: Mengovirus				Replicate no:C			
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ	$2.34x10^5$						
1.2	Seeded water	10ℓ	1mℓ	$2.06x10^5$						
1.3	Seeded water	10ℓ	1mℓ	$2.12x10^5$						
2.1	Flow Through	10ℓ	1mℓ				$1.86x10^5$	0	79.48%	
2.2	Flow Through	10ℓ	1mℓ				$1.74x10^5$	0	84.46%	
2.3	Flow Through	10ℓ	1mℓ				$1.96x10^5$	0	92.45%	85.45%
3.1	Eluate	100 mℓ	1mℓ				$2.62X10^5$	0	111.96%	
3.2	Eluate	100 mℓ	1mℓ				$2.84x10^5$	0	137.86%	
3.3	Eluate	100 mℓ	1mℓ				$2.87x10^5$	0	135.40%	128.41%
4.1	Final concentrate	10 mℓ	1mℓ				$5.44x10^4$	1	23.27%	
4.2	Final concentrate	10 mℓ	1mℓ				$6.06x10^4$	1	29.42%	
4.3	Final concentrate	10 mℓ	1mℓ				$5.74x10^4$	1	27.07%	26.58%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment :Glasswool modification,20g no grid										
Date:25/05/11	Water type:tap			Virus: Mengovirus			Replicate no:A			
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ	$2.98X10^5$						
1.2	Seeded water	10ℓ	1mℓ	$3.04x10^5$						
1.3	Seeded water	10ℓ	1mℓ	$3.00x10^5$						
2.1	Flow Through	10ℓ	1mℓ				$4.38x10^5$	0	146.97%	
2.2	Flow Through	10ℓ	1mℓ				$1.80x10^5$	0	59.21%	
2.3	Flow Through	10ℓ	1mℓ				$1.88x10^5$	0	62.66%	89.61%
3.1	Eluate	100 mℓ	1mℓ				$1.90X10^5$	0	63.76%	
3.2	Eluate	100 mℓ	1mℓ				$1.58x10^5$	0	51.94%	
3.3	Eluate	100 mℓ	1mℓ				$1.64x10^5$	0	54.67%	56.79%
4.1	Final concentrate	10 mℓ	1mℓ				$2.08x10^4$	1	6.97%	
4.2	Final concentrate	10 mℓ	1mℓ				$1.97x10^4$	1	6.48%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.06x10^4$	1	6.87%	6.77%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment :Glasswool modification,20g no grid										
Date:25/05/11	Water type:tap			Virus: Mengovirus			Replicate no:B			
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ	$3.70X10^5$						
1.2	Seeded water	10ℓ	1mℓ	$2.72x10^5$						
1.3	Seeded water	10ℓ	1mℓ	$2.28x10^5$						
2.1	Flow Through	10ℓ	1mℓ				$2.32x10^5$	0	62.70%	
2.2	Flow Through	10ℓ	1mℓ				$1.58x10^5$	0	58.08%	
2.3	Flow Through	10ℓ	1mℓ				$2.05x10^5$	0	89.91%	70.23%
3.1	Eluate	100 mℓ	1mℓ				$1.74X10^5$	0	47.02%	
3.2	Eluate	100 mℓ	1mℓ				$1.26x10^5$	0	46.32%	
3.3	Eluate	100 mℓ	1mℓ				$1.44x10^5$	0	63.16%	52.17%
4.1	Final concentrate	10 mℓ	1mℓ				$2.40x10^4$	1	6.49%	
4.2	Final concentrate	10 mℓ	1mℓ				$2.54x10^4$	1	9.34%	
4.3	Final concentrate	10 mℓ	1mℓ				$2.36x10^4$	1	10.35%	8.72%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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MSc study Vurayai Ruhanya: Seeding experiment :Glasswool modification,20g no grid											
Date:25/05/11		Water type:tap			Virus: Mengovirus			Replicate no:C			
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery	
1.1	Seeded water	10ℓ	1mℓ	$3.08X10^5$							
1.2	Seeded water	10ℓ	1mℓ	$2.88x10^5$							
1.3	Seeded water	10ℓ	1mℓ	$2.96x10^5$							
2.1	Flow Through	10ℓ	1mℓ				$2.64x10^5$	0	85.71%		
2.2	Flow Through	10ℓ	1mℓ				$1.77x10^5$	0	61.46%		
2.3	Flow Through	10ℓ	1mℓ				$4.38x10^5$	0	147.97%	98.38%	
3.1	Eluate	100 mℓ	1mℓ				$2.00X10^5$	0	64.94%		
3.2	Eluate	100 mℓ	1mℓ				$2.04x10^5$	0	70.83%		
3.3	Eluate	100 mℓ	1mℓ				$1.92x10^5$	0	64.86%	66.86%	
4.1	Final concentrate	10 mℓ	1mℓ				$3.48x10^3$	2	1.13%		
4.2	Final concentrate	10 mℓ	1mℓ				$3.44x10^3$	2	1.19%		
4.3	Final concentrate	10 mℓ	1mℓ				$3.88x10^3$	2	1.31%	1.21%	
	Key words										
	Stock virus titre:										
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction										

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A8: Mengovirus: pH Modification

MSc study Vurayai Ruhanya: Seeding experiment -Acidification (Ph3.5) 1:10 dilution										
Date:19/05/11		Water type: Tap			Virus:Mengovirus			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$5.10X10^{10}$					
1.2	Seeded water	10ℓ	1mℓ							
1.3	Seeded water	10ℓ	1mℓ							
2.1	Flow Through	10ℓ	1mℓ				$2.54X10^{8}$	2	0.49%	
2.2	Flow Through	10ℓ	1mℓ							
2.3	Flow Through	10ℓ	1mℓ							0.49%
3.1	Eluate	100 mℓ	1mℓ				$1.00X10^{6}$	4	0.19%	
3.2	Eluate	100 mℓ	1mℓ							
3.3	Eluate	100 mℓ	1mℓ							0.19%
4.1	Final concentrate	10 mℓ	1mℓ				$1.21X10^{7}$		0.02%	
4.2	Final concentrate	10 mℓ	1mℓ							
4.3	Final concentrate	10 mℓ	1mℓ							0.02%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

Date:19/05/11		Water type: Tap			Virus:Mengovirus			Replicate No:B		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	% recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$1.90X10^{5}$					
1.2	Seeded water	10ℓ	1mℓ		$1.10X10^{8}$					
1.3	Seeded water	10ℓ	1mℓ		$2.2X10^{9}$					
2.1	Flow Through	10ℓ	1mℓ							
2.2	Flow Through	10ℓ	1mℓ							
2.3	Flow Through	10ℓ	1mℓ							
3.1	Eluate	100 mℓ	1mℓ				$5.84X10^{8}$		3074.00%	
3.2	Eluate	100 mℓ	1mℓ				$1.16X10^{8}$	0	105.45%	
3.3	Eluate	100 mℓ	1mℓ				$6.28X10^{7}$		2.85%	1060.77%
4.1	Final concentrate	10 mℓ	1mℓ				$3.06X10^{6}$	2	1610.50%	
4.2	Final concentrate	10 mℓ	1mℓ				$1.11X10^{6}$	2	1.01%	
4.3	Final concentrate	10 mℓ	1mℓ				$5.96X10^{6}$	2	0.27%	537.00%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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A9: Hepatitis A virus: pH Modification

MSc study Vurayai Ruhanya: Seeding experiment -pH 3.5										
Date:30/08/2011		Water type: Raw			Virus:HAV			Replicate No:C		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	%recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$7.96X10^{7}$					
1.2	Seeded water	10ℓ	1mℓ		$2.98X10^{7}$					
1.3	Seeded water	10ℓ	1mℓ							
2.1	Flow Through	10ℓ	1mℓ				$4.18X10^{11}$	3	525.10%	
2.2	Flow Through	10ℓ	1mℓ				$4.84X10^{6}$	1	16.24%	
2.3	Flow Through	10ℓ	1mℓ							180.00%
3.1	Eluate	100 mℓ	1mℓ				$3.14X10^{8}$	1	1602.00%	
3.2	Eluate	100 mℓ	1mℓ				$3.3X10^{5}$	2	1.10%	
3.3	Eluate	100 mℓ	1mℓ				$1.15X10^{9}$	2		534.36%
4.1	Final concentrate	10 mℓ	1mℓ				$3.66X10^{4}$	3	0.05%	
4.2	Final concentrate	10 mℓ	1mℓ				$1.14X10^{4}$		0.04%	
4.3	Final concentrate	10 mℓ	1mℓ							0.45%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

MSc study Vurayai Ruhanya: Seeding experiment -pH 6.0										
Date:30/08/2011		Water type: Raw			Virus:HAV			Replicate No:A		
No	Sample type	Volume	Volume of sample removed	Viral titre input	Viral titre input [log_{10}]	Viral titre recovered	Viral titre recovered [log_{10}]	Log difference	%recovery	% mean recovery
1.1	Seeded water	10ℓ	1mℓ		$7.4X10^{8}$					
1.2	Seeded water	10ℓ	1mℓ							
1.3	Seeded water	10ℓ	1mℓ							
2.1	Flow Through	10ℓ	1mℓ				$5.62X10^{8}$	0	75.95%	
2.2	Flow Through	10ℓ	1mℓ							
2.3	Flow Through	10ℓ	1mℓ							75.95%
3.1	Eluate	100 mℓ	1mℓ				$1.22X10^{6}$	2	0.16%	
3.2	Eluate	100 mℓ	1mℓ							
3.3	Eluate	100 mℓ	1mℓ							0.16%
4.1	Final concentrate	10 mℓ	1mℓ				$1.01X10^{8}$	0	13.65%	
4.2	Final concentrate	10 mℓ	1mℓ							
4.3	Final concentrate	10 mℓ	1mℓ							13.65%
	Key words									
	Stock virus titre:									
	Nucleic acid eluted in 100µℓ /5 µℓ per reaction									

