EnviroMail No. 63 June 2012

QUANTITATION OF INFECTIOUS ENTERIC VIRUSES

INTRODUCTION

Following an extensive research and development project, ALS Scoresby (Victoria) now offers NATA accredited adenoviruses and enteroviruses in wastewater, environmental water and biosolids.

LEGISLATION – WHY ANALYSE INFECTIOUS ENTERIC VIRUSES?

Enteric viruses such as adenoviruses and enteroviruses are a major cause of gastroenteritis and other infectious diseases and may pose a significant risk to public health. The Australian Guidelines for Water Recycling recommend the use of adenoviruses and enteroviruses as "representatives of viral pathogens" for the validation monitoring of wastewater treatment processes.

State and USEPA guidelines for the environmental management of biosolids also recommend the validation monitoring of enteric viruses including adenoviruses and enteroviruses.

Adenoviruses and enteroviruses are suitable reference viral pathogens as they are culturable and are usually present in high numbers in raw wastewater.

WHAT ARE APPLICATIONS TO THE WATER INDUSTRY?

- Quantitative microbial risk assessment (QMRA) of wastewater treatment plants and environmental water.
- Validation monitoring of wastewater treatment processes.
- Compliance monitoring of recycled wastewater.
- Challenge testing for the validation of wastewater treatment processes.
- Microbial source tracking further investigation.
- Validation monitoring of biosolids for unrestricted land application.

METHOD INFORMATION

ALS METHOD CODE
W-ENTVIR - Wastewater and Environmental Water
S-ENTVIR - Bio-solids

LIMIT OF DETECTION
1 MPNIU per volume analysed

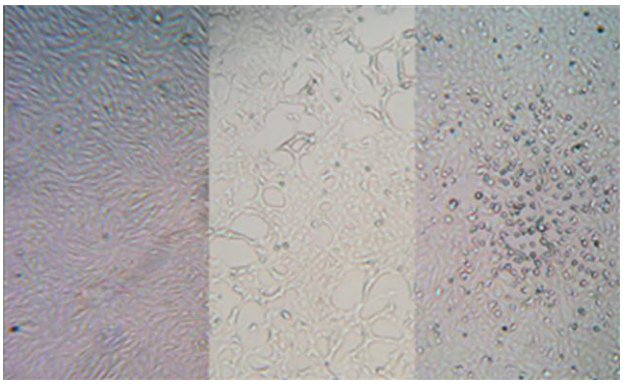
METHOD REFERENCES
In-house

Biosolids Environmental Wastewater

ANALYSIS OF INFECTIOUS ENTERIC VIRUSES

Analysis is via a novel integrated virus cell culture and real time PCR method which has been developed at ALS based on review of the published scientific literature, the USEPA, WHO and the APHA.

The method involves primary concentration by ultrafiltration, elution of solids or bio-solids associated viruses and secondary concentration by precipitation. Adenoviruses and enteroviruses in the sample concentrate are cultured in susceptible cells and the culture of the target viruses is confirmed by real time PCR. Quantitation is based on the number of cell cultures confirmed positive for each virus, which is used to calculate the Most Probable Number of Infectious Units (MPNIU).



