Norovirus

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *norovirus*.

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1 PHLN SUMMARY LABORATORY DEFINITION

1.1 Condition:

Norovirus infection

1.1.1 Definitive Criteria

a Detection of human *norovirus* by antigen detection; OR
b Detection of human *norovirus* by Nucleic Acid Amplification (NAA).

1.1.2 Suggestive Criteria

Detection of *norovirus*-like viral particles by Electron Microscopy (EM).

2 Introduction

Noroviruses (NoV) are a genetically diverse group of single stranded RNA, nonenveloped viruses belonging to the *Caliciviridae* family. They are non-culturable and were originally found in faeces examined using electron microscopy during an outbreak of gastroenteritis in a school in Norwalk, Ohio in 1968 (Kapikian et al, 1972). For decades they were called "small round structured viruses" or "Norwalk-like viruses" until recently when their taxonomy was investigated using modern molecular techniques. They are now one of four genera in the *Caliciviridae* each sharing a similar structure when examined by electron microscopy. A second genus, *Sapovirus*, also causes gastroenteritis in humans. Man is the only known host for these two genera.

Noroviruses are a major cause of acute gastroenteritis worldwide, often causing explosive outbreaks in institutions. They are highly contagious, with an inoculum of as few as ten particles being able to cause infection. Transmission occurs through ingesting contaminated food and water and by person-to-person spread. Transmission is predominantly faecal-oral but may be airborne due to aerosolisation

of vomitus, which typically contains abundant infectious virus particles. Outbreaks may involve several routes of transmission. The illness is acute, usually mild, although it has caused fatalities among the frail elderly, and self-limiting and follows an incubation period of 24-48 hours although cases can occur within 12 hours of exposure. Asymptomatic carriage may be common, especially in outbreak settings (Gallimore et al, 2004) although its role in transmission is not well understood.

Sporadic illness commonly occurs in Australia but it is the ability of *noroviruses* to cause outbreaks in institutions, which has become a major public health issue. In 2004 in NSW alone, there were more than 400 outbreaks of *norovirus* infection associated with restaurants and institutions as diverse as nursing homes, hospitals and elite sporting camps.

Noroviruses commonly isolated in cases of acute gastroenteritis belong to two genogroups: genogroup I (GI) includes *Norwalk viru*s, *Desert Shield virus* and *Southampton virus* and II (GII) which includes *Bristol virus*, L*ordsdale virus, Toronto virus, Mexico virus, Hawaii virus* and *Snow Mountain virus*. Infections with strains of GII tend to be more prevalent (Ando et al 2000, Hansman et al 2004, Kirkwood & Bishop 2001). Interestingly, a study of NoVs detected over a three-year period (1995-1997) in Central Australia (Schnagel et al 2000) showed a predominance of GI strains and contrasts the increased prevalence of GII strains observed in outbreaks investigated in Southeastern Australia during 1980-1996 (Wright et al 1998, White et al 2002). The dominant strain in 2004/5 in Australia was a GII/4 strain, variously referred to as the GII/4 variant strain, Grimsby strain or Farmington Hills strain, which was first detected in 2001 as the cause of a sudden increase in outbreaks in Europe and the US, and has now spread globally.

Until recently diagnosis relied on electron microscopy to visualise the virus in faeces. In the 1990's sensitive Reverse transcription polymerase chain reaction (RT- PCR) techniques and, more recently, commercially available rapid antigen detection kits have become available for diagnosis, allowing rapid recognition of the causative agent and introduction of targeted control measures.

3 Laboratory diaganosis/tests

3.1 Culture

These viruses cannot be cultured in the diagnostic laboratory and there is no simple laboratory animal model available.

3.2 Electron Microscopy

This was the original method used to detect NoV and it remains a useful diagnostic tool when specimens from "typical viral gastroenteritis" outbreaks are negative by RT-PCR and antigen detection. EM can be used to observe NoV in stool samples but its sensitivity is limited because the virus can be shed in low numbers and may be missed unless immune EM is performed or faecal specimens are concentrated by ultracentrifugation. IEM is a sophisticated technique that requires hard to obtain convalescent serum from NoV infected patients, well developed technical skills and expensive equipment. The insensitivity and cumbersome nature of EM make widespread screening impractical. (Kapikian & Chanock 1996).