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APPENDIX B

STATISTICAL ANALYSIS

Statistical Analysis

```
. list  virus_type  vrus source sample replicate dose_pct flowthroughpct recovery_eluate
final_rec_pegnacl_pct , compress table

     +-------------------------------------------------------------------------------
----------+
     |        virus_type      vrus      source   sam~e   rep~e   dos~t   flowt~t
reco~e   fin~t |
     |-------------------------------------------------------------------------------
----------|
  1. |    AdV- Raw water       Adv   Raw water       1       1     100     51.94
12.17    8.23 |
  2. |    AdV- Raw water       Adv   Raw water       1       2     100     93.84
13.70    8.97 |
  3. |    AdV- Raw water       Adv   Raw water       1       3     100     75.00
10.06    8.50 |
  4. |    AdV- Raw water       Adv   Raw water       2       1     100     54.54
12.65    7.42 |
  5. |    AdV- Raw water       Adv   Raw water       2       2     100     97.56
20.51   14.74 |
     |-------------------------------------------------------------------------------
----------|
  6. |    AdV- Raw water       Adv   Raw water       2       3     100     67.84
17.88   10.75 |
  7. |    AdV- Raw water       Adv   Raw water       3       1     100     37.60
6.69    4.02 |
  8. |    AdV- Raw water       Adv   Raw water       3       2     100     63.04
9.86    6.20 |
  9. |    AdV- Raw water       Adv   Raw water       3       3     100     66.66
9.09    5.48 |
 10. |    AdV- Tap Water       Adv   Tap water       1       1     100      3.21
76.07   31.96 |
     |-------------------------------------------------------------------------------
----------|
 11. |    AdV- Tap Water       Adv   Tap water       1       2     100      8.44
191.70   86.02 |
 12. |    AdV- Tap Water       Adv   Tap water       1       3     100      8.44
121.69   55.66 |
 13. |    AdV- Tap Water       Adv   Tap water       2       1     100      7.58
89.04   40.41 |
 14. |    AdV- Tap Water       Adv   Tap water       2       2     100      4.16
54.86   29.73 |
 15. |    AdV- Tap Water       Adv   Tap water       2       3     100      3.93
32.03   14.75 |
     |-------------------------------------------------------------------------------
----------|
 16. |    AdV- Tap Water       Adv   Tap water       3       1     100      0.42
27.38   10.38 |
 17. |    AdV- Tap Water       Adv   Tap water       3       2     100      0.23
33.86   16.48 |
 18. |    AdV- Tap Water       Adv   Tap water       3       3     100      0.00
39.53   20.35 |
 19. |     HAV-Raw Water       HAV   Raw water       1       1     100      1.51
0.97    0.77 |
 20. |     HAV-Raw Water       HAV   Raw water       1       2     100      3.60
0.07    1.09 |
     |-------------------------------------------------------------------------------
----------|
 21. |     HAV-Raw Water       HAV   Raw water       1       3     100      1.60
0.03    0.27 |
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 22. |     HAV-Raw Water       HAV   Raw water       2       1     100    141.40
0.08   26.41 |
 23. |     HAV-Raw Water       HAV   Raw water       2       2     100     55.59
0.30   12.14 |
 24. |     HAV-Raw Water       HAV   Raw water       2       3     100     24.40
0.85    4.64 |
 25. |     HAV-Raw Water       HAV   Raw water       3       1     100     29.45
3.79    0.00 |
     |-------------------------------------------------------------------------------
----------|
 26. |     HAV-Raw Water       HAV   Raw water       3       2     100     52.24
9.49    0.00 |
 27. |     HAV-Raw Water       HAV   Raw water       3       3     100     18.58
2.23    0.00 |
 28. |     HAV-Tap Water       HAV   Tap water       1       1     100     49.55
25.33   14.77 |
 29. |     HAV-Tap Water       HAV   Tap water       1       2     100     71.43
25.58   16.36 |
 30. |     HAV-Tap Water       HAV   Tap water       1       3     100     73.03
31.57   16.52 |
     |-------------------------------------------------------------------------------
----------|
 31. |     HAV-Tap Water       HAV   Tap water       2       1     100     26.95
2.22    0.96 |
 32. |     HAV-Tap Water       HAV   Tap water       2       2     100     22.72
0.43    1.71 |
 33. |     HAV-Tap Water       HAV   Tap water       2       3     100    107.70
3.95    3.40 |
 34. |     HAV-Tap Water       HAV   Tap water       3       1     100     97.22
42.01   17.84 |
 35. |     HAV-Tap Water       HAV   Tap water       3       2     100    117.40
59.13   25.00 |
     |-------------------------------------------------------------------------------
----------|
 36. |     HAV-Tap Water       HAV   Tap water       3       3     100     69.97
45.86   21.70 |
 37. | RV-SA11-Raw Water   RV-SA11   Raw water       1       1     100     65.27
6.82    3.92 |
 38. | RV-SA11-Raw Water   RV-SA11   Raw water       1       2     100    101.88
9.43    4.95 |
 39. | RV-SA11-Raw Water   RV-SA11   Raw water       1       3     100    104.24
9.48    6.84 |
 40. | RV-SA11-Raw Water   RV-SA11   Raw water       2       1     100   1417.00
2.87    0.38 |
     |-------------------------------------------------------------------------------
----------|
 41. | RV-SA11-Raw Water   RV-SA11   Raw water       2       2     100   1505.80
2.38    0.34 |
 42. | RV-SA11-Raw Water   RV-SA11   Raw water       2       3     100   1435.60
3.36    0.37 |
 43. | RV-SA11-Raw Water   RV-SA11   Raw water       3       1     100    553.00
2.94    0.18 |
 44. | RV-SA11-Raw Water   RV-SA11   Raw water       3       2     100    918.30
7.09    0.31 |
 45. | RV-SA11-Raw Water   RV-SA11   Raw water       3       3     100    606.96
5.42    0.25 |
     |-------------------------------------------------------------------------------
----------|
 46. | RV-SA11-Tap Water   RV-SA11   Tap water       1       1     100     93.22
7.45    4.94 |
 47. | RV-SA11-Tap Water   RV-SA11   Tap water       1       2     100     98.29
10.11    5.14 |
 48. | RV-SA11-Tap Water   RV-SA11   Tap water       1       3     100     89.14
7.60    5.43 |
 49. | RV-SA11-Tap Water   RV-SA11   Tap water       2       1     100     90.96
13.33    6.84 |
 50. | RV-SA11-Tap Water   RV-SA11   Tap water       2       2     100    228.30
27.55    6.84 |
     |-------------------------------------------------------------------------------
----------|
 51. | RV-SA11-Tap Water   RV-SA11   Tap water       2       3     100     88.52
10.87    5.36 |
 52. | RV-SA11-Tap Water   RV-SA11   Tap water       3       1     100     65.27
6.82    3.92 |
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 53. | RV-SA11-Tap Water   RV-SA11   Tap water       3       2     100    101.88
9.43    4.95 |
 54. | RV-SA11-Tap Water   RV-SA11   Tap water       3       3     100    104.24
9.48    6.84 |
 55. |   Mengo-Raw Water     Mengo   Raw water       1       1     100     70.77
2.97    0.14 |
     |-------------------------------------------------------------------------------
----------|
 56. |   Mengo-Raw Water     Mengo   Raw water       1       2     100     68.55
2.83    0.12 |
 57. |   Mengo-Raw Water     Mengo   Raw water       1       3     100    102.17
2.30    0.20 |
 58. |   Mengo-Raw Water     Mengo   Raw water       2       1     100    117.48
3.98    0.11 |
 59. |   Mengo-Raw Water     Mengo   Raw water       2       2     100    116.06
4.41    0.17 |
 60. |   Mengo-Raw Water     Mengo   Raw water       2       3     100     68.65
3.71    0.18 |
     |-------------------------------------------------------------------------------
----------|
 61. |   Mengo-Raw Water     Mengo   Raw water       3       1     100    104.15
3.56    0.18 |
 62. |   Mengo-Raw Water     Mengo   Raw water       3       2     100     93.89
4.11    0.17 |
 63. |   Mengo-Raw Water     Mengo   Raw water       3       3     100     94.06
4.70    0.15 |
 64. |   Mengo-Tap Water     Mengo   Tap water       1       1     100     64.36
88.83   24.47 |
 65. |   Mengo-Tap Water     Mengo   Tap water       1       2     100     44.32
34.95   14.42 |
     |-------------------------------------------------------------------------------
----------|
 66. |   Mengo-Tap Water     Mengo   Tap water       1       3     100     56.91
57.61   17.17 |
 67. |   Mengo-Tap Water     Mengo   Tap water       2       1     100     40.00
55.68    7.89 |
 68. |   Mengo-Tap Water     Mengo   Tap water       2       2     100     71.03
47.50   17.50 |
 69. |   Mengo-Tap Water     Mengo   Tap water       2       3     100     34.88
26.78   10.00 |
 70. |   Mengo-Tap Water     Mengo   Tap water       3       1     100     33.79
60.99   16.77 |
     |-------------------------------------------------------------------------------
----------|
 71. |   Mengo-Tap Water     Mengo   Tap water       3       2     100     55.43
64.43   19.14 |
 72. |   Mengo-Tap Water     Mengo   Tap water       3       3     100     52.10
56.30   11.42 |
 73. |  NoVGII Raw water    NoVG11   Raw water       1       1     100     25.09
0.51       . |
 74. |  NoVGII Raw water    NoVG11   Raw water       1       2     100         .
.       . |
 75. |  NoVGII Raw water    NoVG11   Raw water       1       3     100         .
.       . |
     |-------------------------------------------------------------------------------
----------|
 76. |  NoVGII Raw water    NoVG11   Raw water       2       1     100     35.50
0.45    0.03 |
 77. |  NoVGII Raw water    NoVG11   Raw water       2       2     100     39.53
0.32    0.01 |
 78. |  NoVGII Raw water    NoVG11   Raw water       2       3     100         .
.       . |
 79. |  NoVGII Raw water    NoVG11   Raw water       3       1     100         .
1.09       . |
 80. |  NoVGII Raw water    NoVG11   Raw water       3       2     100         .
8.25    0.08 |
     |-------------------------------------------------------------------------------
----------|
 81. |  NoVGII Raw water    NoVG11   Raw water       3       3     100         .
.       . |
 82. |  NoVGII Tap water    NoVG11   Tap water       1       1     100     85.25
4.39    0.93 |
 83. |  NoVGII Tap water    NoVG11   Tap water       1       2     100    261.11
23.33    3.52 |
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 84. |  NoVGII Tap water    NoVG11   Tap water       1       3     100    152.70
14.26    3.80 |
 85. |  NoVGII Tap water    NoVG11   Tap water       2       1     100         .
1.57    0.54 |
     |-------------------------------------------------------------------------
----------|
 86. |  NoVGII Tap water    NoVG11   Tap water       2       2     100         .
2.86    0.71 |
 87. |  NoVGII Tap water    NoVG11   Tap water       2       3     100         .
2.42    1.12 |
 88. |  NoVGII Tap water    NoVG11   Tap water       3       1     100      8.64
1.68    0.57 |
 89. |  NoVGII Tap water    NoVG11   Tap water       3       2     100     63.32
2.51    0.66 |
 90. |  NoVGII Tap water    NoVG11   Tap water       3       3     100     90.64
15.07    1.28 |
     |-------------------------------------------------------------------------
----------|
 91. |  Cox B6 Raw water    Cox_B6   Raw water       1       1     100     55.37
2.03    0.87 |
 92. |  Cox B6 Raw water    Cox_B6   Raw water       1       2     100     39.03
1.66    0.58 |
 93. |  Cox B6 Raw water    Cox_B6   Raw water       1       3     100    110.24
4.22    1.67 |
 94. |  Cox B6 Raw water    Cox_B6   Raw water       2       1     100     36.36
1.63    1.62 |
 95. |  Cox B6 Raw water    Cox_B6   Raw water       2       2     100     85.42
9.44    3.58 |
     |-------------------------------------------------------------------------
----------|
 96. |  Cox B6 Raw water    Cox_B6   Raw water       2       3     100     72.62
6.67    3.26 |
 97. |  Cox B6 Raw water    Cox_B6   Raw water       3       1     100     49.70
3.81    0.78 |
 98. |  Cox B6 Raw water    Cox_B6   Raw water       3       2     100     31.48
1.96    0.59 |
 99. |  Cox B6 Raw water    Cox_B6   Raw water       3       3     100     78.95
3.01    1.15 |
100. |  Cox B6 Tap water    Cox_B6   Tap water       1       1     100     27.78
36.24   40.68 |
     |-------------------------------------------------------------------------
----------|
101. |  Cox B6 Tap water    Cox_B6   Tap water       1       2     100     66.21
45.52   55.72 |
102. |  Cox B6 Tap water    Cox_B6   Tap water       1       3     100     43.80
26.32   45.05 |
103. |  Cox B6 Tap water    Cox_B6   Tap water       2       1     100     51.27
1.90   27.08 |
104. |  Cox B6 Tap water    Cox_B6   Tap water       2       2     100     43.70
35.09   17.96 |
105. |  Cox B6 Tap water    Cox_B6   Tap water       2       3     100     67.43
56.68   22.68 |
     |-------------------------------------------------------------------------
----------|
106. |  Cox B6 Tap water    Cox_B6   Tap water       3       1     100     24.41
75.93   60.00 |
107. |  Cox B6 Tap water    Cox_B6   Tap water       3       2     100     62.66
115.30   87.07 |
108. |  Cox B6 Tap water    Cox_B6   Tap water       3       3     100     44.34
64.35   64.90 |
     +-------------------------------------------------------------------------
----------+
```