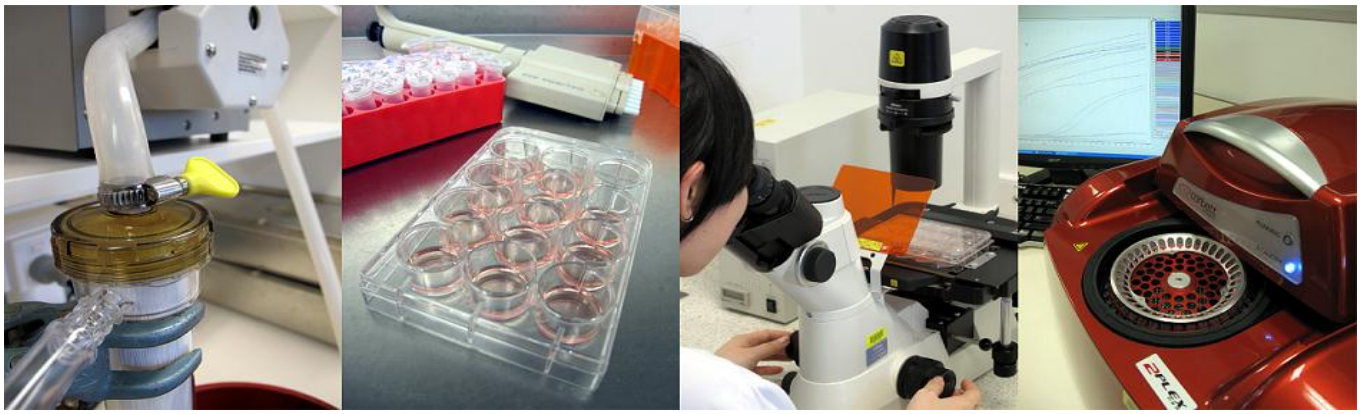
Control Adenovirus Enterovirus

The figure above shows the cytopathic changes associated with the culture of adenovirus and enterovirus in susceptible cells as observed under the microscope. Uninfected cells in the control appear as a confluent monolayer. Cells infected with adenovirus typically become enlarged and refractile and a web-like degeneration of the monolayer can be observed. Cells infected with enterovirus typically become small and rounded and distinct foci of cytopathic changes may be observed.

ENTERIC VIRUS RECOVERY AND MEASUREMENT OF UNCERTAINTY

The performance of the analysis as defined by the mean recovery of adenoviruses and enteroviruses from spiked wastewater samples and the measurement of uncertainty in the quantitation of indigenous adenoviruses and enteroviruses are described below. The mean recoveries achieved by ALS are exceptionally high compared to the acceptance criteria described by NATA and the USEPA for other microorganisms.

Enteric Virus	Mean Recovery (%)		Measurement of Uncertainty (%)
	Raw Wastewater	Secondary Wastewater	
Enteroviruses	35	60	16
Adenoviruses	13	33	6



SAMPLING REQUIREMENTS

Holding Time:	Two days (48 hours)
Sample Shipping and Storage:	≤10ºC [1]
Sample Containers:	Clean Plastic Containers preserved with Sodium Thiosulphate (as required) to neutralise chlorine[1]

[1]ALS recommends that sample containers be immediately placed in an esky upon sampling and covered with sufficient ice to chill each sample well below 10˚C. In hot conditions for remote/overnight air-freight, samples should have ice/free water removed from the esky and replaced with ice in zip seal bags or ice bricks immediately prior to submission to couriers. ALS OH&S requirements are for no more than one 10L sample per esky and where 50L samples are provided eskies and containers should be marked accordingly (e.g. 1 of 5, 2 of 5 etc).

The volume of wastewater that can be analysed depends on the level of suspended solids as this can foul the ultra-filtration membrane. The limit of detection is always 1 MPNIU per volume analysed. Therefore, as the sample volume increases the limit of detection per unit volume decreases. State guidelines for the management of the environment and public health define acceptance criteria for the limit of detection of enteric viruses in recycled wastewater and biosolids. These guidelines and conservative estimates of the sample volume requirements for different water samples are described below.

Water sample	Sample volume or weight	Expected concentration of enteric viruses (MPNIU/L)
Raw and primary wastewater	1 L	10 - 10000
Secondary wastewater	10 L	0 - 1000
Recycled wastewater (Class A)	50 L[2]	0
Environmental water	10 L	0 - 1000
Biosolid	≤100 g (dry weight)[3] (150mL jar)	0

[2] EPA VIC publication 464.2. [3] EPA VIC publication 943

For further information please contact your ALS Scoresby or local ALS client services team.