3.2.1 Suitable specimens

Stool or vomitus are suitable specimens for diagnostic testing. Water may be tested after concentration of virus by centrifugation or filtration. Foodstuffs are extremely difficult to examine for

NoV but there has been limited success testing molluscs, either by macerating the shellfish flesh or by depurating the shellfish in water and subsequently concentrating the water for examination.

3.2.2 Test Sensitivity

The analytical sensitivity of EM is approximately 10E6 particles/ml and approximately 10E3ml for IEM, or EM after ultracentrifugation. Clinical sensitivity is approximately 20% for EM and 80% for IEM by experienced operators. The low numbers of virus excreted or present account for the low sensitivity.

3.2.3 Test specificity

Immune EM specificity approaches 100% for the virus sought. Because of the large number of different strains there is a need for the development of polyclonal antisera, which will detect all strains (Doan & Anderson 1987).

3.2.4 Suitable external QC programmes

None.

3.3 Nucleic Acid Diagnosis

Noroviruses are genetically diverse complicating the search for sequences, which can be used to design broadly reactive primers for nucleic acid detection. The RT-PCR assays commonly used to detect NoVs generally use primers targeting the polymerase region of ORF 1. More recently primers targeting conserved regions of ORF 2 (capsid gene) (Kojima et al. 2002, Noel et al 1997, Vinje et al 2004) have been developed to provide increased phylogenetic information. There are various protocols and primer sets used by Australian laboratories. Two published nested RT-PCR protocols for the detection of conserved sequences of common NoVs using primers listed in Table 1 (McIver et al 2005). Another laboratory has published a hanging drop, single tube, nested RT-PCR method, which might have application with other nested primer sets in reducing lab contamination (Ratcliff et al 2002). Highly sensitive real time PCR assays have also been published (Kageyama et al. 2003).

3.3.1 Suitable specimens

Faeces collected in the acute phase of infection.

3.3.2 Test sensitivity

RT-PCR is regarded as the optimal approach to detection. Sensitivity is dependent on the primers chosen and the phase of illness when specimens are collected. False negatives due to the sequence diversity of *Noroviruses* may occur as no primer pair has been shown to be universally active. Negative results from patients involved in a "typical" outbreak should prompt further testing with other primers. Ideally specimens should be collected during the symptomatic phase when large numbers of virus particles are present.

3.3.3 Test specificity

Cross-reactions with other genetic species have not been documented using the primers listed in Table 1. Regular sequencing and BLAST analysis of the ORF 1 and ORF 2 of recent isolates ensure veracity of identification. Further, this approach allows detection of emerging strains that may be refractory to detection by primer pairs in use.

3.3.4 Predictive values

The predictive value of a positive finding can be potentially compromised by the presence of NoVs in asymptomatic individuals as has been reported in case control studies (Koopmans et al., 2001). Negative predictive values depend on the primer pair chosen and the phase of illness when specimens are collected. Negative results from patients involved in a "typical" outbreak should prompt further testing with other primers.

3.3.5 Suitable validation criteria

Novel methods should be tested against endemic strains representative of the genetic diversity prevailing in the Australian epidemiologic setting.

3.3.6 Suitable external QC programmes

A QC programme for the detection of NoVs by RT-PCR is not available in Australia.

3.4 Direct Antigen Detection

In-house and commercial ELISA based assays have been developed to provide cheaper and more rapid detection of genetically diverse HuNoV in stools. All use recombinant-expressed major capsid proteins (VP1), which spontaneously assemble into virus-like particles (VLPs) and polyclonal and monoclonal antibodies against the VLPs. Two commercial kits are available in Australia: SRSV (II)-AD (Denka Seiken Co. Ltd. Tokyo, Japan) and IDEIA NLV (DakoCytomation Ltd., Ely, United Kingdom). These kits were recently evaluated by CDC (Burton-MacLeod et al 2004) and their performance characteristics compared with RT-PCR using strongly positive samples.