tab vrus

Vrus	Freq.	Percent	Cum.
Adv	18	16.67	16.67
HAV	18	16.67	33.33
RV-SA11	18	16.67	50.00
Mengo	18	16.67	66.67
NoVG11	18	16.67	83.33
Cox_B6	18	16.67	100.00

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```
      Total |        108      100.00
```

Summary statistics for the parameters

```
. tabstat flowthroughpct recovery_eluate final_rec_pegnacl_pct,
statistics(n mean sd min max p50) by( virus_type)

Summary statistics: N, mean, sd, min, max, p50
  by categories of: virus_type (Virus)

      virus_type |  flowth~t  recove~e  final_~t
-----------------+------------------------------
  AdV- Raw water |          9          9          9
                 |   67.55778   12.51222   8.256667
                 |   19.29513   4.372956   3.150559
                 |       37.6       6.69       4.02
                 |      97.56      20.51      14.74
                 |      66.66      12.17       8.23
-----------------+------------------------------
  AdV- Tap Water |          9          9          9
                 |   4.045555   74.01778   33.97111
                 |   3.462456   54.15113    24.1362
                 |          0      27.38      10.38
                 |       8.44      191.7      86.02
                 |       3.93      54.86      29.73
-----------------+------------------------------
Cox B6 Raw water |          9          9          9
                 |      62.13   3.825555   1.566667
                 |   26.42744    2.66102   1.125811
                 |      31.48       1.63        .58
                 |     110.24       9.44       3.58
                 |      55.37       3.01       1.15
-----------------+------------------------------
Cox B6 Tap water |          9          9          9
                 |   47.95556   50.81444   46.79333
                 |   15.61082   32.57127    22.4889
                 |      24.41        1.9      17.96
                 |      67.43      115.3      87.07
                 |      44.34      45.52      45.05
-----------------+------------------------------
   HAV-Raw Water |          9          9          9
                 |   36.48556   1.978889   5.035556
                 |   44.24102   3.080558    8.94455
                 |       1.51        .03          0
                 |      141.4       9.49      26.41
                 |       24.4        .85        .77
-----------------+------------------------------
   HAV-Tap Water |          9          9          9
                 |   70.66333   26.23111      13.14
                 |   33.39751    20.8717   8.904618
                 |      22.72        .43        .96
                 |      117.4      59.13         25
                 |      71.43      25.58      16.36
-----------------+------------------------------
 Mengo-Raw Water |          9          9          9
                 |   92.86444   3.618889   .1577778
                 |   19.44895   .7874713   .0299073
                 |      68.55        2.3        .11
```


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	117.48	4.7	.2
	94.06	3.71	.17
Mengo-Tap Water	9	9	9
	50.31333	54.78556	15.42
	13.00419	17.79201	5.099382
	33.79	26.78	7.89
	71.03	88.83	24.47
	52.1	56.3	16.77
NoVGII Raw water	3	5	3
	33.37333	2.124	.04
	7.451203	3.437249	.0360555
	25.09	.32	.01
	39.53	8.25	.08
	35.5	.51	.03
NoVGII Tap water	6	9	9
	110.2767	7.565556	1.458889
	87.28952	7.941176	1.27422
	8.64	1.57	.54
	261.11	23.33	3.8
	87.945	2.86	.93
RV-SA11-Raw Wate	9	9	9
	745.3389	5.532222	1.948889
	599.9468	2.813747	2.575337
	65.27	2.38	.18
	1505.8	9.48	6.84
	606.96	5.42	.37
RV-SA11-Tap Wate	9	9	9
	106.6467	11.40444	5.584444
	46.99909	6.374543	1.036172
	65.27	6.82	3.92
	228.3	27.55	6.84
	93.22	9.48	5.36
Total	99	104	102
	124.4221	21.93462	11.76588
	264.8595	30.93788	17.58078
	0	.03	0
	1505.8	191.7	87.07
	65.27	9.26	4.95

Plotting the data showed that the parameters are not normally distributed and as such. I have analysed the parameters using generalized linear model using negative binomial as the family.

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```
. tabstat flowthroughpct recovery_eluate final_rec_pegnacl_pct,
statistics(n mean sd min max p50) by( source )

Summary statistics: N, mean, sd, min, max, p50
  by categories of: source

   source |  flowth~t  recove~e  final_~t
----------+---------------------------
Raw water |        48         50        48
          |  190.4065     5.1566  3.183542
          |  367.1383   4.658572  5.041204
          |      1.51        .03         0
          |    1505.8      20.51     26.41
          |      68.6       3.75       .68
----------+---------------------------
Tap water |        51         54        54
          |  62.31922   37.46981  19.39463
          |  51.75269   36.44994  20.98734
          |         0        .43       .54
          |    261.11      191.7     87.07
          |     56.91      29.56     14.76
----------+---------------------------
    Total |        99        104       102
          |  124.4221   21.93462  11.76588
          |  264.8595   30.93788  17.58078
          |         0        .03         0
          |    1505.8      191.7     87.07
          |     65.27       9.26      4.95
-------------------------------------------

. tabstat flowthroughpct recovery_eluate final_rec_pegnacl_pct,
statistics(n mean sd min max p50) by( sample )

Summary statistics: N, mean, sd, min, max, p50
  by categories of: sample (Sample)

  sample |  flowth~t  recove~e  final_~t
---------+---------------------------
       1 |        34         34        33
         |    66.685       26.7  14.83879
         |  50.30169   40.30203  20.40442
         |      1.51        .03       .12
         |    261.11      191.7     86.02
         |     65.74     10.085      5.43
---------+---------------------------
       2 |        32         35        35
         |    195.64   15.89286     8.618
         |  413.4455   21.59013  10.28178
         |      3.93        .08       .01
         |    1505.8      89.04     40.41
         |    67.635       3.98      4.64
---------+---------------------------
       3 |        33         35        34
         |  114.8491   23.34714  12.02382
         |  194.7381    28.1896  20.33029
         |         0       1.09         0
         |     918.3      115.3     87.07
```


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```
         |     63.04      9.09      3.97
---------+--------------------------------
   Total |        99       104       102
         |  124.4221  21.93462  11.76588
         |  264.8595  30.93788  17.58078
         |         0       .03         0
         |    1505.8     191.7     87.07
         |     65.27      9.26      4.95
----------------------------------------

. glm flowthroughpct  vrus##source##sample , family(nbinomial)
link(nbinomial)  robust
Iteration 0:   log pseudolikelihood = -516.34493
Iteration 1:   log pseudolikelihood = -502.71267
Iteration 2:   log pseudolikelihood = -498.17442
Iteration 3:   log pseudolikelihood = -495.47225
Iteration 4:   log pseudolikelihood = -494.57184
Iteration 5:   log pseudolikelihood = -494.36621
Iteration 6:   log pseudolikelihood = -494.36071
Iteration 7:   log pseudolikelihood =  -494.3607

Generalized linear models                         No. of obs
=        99
Optimization     : ML                             Residual df
=        66
                                                  Scale parameter
=         1
Deviance         =  12.70040768                   (1/df) Deviance
=  .1924304
Pearson          =  11.21156822                   (1/df) Pearson
=  .1698722

Variance function: V(u) = u+(1)u^2                [Neg. Binomial]
Link function    : g(u) = ln(u/(u+(1/1)))         [Neg. Binomial]

