

# Diagnosis and Typing of Norovirus

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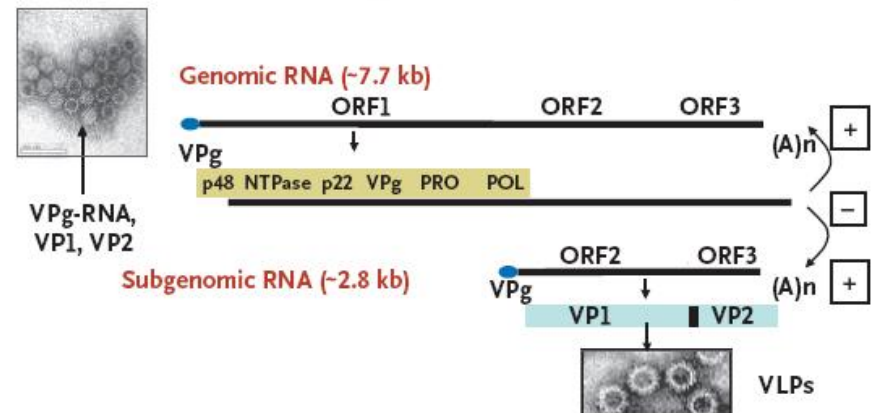
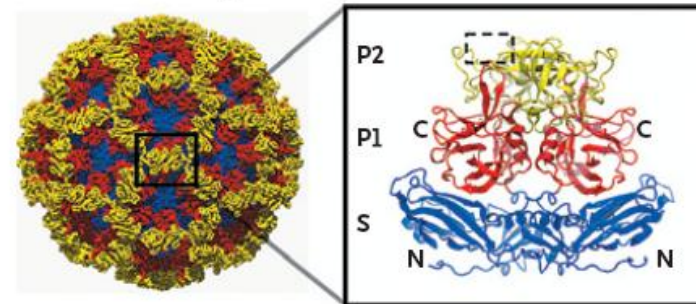
## Objectives

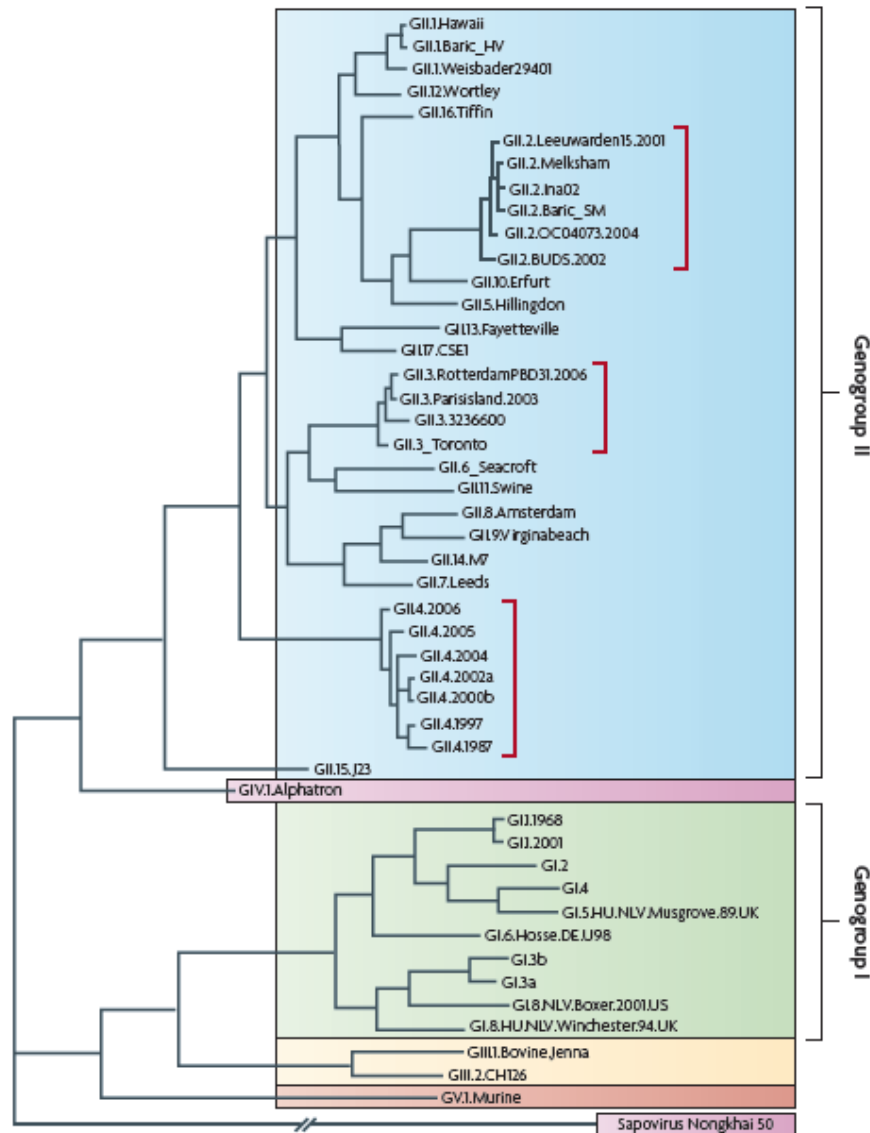
- Characteristics of Norovirus
- Methods for detection of Norovirus
- Rapid test for detection of Norovirus
- Current algorithm at OAHPP-PHL
- Revision of Norovirus algorithm
- Utility of Norovirus typing
- Multiplex testing