3.4.1 Suitable specimens

Faeces collected as early in the illness as possible

3.4.2 Test sensitivity

Denka: GI 81% Dako: GI 60%
GII 69% GII 28%
Both 80% Both 39%

3.4.3 Test specificity

Denka: GI 72% Dako: GI 100%
GII 85% GII 100%
Both 69% Both 100%

3.4.4 Predictive values

Positive predictive value: Denka kit found to have low value owing to non-specific cross reactions with non HuNV. Dako Kit has a high positive predictive value (~100%).
Negative predictive value: Both kits are insensitive compared with RT-PCR.

3.4.5 Suitable test criteria

See Manufacturer's instruction leaflet.

3.4.6 Suitable internal controls

Internal controls are supplied with the test kit.

3.4.7 Suitable validation criteria

See manufacturer's instruction leaflet.

3.4.8 External QC programmes

None available.

Note: Although these kits are markedly less sensitive than RT-PCR they are very useful in an outbreak investigation where numerous specimens can be collected and tested early in disease. Their ability to detect a range of genotypes, short test time (less than one day), ease of use and relative cheapness is a major advantage compared with RT-PCR. Denka Seiken is presently working to improve the specificity of their kit.

3.5 Serological Diagnosis

Immunity to *norovirus* infection and correlates of antibody with protection are not well understood. Pre-existing serum antibody does not always correlate with protection from reinfection. Although there is some evidence that the magnitude of the immune response may relate to the severity of the illness. Currently available antibody tests do not measure neutralising antibody. These tests are not deemed diagnostically useful.

4 Subtyping

Genotyping using polymerase and capsid sequences is now the universally available method of subtyping *noroviruses*.
The Health Protection Agency of Great Britain hosts a *norovirus* database for polymerase and capsid gene sequences and associated epidemiological data. The database can be searched by entering polymerase gene sequences and the HPA proposes to add a search facility for capsid sequences in the near future.

5 References

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Table 1 List of primers for the detection of norovirus and sapovirus.

Primer	Function[a]	Sequence (5´ to 3´)	Size (bp)	Reference
Norovirus genogroup I				
Capsid gene region (ORF 2)				
COG1F	A	CGY TGG ATG CGN TTY CAT GA	381	Kageyama et al., 2003
G1SKR	B	CCA ACC CAR CCA TTR TAC A		Kojima et al., 2002
Norovirus genogroup II				
RNA polymerase region (ORF 1)				
CB1	A	GGC CCC ATC ATC TTC GAG AG	433	White et al., 2002
CB2	B	GTT TYA RCC CGT ATT CCT TG		White et al., 2002
CB3	C	AGC AGC CCT AGA AAT CAT GG	189	White et al., 2002
CB4	D	CAG AGA GTG AGG AGC AGT G		White et al., 2002
3' end of the polymerase region and the 5' end of the capsid gene region (ORF 2)				
NV2oF2	A	GG GAG GGC GAT CGC AAT C		Bull, 2003
NV2oR	B	GTR AAC GCR TTY CCM GC	380	Bull, 2003

Primer	Function[a]	Sequence (5′ to 3′)	Size (bp)	Reference
G2F3	C	TTG TGA ATG AAG ATG GCG TCG A		Hansman et al. 2004
G2SKR	D	CCR CCN GCA TRH CCR TTR TAC AT	311	Kojima et al. 2002
Sapovirus				
SV5317	A	CTC GCC ACC TAC RAW GCB TGG TT		Hansman et al. 2004
SV5749	B	CGG RCY TCA AAV STA CCB CCC CA	437	Hansman et al. 2004

B = C, G, or T; H = A, C or T; M = A or C; N = A, C, G or T; R = A or G; S = C or G; V = A, C, or G; W = A or T; Y = C or T.

aA = Outer sense primer; B = Outer anti-sense primer; C = Inner sense primer; D = Inner antisense primer. Adapted from McIver et al., 2005.