                                                  AIC
=  10.65375
Log pseudolikelihood = -494.3607022               BIC
= -290.5775

------------------------------------------------------------------------
-----------------
                    |               Robust
    flowthroughpct |      Coef.   Std. Err.      z    P>|z|
[95% Conf. Interval]
-------------------+----------------------------------------------------
-----------------
              vrus |
                2  |  -.3560606    .077351    -4.60   0.000    -
.5076659   -.2044554
                3  |   .0025031   .0022014     1.14   0.256    -
.0018115    .0068178
                4  |   .0011504   .0022638     0.51   0.611    -
.0032866    .0055873
                5  |   -.025586   .0018112   -14.13   0.000     -
.029136   -.0220361
```


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```
               6  |  -.0010568   .0041586    -0.25   0.799    -
.0092075    .0070939
                  |
          2.source |  -.1256808   .0278164    -4.52   0.000    -
.1801999   -.0711616
                  |
       vrus#source |
             2 2  |   .4798933   .0821936     5.84   0.000
.3187968    .6409899
             3 2  |   .1260416   .0278456     4.53   0.000
.0714652    .1806179
             4 2  |   .1200722   .0278925     4.30   0.000
.065404    .1747404
             5 2  |   .1587702   .0278573     5.70   0.000
.1041708    .2133696
             6 2  |   .1186956   .0283861     4.18   0.000
.0630599    .1743312
                  |
            sample |
                2  |  -.0000512   .0026362    -0.02   0.985    -
.0052182    .0051158
                3  |  -.0042763   .0029831    -1.43   0.152     -
.010123    .0015704
                  |
       vrus#sample |
             2 2  |   .3561488   .0775493     4.59   0.000
.2041549    .5081426
             2 3  |   .3443532     .07771     4.43   0.000
.1920445     .496662
             3 2  |   .0103567   .0029181     3.55   0.000
.0046372    .0160761
             3 3  |   .0138274   .0032407     4.27   0.000
.0074758     .020179
             4 2  |    .002519   .0032319     0.78   0.436    -
.0038154    .0088534
             4 3  |   .0064046   .0032905     1.95   0.052    -
.0000447    .0128538
             5 2  |    .012827   .0028164     4.55   0.000
.007307    .0183471
             5 3  |   1.574032   .3790871     4.15   0.000
.8310345    2.317029
             6 2  |  -.0007096   .0053814    -0.13   0.895    -
.0112569    .0098378
             6 3  |   .0002683   .0061802     0.04   0.965    -
.0118446    .0123812
                  |
     source#sample |
             2 2  |  -.0359412   .0408065    -0.88   0.378    -
.1159204    .0440379
             2 3  |  -1.582044   .3790381    -4.17   0.000    -
2.324945   -.8391429
                  |
vrus#source#sample |
            2 2 2  |  -.3236955   .0879757    -3.68   0.000    -
.4961247   -.1512662
            2 2 3  |   1.246826   .3869154     3.22   0.001
.4884853    2.005166
```


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```
            3 2 2  |   .0289385   .0408772     0.71   0.479    -
.0511792    .1090563
            3 2 3  |   1.572132   .3790424     4.15   0.000
.8292227    2.315042
            4 2 2  |   .0310763   .0410584     0.76   0.449    -
.0493968    .1115493
            4 2 3  |   1.576864   .3790517     4.16   0.000
.8339368    2.319792
            5 1 3  |          0  (empty)
            5 2 2  |          0  (empty)
            5 2 3  |          0  (omitted)
            6 2 2  |   .0399363   .0413386     0.97   0.334     -
.041086    .1209585
            6 2 3  |   1.585018   .3791287     4.18   0.000
.8419395    2.328097
                   |
             _cons |  -.0134967   .0018112    -7.45   0.000    -
.0170467   -.0099467
------------------------------------------------------------------------------

. glm, eform

Generalized linear models                         No. of obs
=        99
Optimization     : ML                             Residual df
=        66
                                                  Scale parameter
=         1
Deviance         =  12.70040768                   (1/df) Deviance
=  .1924304
Pearson          =  11.21156822                   (1/df) Pearson
=  .1698722

Variance function: V(u) = u+(1)u^2                [Neg. Binomial]
Link function    : g(u) = ln(u/(u+(1/1)))         [Neg. Binomial]

                                                  AIC
=  10.65375
Log pseudolikelihood = -494.3607022               BIC
= -290.5775

------------------------------------------------------------------------------
                 |               Robust
    flowthroughpct |     exp(b)   Std. Err.      z    P>|z|
[95% Conf. Interval]
-----------------+------------------------------------------------------------
             vrus |
               2  |   .7004301    .054179    -4.60   0.000
.6018989    .8150911
               3  |   1.002506   .0022069     1.14   0.256
.9981901    1.006841
               4  |   1.001151   .0022664     0.51   0.611
.9967188    1.005603
```


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```
                5  |   .9747385   .0017655   -14.13   0.000
.9712843     .978205
                6  |   .9989438   .0041542    -0.25   0.799
.9908348    1.007119
                   |
          2.source |   .8818963   .0245312    -4.52   0.000
.8351033    .9313113
                   |
       vrus#source |
              2 2  |   1.615902   .1328168     5.84   0.000
1.375472    1.898359
              3 2  |   1.134329   .0315861     4.53   0.000
1.074081    1.197957
              4 2  |   1.127578   .0314509     4.30   0.000
1.06759     1.190937
              5 2  |   1.172069   .0326507     5.70   0.000
1.10979     1.237842
              6 2  |   1.126027   .0319635     4.18   0.000
1.065091     1.19045
                   |
            sample |
                2  |   .9999488   .0026361    -0.02   0.985
.9947954    1.005129
                3  |   .9957328   .0029703    -1.43   0.152
.9899281    1.001572
                   |
       vrus#sample |
              2 2  |    1.42782   .1107265     4.59   0.000
1.226488    1.662201
              2 3  |   1.411077   .1096548     4.43   0.000
1.211724    1.643227
              3 2  |    1.01041   .0029485     3.55   0.000
1.004648    1.016206
              3 3  |   1.013923   .0032858     4.27   0.000
1.007504    1.020384
              4 2  |   1.002522     .00324     0.78   0.436
.9961919    1.008893
              4 3  |   1.006425   .0033116     1.95   0.052
.9999553    1.012937
              5 2  |    1.01291   .0028528     4.55   0.000
1.007334    1.018516
              5 3  |   4.826066   1.829499     4.15   0.000
2.295693    10.14548
              6 2  |   .9992907   .0053776    -0.13   0.895
.9888062    1.009886
              6 3  |   1.000268   .0061818     0.04   0.965
.9882253    1.012458
                   |
     source#sample |
              2 2  |    .964697   .0393659    -0.88   0.378
.8905461    1.045022
              2 3  |   .2055545    .077913    -4.17   0.000
.0977888    .4320807
                   |
vrus#source#sample |
            2 2 2  |   .7234705   .0636478    -3.68   0.000
.6088857    .8596188
```


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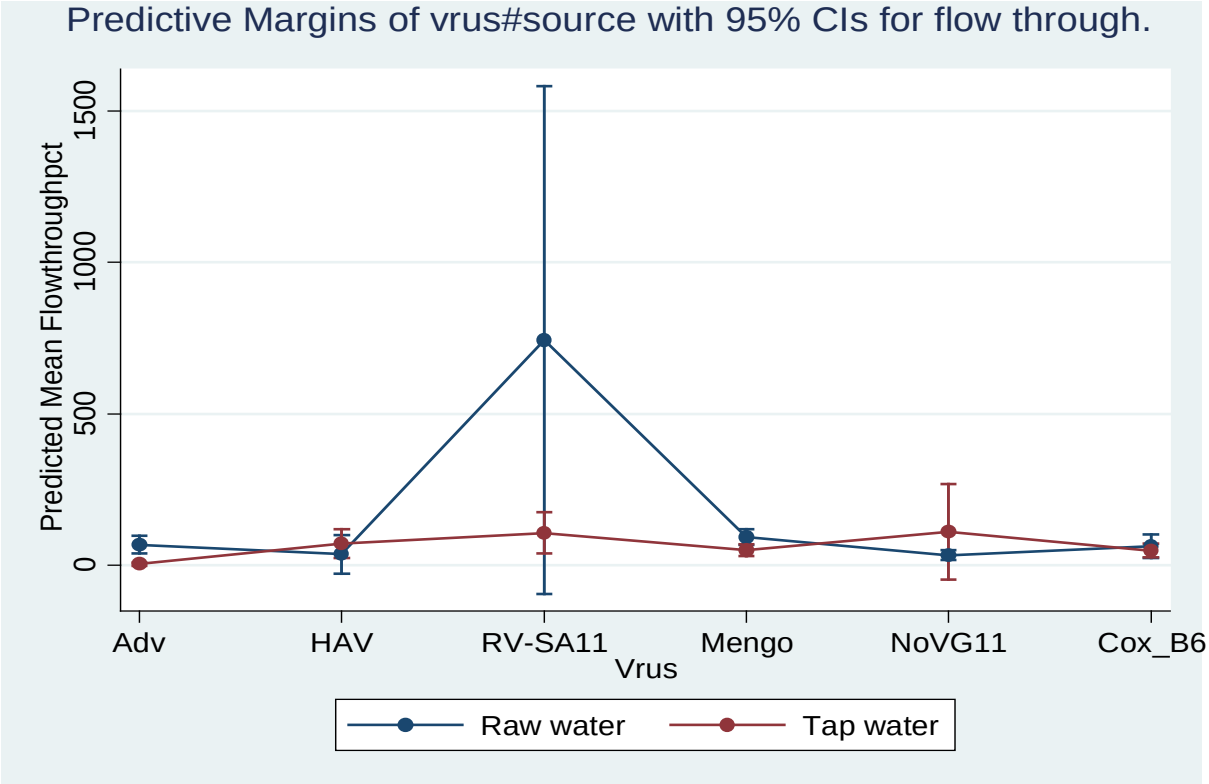
```
             2 2 3  |   3.479281   1.346187     3.22   0.001
1.629846    7.427325
             3 2 2  |   1.029361   .0420774     0.71   0.479
.9501084    1.115225
             3 2 3  |   4.816908   1.825812     4.15   0.000
2.291537    10.12534
             4 2 2  |   1.031564   .0423544     0.76   0.449
.9518034    1.118009
             4 2 3  |   4.839756   1.834518     4.16   0.000
2.302365    10.17356
             5 1 3  |          1  (empty)
             5 2 2  |          1  (empty)
             5 2 3  |          1  (omitted)
             6 2 2  |   1.040744    .043023     0.97   0.334
.9597466    1.128578
             6 2 3  |   4.879379   1.849912     4.18   0.000
2.320864     10.2584
                    |
              _cons |    .986594    .001787    -7.45   0.000
.9830978    .9901026
------------------------------------------------------------------------------

. margins vrus#source#sample
```