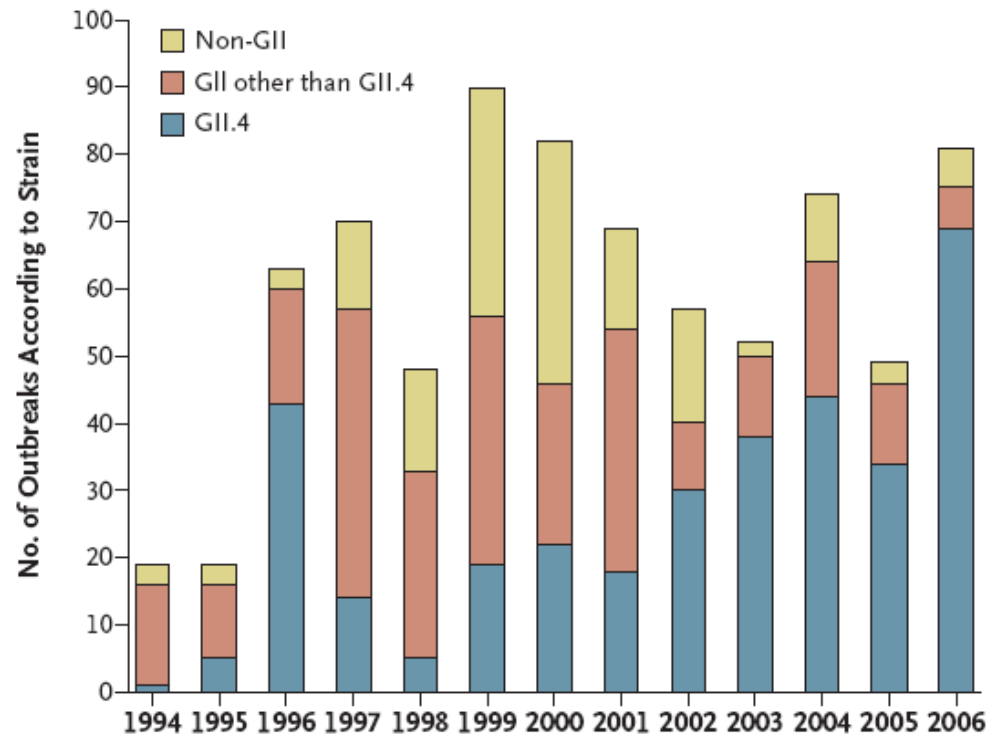
# Viral characteristics

- Family of Caliciviridae
- Single + strand RNA, Non-enveloped
- Huge diversity

180 molecules (90 dimers) of VP1





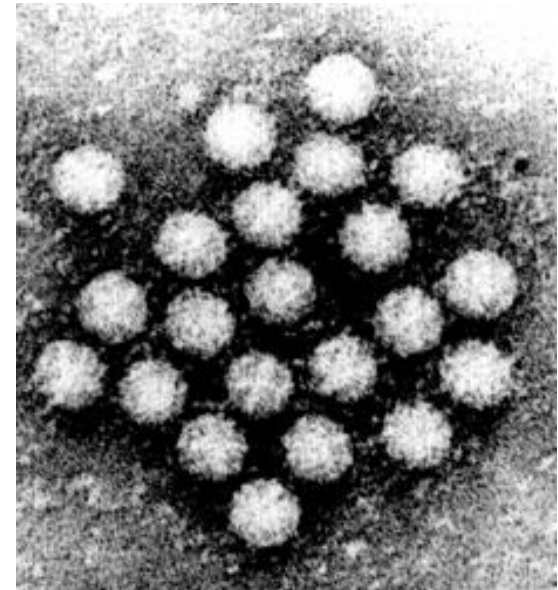


**Figure 3. Outbreaks of Noroviruses in the United States, 1994 to 2006, According to Genotype and Genogroup.**

The original Norwalk virus strain, GI.1, is rarely present, whereas GII.4 has become the pandemic strain. The increase in outbreaks after 1996 reflects the application of new polymerase-chain-reaction diagnostics and not an absolute increase in the number of outbreaks that occurred.

## Methods for Detecting Norovirus

- **EM**
  - Low sensitivity ( $10^6$  particles/ml) required
  - High specificity
  - Laborious and Time consuming
  - Expensive
- **Rapid assay**
  - ICT and ELISA
  - Fast and easy to perform
  - Ok sensitivity
  - Very good specificity
  - Reasonable cost
- **PCR**
  - Highly sensitive
  - Highly specific
  - Relatively quick
  - Expensive



# RIDA QUICK Norovirus

## INTERPRETATION:

Figure. 1  
 Test cassette and  
 interpretation of  
 results.



T = blue, Norovirus positive  
 C = blue, control line