There is clear evidence of significant differences between viruses, Source of water with respect to flow through. There is also clear evidence that the flow through is also influenced by the type of virus, source of water. The same pattern was observed for all the three measures

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```
  Variables that uniquely identify margins: vrus source sample

. graph save Graph "H:\Dudu_2012\UP_2012\Ruhaya_data\Adjusted
Predictions of vrus#source#sample with 95% CIs for flow through
analysis.gph
> "
(file H:\Dudu_2012\UP_2012\Ruhaya_data\Adjusted Predictions of
vrus#source#sample with 95% CIs for flow through analysis.gph
saved)
```

```
. glm recovery_eluate sample vrus##source  , family(nbinomial)
link(nbinomial)  robust
note: recovery_eluate has noninteger values

Iteration 0:   log pseudolikelihood = -374.75951
Iteration 1:   log pseudolikelihood = -363.58991
Iteration 2:   log pseudolikelihood = -362.99251
Iteration 3:   log pseudolikelihood = -362.97437
Iteration 4:   log pseudolikelihood = -362.97436

Generalized linear models                         No. of obs
=       104
Optimization     : ML                             Residual df
=        91
                                                  Scale parameter
=         1
Deviance         =  52.10294208                   (1/df) Deviance
=  .5725598
Pearson          =  49.23881045                   (1/df) Pearson
=  .5410858

Variance function: V(u) = u+(1)u^2                [Neg. Binomial]
Link function    : g(u) = ln(u/(u+(1/1)))         [Neg. Binomial]

                                                  AIC
=  7.230276
Log pseudolikelihood = -362.9743589               BIC
= -370.5366
```


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```
------------------------------------------------------------------------------
                |               Robust
recovery_eluate |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
----------------+-------------------------------------------------------------
         sample |  -.0012483   .0022341    -0.56   0.576    -.005627    .0031304
                |
           vrus |
              2 |  -.3321149   .1654514    -2.01   0.045    -.6563937   -.0078362
              3 |  -.0892617   .0257046    -3.47   0.001    -.1396418   -.0388815
              4 |  -.1670892   .0171574    -9.74   0.000    -.200717   -.1334614
              5 |  -.3086635   .2085998    -1.48   0.139    -.7175115    .1001845
              6 |  -.1553245   .0462456    -3.36   0.001    -.2459641   -.0646848
                |
       2.source |   .0634052   .0085066     7.45   0.000     .0467325    .0800778
                |
    vrus#source |
            2 2 |   .3081702   .1657442     1.86   0.063    -.0166825    .6330229
            3 2 |   .0186946   .0295078     0.63   0.526    -.0391395    .0765288
            4 2 |   .1624405   .0174818     9.29   0.000     .1281769    .1967041
            5 2 |   .1980089   .2121554     0.93   0.351    -.217808    .6138259
            6 2 |   .1492799   .0465103     3.21   0.001     .0581214    .2404385
                |
          _cons |  -.0744057   .0091092    -8.17   0.000    -.0922594   -.0565519
------------------------------------------------------------------------------
```

Result showed no evidence of differences between samples. There is however clear evidence between Viruses and source of the water. With respect to viruses, NoVG11 does not differ significantly from Adv Others differ

```
. glm, eform

Generalized linear models                         No. of obs      =       104
Optimization     : ML                             Residual df     =        91
                                                  Scale parameter =         1
Deviance         =  52.10294208                   (1/df) Deviance =  .5725598
```


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```
Pearson           =  49.23881045                  (1/df) Pearson
=  .5410858

Variance function: V(u) = u+(1)u^2               [Neg. Binomial]
Link function    : g(u) = ln(u/(u+(1/1)))        [Neg. Binomial]

                                                 AIC
=  7.230276
Log pseudolikelihood = -362.9743589              BIC
= -370.5366

------------------------------------------------------------------------------
                 |               Robust
recovery_eluate |      exp(b)   Std. Err.      z    P>|z|     [95% Conf. Interval]
-----------------+------------------------------------------------------------
         sample |    .9987525   .0022313    -0.56   0.576NS    .9943888    1.003135
                |
           vrus |
             2  |    .7174049   .1186956    -2.01   0.045    .5187186    .9921945
             3  |    .9146062   .0235096    -3.47   0.001    .8696697    .9618647
             4  |    .8461241   .0145173    -9.74   0.000    .8181439    .8750612
             5  |    .7344279   .1532015    -1.48   0.139     .487965    1.105375
             6  |    .8561373   .0395926    -3.36   0.001    .7819503    .9373628
                |
       2.source |    1.065458   .0090634     7.45   0.000    1.047842    1.083371
                |
    vrus#source |
          2 2  |    1.360933   .2255667     1.86   0.063    .9834559    1.883295
          3 2  |     1.01887   .0300646     0.63   0.526    .9616165    1.079533
          4 2  |    1.176378   .0205652     9.29   0.000    1.136754    1.217384
          5 2  |    1.218973   .2586118     0.93   0.351    .8042798    1.847486
          6 2  |    1.160998   .0539984     3.21   0.001    1.059844    1.271807
                |
          _cons |     .928295    .008456    -8.17   0.000    .9118686    .9450174
------------------------------------------------------------------------------

.


. margins vrus#source
```


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```
Predictive margins                                  Number of obs   =
104
Model VCE    : Robust

Expression   : Predicted mean recovery_eluate, predict()

------------------------------------------------------------------------
-----------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95%
Conf. Interval]
-------------+----------------------------------------------------------
-----------
 vrus#source |
        1 1  |   12.51018     1.3625     9.18   0.000      9.83973
15.18063
        1 2  |   73.94928   15.59737     4.74   0.000     43.37899
104.5196
        2 1  |    1.97882    .974122     2.03   0.042      .069576
3.888064
        2 2  |   26.22245   6.698217     3.91   0.000     13.09419
39.35071
        3 1  |   5.531788   .8819349     6.27   0.000     3.803227
7.260349
        3 2  |   11.40274   2.013933     5.66   0.000     7.455502
15.34998
        4 1  |   3.618688   .2531481    14.29   0.000     3.122527
4.114849
        4 2  |   54.74811   5.682567     9.63   0.000     43.61049
65.88574
        5 1  |   2.125581    1.38485     1.53   0.125    -.5886748
4.839837
        5 2  |   7.564778   2.498085     3.03   0.002     2.668622
12.46093
        6 1  |   3.825334   .8405716     4.55   0.000     2.177844
5.472823
        6 2  |   50.78223   10.88535     4.67   0.000     29.44734
72.11712
------------------------------------------------------------------------
-----------
vrus#source#sample with 95% CIs for recovery data.gph", replace
(file H:\Dudu_2012\UP_2012\Ruhaya_data\Adjusted Predictions of
vrus#source#sample with 95% CIs for recovery data.gph saved)
```


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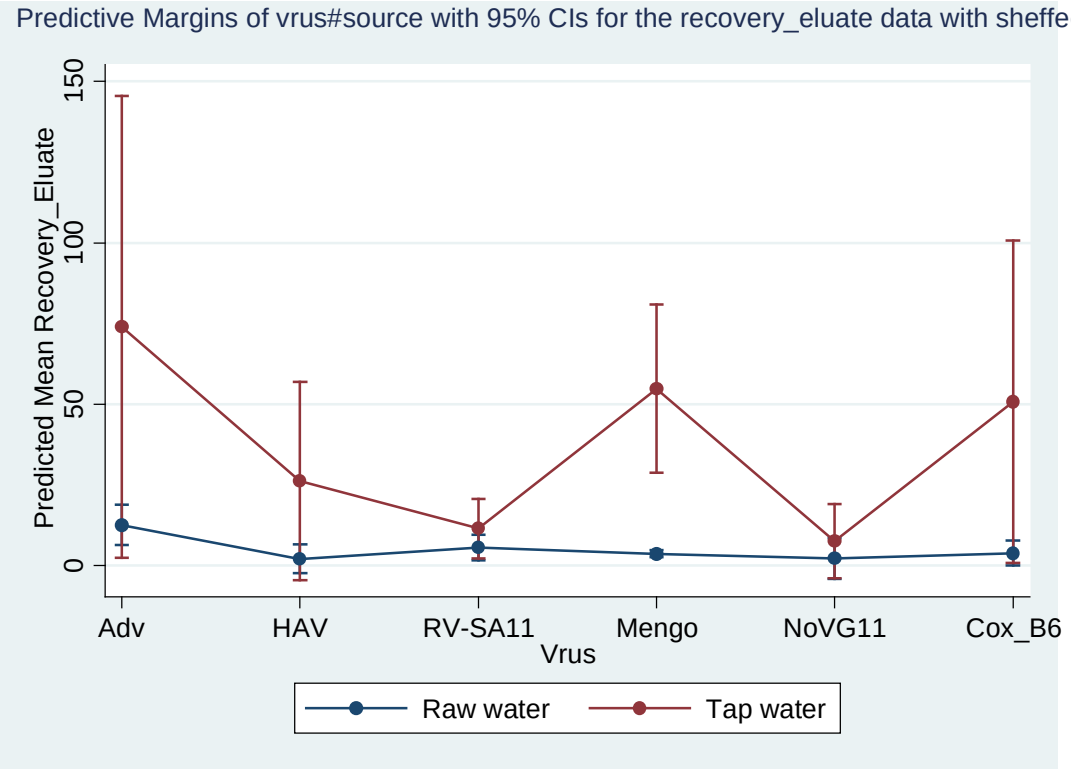

Result indicated clearly that the predicted mean recovery varied for each virus. It is clearly noticeable for Adv, HAV, Mengo and Cox_B6 viruses compared to RV_SA11, HAV and NoVG11 as far as recovery_eluate is concerned.

```
glm final_rec_pegnacl_pct sample vrus##source  , family(nbinomial)
link(nbinomial)  robust
note: final_rec_pegnacl_pct has noninteger values

Iteration 0:   log pseudolikelihood = -301.77824
Iteration 1:   log pseudolikelihood = -285.09359
Iteration 2:   log pseudolikelihood = -281.85228
Iteration 3:   log pseudolikelihood =  -281.1077
Iteration 4:   log pseudolikelihood =  -281.0591
Iteration 5:   log pseudolikelihood =  -281.0574
Iteration 6:   log pseudolikelihood =  -281.0574

Generalized linear models                         No. of obs
=       102
Optimization     : ML                             Residual df
=        89
                                                  Scale parameter
=         1
Deviance         =  49.90184575                   (1/df) Deviance
=  .5606949
Pearson          =  47.73835537                   (1/df) Pearson
=   .536386
```


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```
Variance function: V(u) = u+(1)u^2                [Neg. Binomial]
Link function    : g(u) = ln(u/(u+(1/1)))          [Neg. Binomial]

                                                   AIC
=  5.765831
Log pseudolikelihood = -281.0573953                BIC
= -361.7207

------------------------------------------------------------------------------
                     |               Robust
final_rec_pegnacl_pct |      Coef.   Std. Err.      z    P>|z|     [95% Conf.
Interval]
---------------------+--------------------------------------------------------
               sample |  -.0033746   .0039139    -0.86   0.389NS   -.0110457
.0042964
                     |
                 vrus |
                   2  |  -.0667965   .0937599    -0.71   0.476    -.2505625
.1169694
                   3  |  -.2997981   .1413126    -2.12   0.034    -.5767656
-.0228306
                   4  |  -1.878686    .053408   -35.18   0.000    -1.983363
-1.774008
                   5  |  -3.142583   .4116767    -7.63   0.000    -3.949455
-2.335712
                   6  |   -.379284   .0892545    -4.25   0.000    -.5542196
-.2043484
                     |
              2.source |   .0851172   .0137962     6.17   0.000     .0580771
.1121572
                     |
          vrus#source |
                 2 2  |   .0226188   .0951742     0.24   0.812    -.1639193
.2091568
                 3 2  |   .1642993   .1416863     1.16   0.246    -.1134008
.4419994
                 4 2  |   1.845002   .0540566    34.13   0.000     1.739054
1.950951
                 5 2  |   2.649806   .4265629     6.21   0.000     1.813758
3.485854
                 6 2  |   .3870549   .0895216     4.32   0.000     .2115958
 .562514
                     |
                _cons |  -.1076402   .0145419    -7.40   0.000    -.1361419
-.0791386
------------------------------------------------------------------------------

. glm, eform

Generalized linear models                          No. of obs      =
102
Optimization     : ML                              Residual df     =
89
                                                   Scale parameter =
1
```


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```
Deviance         =  49.90184575                  (1/df) Deviance =
.5606949
Pearson          =  47.73835537                  (1/df) Pearson  =
.536386

Variance function: V(u) = u+(1)u^2               [Neg. Binomial]
Link function    : g(u) = ln(u/(u+(1/1)))        [Neg. Binomial]

                                                 AIC             =
5.765831
Log pseudolikelihood = -281.0573953              BIC             = -
361.7207

------------------------------------------------------------------------------
                     |               Robust
final_rec_pegnacl_pct |     exp(b)   Std. Err.      z    P>|z|
[95% Conf. Interval]
---------------------+--------------------------------------------------------
              sample |    .996631   .0039007    -0.86   0.389NS
.9890151    1.004306
                     |
                vrus |
                   2 |   .9353855   .0877016    -0.71   0.476
.7783628    1.124085
                   3 |   .7409678   .1047081    -2.12   0.034
.5617122    .9774281
                   4 |   .1527908   .0081603   -35.18   0.000
.1376056    .1696517
                   5 |   .0431711   .0177725    -7.63   0.000
.0192652    .0967416
                   6 |   .6843512   .0610814    -4.25   0.000
.5745205    .8151783
                     |
             2.source |   1.088845   .0150219     6.17   0.000
1.059797    1.118689
                     |
          vrus#source |
                 2 2 |   1.022877   .0973515     0.24   0.812
.8488105    1.232638
                 3 2 |   1.178567   .1669868     1.16   0.246
.8927927    1.555815
                 4 2 |   6.328116   .3420761    34.13   0.000
5.691954    7.035378
                 5 2 |    14.1513   6.036419     6.21   0.000
6.133456    32.65031
                 6 2 |   1.472637   .1318328     4.32   0.000
1.235648    1.755079
                     |
                _cons |   .8979506   .0130579    -7.40   0.000
.8727188    .9239119
------------------------------------------------------------------------------
From the result, it is clear that there is no evidence of
differences between samples. The result also showed difference
between raw and tap water with respect to final_recovery.
.

. margins vrus#source
```


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```
Predictive margins                                  Number of obs   =
102
Model VCE    : Robust

Expression   : Predicted mean final_rec_pegnacl_pct, predict()

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf.
Interval]
-------------+----------------------------------------------------------------
 vrus#source |
        1 1  |   8.254064   .9650766     8.55   0.000     6.362549
10.14558
        1 2  |   33.92747   6.789388     5.00   0.000     20.62052
47.23443
        2 1  |   5.034531   2.823248     1.78   0.075    -.4989321
10.56799
        2 2  |   13.13358   2.857974     4.60   0.000      7.53205
18.7351
        3 1  |   1.948697   .8087483     2.41   0.016     .3635796
3.533815
        3 2  |   5.583203   .3300463    16.92   0.000     4.936324
6.230082
        4 1  |   .1577717   .0094789    16.64   0.000     .1391934
.1763501
        4 2  |   15.41118   1.563336     9.86   0.000      12.3471
18.47526
        5 1  |   .0400456   .0171379     2.34   0.019      .006456
.0736352
        5 2  |   1.458769   .4002204     3.64   0.000     .6743518
2.243187
        6 1  |   1.566533   .3552528     4.41   0.000     .8702501
2.262815
        6 2  |   46.70845   8.142532     5.74   0.000     30.74938
62.66752
------------------------------------------------------------------------------


. marginsplot,  mcompare(scheffe)
```


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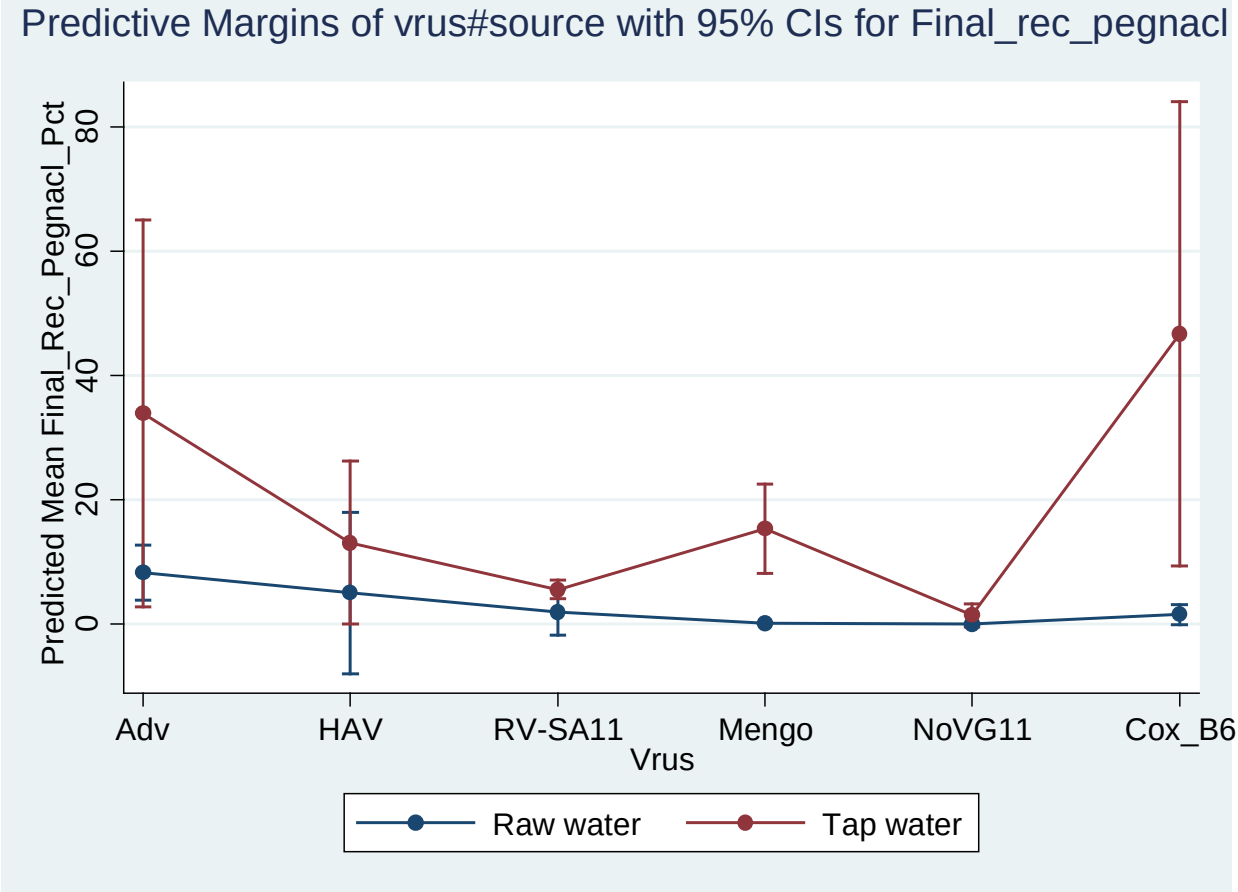

GLASS WOOL MODIFICATION : 15g + Grid (Standard Method) SUMMARY STATISTICS

. summarize

Variable	Obs	Mean	Std. Dev.	Min	Max
ggridinput	9	176444.4	31930.05	136000	242000
flowthrough	9	87066.67	19331.32	54400	121000
pctflowthr~h	9	50.31333	13.00419	33.79	71.03
eluate	9	94600	31759.72	64600	167000
pcteluate	9	54.78556	17.79201	26.78	88.83

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finrecovery 9 26477.78 8912.881 14600 46000

pctfinalre~y 9 15.42 5.099382 7.89 24.47

. sum,detail

15g +grid input

Percentiles		Smallest		
1%	136000	136000		
5%	136000	140000		
10%	136000	161000	Obs	9
25%	161000	162000	Sum of Wgt.	9
50%	184000	Mean		176444.4
	Largest	Std. Dev.		31930.05
75%	188000	185000		
90%	242000	188000	Variance	1.02e+09
95%	242000	190000	Skewness	.6742772
99%	242000	242000	Kurtosis	3.121024

flow through

Percentiles		Smallest		
1%	54400	54400		
5%	54400	74000		
10%	54400	77600	Obs	9
25%	77600	84200	Sum of Wgt.	9

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50%	84400	Mean	87066.67		
	Largest	Std. Dev.	19331.32		
75%	96600	84400			
90%	121000	96600	Variance	3.74e+08	
95%	121000	107000	Skewness	.1809611	
99%	121000	121000	Kurtosis	2.686696	

%flow throgh

Percentiles		Smallest		
1%	33.79	33.79		
5%	33.79	34.88		
10%	33.79	40	Obs	9
25%	40	44.32	Sum of Wgt.	9

50%	52.1	Mean	50.31333		
	Largest	Std. Dev.	13.00419		
75%	56.91	55.43			
90%	71.03	56.91	Variance	169.1088	
95%	71.03	64.36	Skewness	.1561444	
99%	71.03	71.03	Kurtosis	1.817413	

eluate

Percentiles		Smallest		
1%	64600	64600		

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5%	64600	64800		
10%	64600	66400	Obs	9
25%	66400	90200	Sum of Wgt.	9
50%	91200	Mean	94600	
	Largest	Std. Dev.	31759.72	
75%	103000	98200		
90%	167000	103000	Variance	1.01e+09
95%	167000	106000	Skewness	1.264807
99%	167000	167000	Kurtosis	4.109084

% eluate

Percentiles		Smallest		
1%	26.78	26.78		
5%	26.78	34.95		
10%	26.78	47.5	Obs	9
25%	47.5	55.68	Sum of Wgt.	9
50%	56.3	Mean	54.78556	
	Largest	Std. Dev.	17.79201	
75%	60.99	57.61		
90%	88.83	60.99	Variance	316.5557
95%	88.83	64.43	Skewness	.2463468
99%	88.83	88.83	Kurtosis	2.983924

fin recovery

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Percentiles		Smallest		
1%	14600	14600		
5%	14600	18500		
10%	14600	22600	Obs	9
25%	22600	23800	Sum of Wgt.	9
50%	26800	Mean	26477.78	
	Largest	Std. Dev.	8912.881	
75%	27400	27000		
90%	46000	27400	Variance	7.94e+07
95%	46000	31600	Skewness	.9951847
99%	46000	46000	Kurtosis	3.79208

% fin recovery

Percentiles		Smallest		
1%	7.89	7.89		
5%	7.89	10		
10%	7.89	11.42	Obs	9
25%	11.42	14.42	Sum of Wgt.	9
50%	16.77	Mean	15.42	
	Largest	Std. Dev.	5.099382	
75%	17.5	17.17		
90%	24.47	17.5	Variance	26.0037
95%	24.47	19.14	Skewness	.1583129

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99% 24.47 24.47

Summarise 15g no grid: Virus quantities at each stage

Variable	Obs	Mean	Std. Dev.	Min	Max
gnogridinput	9	137711.1	73443.86	40400	234000
flowthrough	9	130133.3	56181.85	47400	196000
pctflothro~h	9	104.1233	28.66924	76.45	163.4
eluate	9	240862.8	343930.8	4.92	1100000
pcteluate	9	71.56333	54.6651	3.62	137.86
finalrecov~y	9	29103.11	24285.36	538	60600
pctfinreco~y	9	17.05556	10.62992	.86	29.42

Glass wool Modification: 20 g No grid Summary Statistics

. sum,detail

20g no grid input

Percentiles		Smallest	
1%	228000	228000	
5%	228000	272000	
10%	228000	288000	Obs 9
25%	288000	296000	Sum of Wgt. 9

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50%	298000	Mean	296000		
	Largest	Std. Dev.	37027.02		
75%	304000	300000			
90%	370000	304000	Variance	1.37e+09	
95%	370000	308000	Skewness	.2057216	
99%	370000	370000	Kurtosis	3.87009	

flow through

Percentiles		Smallest			
1%	158000	158000			
5%	158000	177000			
10%	158000	180000	Obs	9	
25%	180000	188000	Sum of Wgt.	9	
50%	205000	Mean	253333.3		
	Largest	Std. Dev.	109356.1		
75%	264000	232000			
90%	438000	264000	Variance	1.20e+10	
95%	438000	438000	Skewness	1.063394	
99%	438000	438000	Kurtosis	2.453589	

%flow through

Percentiles		Smallest			
1%	58.08	58.08			
5%	58.08	59.21			

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10%	58.08	61.46	Obs	9
25%	61.46	62.66	Sum of Wgt.	9
50%	62.7	Mean	86.07445	
	Largest	Std. Dev.	36.6743	
75%	89.91	85.71		
90%	147.97	89.91	Variance	1345.004
95%	147.97	146.97	Skewness	1.024468
99%	147.97	147.97	Kurtosis	2.373217

Eluate

Percentiles		Smallest		
1%	126000	126000		
5%	126000	144000		
10%	126000	158000	Obs	9
25%	158000	164000	Sum of Wgt.	9
50%	174000	Mean	172444.4	
	Largest	Std. Dev.	26679.16	
75%	192000	190000		
90%	204000	192000	Variance	7.12e+08
95%	204000	200000	Skewness	-.4299211
99%	204000	204000	Kurtosis	1.988952

%eluate

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Percentiles		Smallest		
1%	46.32	46.32		
5%	46.32	47.02		
10%	46.32	51.94	Obs	9
25%	51.94	54.67	Sum of Wgt.	9
50%	63.16	Mean	58.61111	
	Largest	Std. Dev.	8.810999	
75%	64.86	63.76		
90%	70.83	64.86	Variance	77.6337
95%	70.83	64.94	Skewness	-.2364304
99%	70.83	70.83	Kurtosis	1.625028

final recovery

Percentiles		Smallest		
1%	2360	2360		
5%	2360	3440		
10%	2360	3480	Obs	9
25%	3480	3880	Sum of Wgt.	9
50%	19700	Mean	13740	
	Largest	Std. Dev.	10073.04	
75%	20800	20600		
90%	25400	20800	Variance	1.01e+08
95%	25400	24000	Skewness	-.1366457
99%	25400	25400	Kurtosis	1.148524

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Fin Recovery%

Percentiles		Smallest		
1%	1.13	1.13		
5%	1.13	1.19		
10%	1.13	1.31	Obs	9
25%	1.31	6.48	Sum of Wgt.	9
50%	6.49	Mean	5.57	
	Largest	Std. Dev.	3.525468	
75%	6.97	6.87		
90%	10.35	6.97	Variance	12.42893
95%	10.35	9.34	Skewness	-.2408318
99%	10.35	10.35	Kurtosis	1.655111

. summarize

Variable	Obs	Mean	Std. Dev.	Min	Max
gnogridinput	9	296000	37027.02	228000	370000
flowthrough	9	253333.3	109356.1	158000	438000
%flothru	9	86.07445	36.6743	58.08	147.97
eluate	9	172444.4	26679.16	126000	204000
%eluate	9	58.61111	8.810999	46.32	70.83
finalrecov~y	9	13740	10073.04	2360	25400

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%finrecovery 9 5.57 3.525468 1.13 10.35

1. 15g No grid Mengovirus Eluate Vs PEG/NaCl

. ttesti 9 71.56 54.66 9 17.06 10.63

Two-sample t test with equal variances

Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
x	9	71.56	18.22	54.66	29.5446	113.5754
y	9	17.06	3.543333	10.63	8.889059	25.23094
combined	18	44.31	11.16891	47.38567	20.74566	67.87434
diff		54.5	18.56135		15.1517	93.8483

Degrees of freedom: 16

Ho: mean(x) - mean(y) = diff = 0

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0

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t = 2.9362 t = 2.9362 t = 2.9362

P < t = 0.9952 P > |t| = 0.0097 P > t = 0.0048

Flowthrough 15g nogrid vs 15+grid

ttesti 9 104.12 28.66 9 50.31 13.00, unequal

Two-sample t test with unequal variances

Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
x	9	104.12	9.553333	28.66	82.08997	126.15
y	9	50.31	4.333333	13	40.31732	60.30268
combined	18	77.215	8.274891	35.10739	59.75651	94.67349
diff		53.81	10.49018		30.76115	76.85885

Satterthwaite's degrees of freedom: 11.1583

Ho: mean(x) - mean(y) = diff = 0

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0

t = 5.1296 t = 5.1296 t = 5.1296

P < t = 0.9998 P > |t| = 0.0003 P > t = 0.0002

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ELUATE (15g no grid vs 15g + grid)

. ttesti 9 71.56 54.66 9 54.78 17.92, unequal

Two-sample t test with unequal variances

	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
x	9	71.56	18.22	54.66	29.5446	113.5754
y	9	54.78	5.973333	17.92	41.00547	68.55453
combined	18	63.17	9.52084	40.3935	43.08278	83.25722
diff		16.78	19.17418		-26.12247	59.68247

Satterthwaite's degrees of freedom: 9.70008

Ho: mean(x) - mean(y) = diff = 0

Ha: diff < 0	Ha: diff != 0	Ha: diff > 0
t = 0.8751	t = 0.8751	t = 0.8751
P < t = 0.7987	P > |t| = 0.4026	P > t = 0.2013

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Final recovery (15g no grid vs 15g + grid)

ttesti 9 17.06 10.63 9 15.42 5.09, unequal

Two-sample t test with unequal variances

	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
x	9	17.06	3.543333	10.63	8.889059 25.23094
y	9	15.42	1.696667	5.09	11.50748 19.33252
combined	18	16.24	1.916	8.1289	12.19759 20.28241
diff		1.64	3.928599		-6.962421 10.24242

Satterthwaite's degrees of freedom: 11.4853

Ho: mean(x) - mean(y) = diff = 0

Ha: diff < 0	Ha: diff != 0	Ha: diff > 0
t = 0.4175	t = 0.4175	t = 0.4175
P < t = 0.6580	P > |t| = 0.6840	P > t = 0.3420

Flowthru (15g no grid vs 20g no grid)

ttesti 9 104.12 28.66 9 86.07 36.67, unequal

Two-sample t test with unequal variances

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```
------------------------------------------------------------------------------
    |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    x |       9     104.12    9.553333      28.66    82.08997     126.15
    y |       9      86.07    12.22333      36.67    57.88294    114.2571
---------+--------------------------------------------------------------------
combined |      18     95.095    7.837147    33.2502    78.56007    111.6299
---------+--------------------------------------------------------------------
   diff |            18.05    15.51374               -14.99434    51.09434
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  15.1177

                 Ho: mean(x) - mean(y) = diff = 0

     Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
      t =   1.1635                 t =   1.1635                 t =   1.1635
   P < t =   0.8687         P > |t| =   0.2627         P > t =   0.1313
```

Eluate (15g no grid vs 20g no grid)

```
. ttesti 9 71.56 58.61 9 54.66 8.81, unequal

Two-sample t test with unequal variances

------------------------------------------------------------------------------
    |     Obs       Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
```


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---------+--

x | 9 71.56 19.53667 58.61 26.50837 116.6116

y | 9 54.66 2.936667 8.81 47.88803 61.43197

---------+--

combined | 18 63.11 9.799832 41.57716 42.43416 83.78584

---------+--

diff | 16.9 19.75615 -28.31733 62.11733

Satterthwaite's degrees of freedom: 8.36133

Ho: mean(x) - mean(y) = diff = 0

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0

t = 0.8554 t = 0.8554 t = 0.8554

P < t = 0.7919 P > |t| = 0.4162 P > t = 0.2081

Final recovery (15g no grid vs 20g no grid)

. ttesti 9 17.06 5.57 9 10.63 3.52, unequal

Two-sample t test with unequal variances

| Obs Mean Std. Err. Std. Dev. [95% Conf. Interval]

---------+--

x | 9 17.06 1.856667 5.57 12.77852 21.34148

y | 9 10.63 1.173333 3.52 7.924288 13.33571

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---------+--

combined | 18 13.845 1.320248 5.601336 11.05952 16.63048

---------+--

diff | 6.43 2.196343 1.703266 11.15673

Satterthwaite's degrees of freedom: 13.5109

Ho: mean(x) - mean(y) = diff = 0

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0

t = 2.9276 t = 2.9276 t = 2.9276

P < t = 0.9943 P > |t| = 0.0114 P > t = 0.0057

Flow through (15g +grid vs 20g no grid)

ttesti 9 50.31 13.00 9 86.07 36.67, unequal

Two-sample t test with unequal variances

| Obs Mean Std. Err. Std. Dev. [95% Conf. Interval]

---------+--

x | 9 50.31 4.333333 13 40.31732 60.30268

y | 9 86.07 12.22333 36.67 57.88294 114.2571

---------+--

combined | 18 68.19 7.640623 32.41642 52.06969 84.31031

---------+--

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```
   diff |          -35.76    12.96872           -64.66411   -6.855888
------------------------------------------------------------------------------
Satterthwaite's degrees of freedom:  9.97961

                Ho: mean(x) - mean(y) = diff = 0

    Ha: diff < 0           Ha: diff != 0          Ha: diff > 0
     t =  -2.7574            t =  -2.7574           t =  -2.7574
   P < t =   0.0101        P > |t| =   0.0203       P > t =   0.9899
```


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APPENDIX C

C.1: Ruhanya V, Olorunju S, Taylor MB. Efficiency of glass wool adsorption-elution technique for the recovery of selected enteric viruses from raw and treated water [Poster]. Faculty Day, Faculty of Health Sciences, University of Pretoria 28-29 August 2012: HW Snyman Building North, Pretoria.

Introduction/Aim: Water quality and human health is affected by human pathogenic enteric viruses derived from sewage and discharged into the environment. To determine the public health risk caused by human enteric viruses in water a reliable and practical method for concentrating and detecting small titers of virus is needed. Sodocalcic glass wool adsorption-elution technique is a cost effective and easy-to-use method to recover and concentrate viruses from water but more data is required on its efficiency and reliability. This study assessed the performance of glass wool adsorption-elution to recover and concentrate selected enteric viruses.
Methods: Treated and turbid water samples were seeded with known titres of selected enteric viruses which were then recovered using a modified glass wool adsorption-elution technique optimized in the Department of Medical Virology. Recovered viruses were further concentrated and precipitated by polyethylene glycol in the presence of sodium chloride. Viral nucleic acid was extracted using the automated MagNA Pure platform and enumerated by quantitative reverse-transcription PCR. The percentage recovery efficiency was calculated as genome copies obtained after adsorption-elution and secondary concentration in relation to input titre.
Results: In tap water final recovery efficiencies averaged 46.9% for coxsackievirus B6, 34% for adenovirus, 15.4% for mengovirus, 13.1% for hepatitis A virus, 5.9% for rotavirus and 1.5% for norovirus GII.4. In turbid water, efficiencies of recovery were lower averaging 8.3% for adenovirus, 5.0% for hepatitis A virus, 2.0% for rotavirus, 1.6% for coxsackievirus B6, 0.2% for mengovirus and 0.1% for norovirus. Higher recoveries were obtained in the eluate in both tap (7.6% for norovirus and 74% for adenovirus) and raw water (1.9% for norovirus and 12.51% for adenovirus). More viruses were lost in the flow through from the turbid water (31.3% for noroviruses to 92.9% for mengovirus) than in tap water (4% for adenovirus to 70% in hepatitis A virus).
Conclusion: These results support a previous assumption that, for all viruses, the efficiency of recovery was higher from treated tap water than from turbid raw water and it varied among different virus genera. Significant numbers of viruses were lost in the flow through and the secondary concentration procedure.

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APPENDIX D

ETHICAL APPROVAL

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The Research Ethics Committee, Faculty of Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
* FWA 00002567. Approved dd 22 May 2002 and Expires 13 Jan 2012.
* IRB 0000 2235 IORG0001762 Approved dd 13/04/2011 and Expires 13/04/2014.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee
Fakulteit Gesondheidswetenskappe Navorsingsetiekkomitee

DATE: 24/06/2011

NUMBER	**S 112/2011**
TITLE	Efficiency of glass wool adsorption-elution technique for the recovery of enteric viruses from water.
INVESTIGATOR	Vurayai Ruhanya **Dept:** Medical Virology; University of Pretoria. **Cell:** 00263 77 211 6510 **E-Mail:** vruhanya@yahoo.com / s10663968@tuks.co.za
SUPERVISOR	Prof Maureen Taylor **E-Mail:** maureen.taylor@up.ac.za
SPONSOR	None
STUDY DEGREE	MSc (Medical Virology)

This Student Protocol was approved by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria on 21/06/2011. The approval is valid for a period of 3 years.

Members of the Main Research Ethics Committee:

Prof M J Bester	(female)BSc (Chemistry and Biochemistry); BSc (Hons)(Biochemistry); MSc(Biochemistry); PhD (Medical Biochemistry)
Prof R Delport	(female)BA et Scien, B Curationis (Hons) (Intensive care Nursing), M Sc (Physiology), PhD (Medicine), M Ed Computer Assisted Education
Prof JA Ker	MBChB; MMed(Int); MD – Vice-Dean (ex officio)
Dr NK Likibi	MBB HM – Representing Gauteng De partment of Health) MPH
Dr MP Mathebula	(female)Deputy CEO: Steve Biko Academic Hospital; MBCHB, PDM, HM
Prof A Nienaber	(female) BA(Hons)(Wits); LLB; LLM; LLD(UP); PhD; Dipl.Datametrics(UNISA) – Legal advisor
Mrs MC Nzeku	(female) BSc(NUL); MSc(Biochem)(UCL, UK) – Community representative
Prof L M Ntlhe	MbChB (Natal) FCS (SA)
Snr Sr J Phatoli	(female) BCur(Eet.A); BTec(Oncology Nursing Science) – Nursing representative
Dr R Reynders	MBChB (Prêt), FCPaed (CMSA) MRCPCH (Lon) Cert Med. Onc (CMSA)
Dr T Rossouw	(female) MBChB (cum laude); M.Phil (Applied Ethics) (cum laude), MPH (Biostatistics and Epidemiology (cum laude), D.Phil
Dr L Schoeman	(female) B.Pharm, BA(Hons)(Psych), PhD – Chairperson: Subcommittee for students' research
Mr Y Sikweyiya	MPH; SARETI Fellowship in Research Ethics; SARETI ERCTP; BSc(Health Promotion)Postgraduate Dip (Health Promotion) – Community representative
Dr R Sommers	(female) MBChB; MMed(Int); MPharmMed – **Deputy Chairperson**
Prof TJP Swart	BChD, MSc (Odont), MChD (Oral Path), PGCHE – School of Dentistry representative
Prof C W van Staden	MBChB; MMed (Psych); MD; FCPsych; FTCL; UPLM - **Chairperson**

Members of the Student Research Ethics Committee:

Prof R S K Apatu	MBChB (Legon,UG); PhD (Cantab); PGDip International Research Ethics (UCT)
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Dr S I Cronje	BA (Pretoria); BD (Pretoria); DD (Pretoria)
Prof M M Ehlers	(female) BSc (Agric) Microbiology (Pret); BSc (Agric) Hons Microbiology (Pret); MSc (Agric) Microbiology (Pret); PhD Microbiology (Pret); Post Doctoral Fellow (Pret)
Dr R Leech	(female) PhD Nursing Science
Prof D Millard	(female) B.Iur (Pretoria); LLB (Pretoria); LLM (Pretoria); AIPSA Diploma in Insolvency Law (Pretoria); LLD (UJ)

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Prof D Millard	(female) B.Iur (Pretoria); LLB (Pretoria); LLM (Pretoria); AIPSA Diploma in Insolvency Law (Pretoria); LLD (UJ)
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Dr L Schoeman	**Chairperson:** (female) BPharm (North West); BAHons (Psychology)(Pretoria); PhD (KwaZulu-Natal); International Diploma in Research Ethics (UCT)
Dr R Sommers	**Vice-Chair** (female) MBChB; MMed(Int); MPharmMed
Prof L Sykes	(female) BSc, BDS, MDent (Pros)

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CHAIRPERSON of the Faculty of Health Sciences
Student Research Ethics Committee,
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