Health Canada Approved

# IDEIA NOROVIRUS ELISA



## Evaluation of Rapid Test

**Performance characteristics of 3 norovirus antigen detection kits compared to 50 positive and 110 negative samples tested by norovirus rRT-PCR**

	Rida <sup>®</sup> quick Norovirus ICT (R-Biopharm AG, Germany)	IDEIA Norovirus <sup>™</sup> EIA (Oxoid, UK)	Ridascreen <sup>®</sup> Norovirus EIA (R-Biopharm AG, Germany)
Detection Method	ICT	EIA, visual or plate reader	EIA plate reader
Sensitivity	67.33%	66%	66.33%
Specificity	98.16%	97.23%	97.53%
Agreement with PCR	423/478 (88.5%)	417/478 (87.2%)	420/478 (87.9%)
Time to Complete Test	15 minutes	90 minutes	105 minutes

Murphey, M et al, 2010 abstract at CVS

**Table 2: Estimated Characteristics of Three Testing Methodologies for Norovirus, Based On Latent Class Analysis and Composite Reference Standard.**

	<b>Sensitivity (95% CI)</b>	<b>Specificity (95% CI)</b>	<b>Positive Predictive Value (95% CI)</b>	<b>Negative Predictive Value (95% CI)</b>
<b>Latent Class Model, prevalence (95% CI) = 0.42 (0.35, 0.49)</b>				
<b>RT<sup>2</sup>-PCR</b>	100% (100%, 100%)	86% (76%, 95%)	88% (74%, 93%)	100% (100%, 100%)
<b>EIA</b>	86% (75%, 95%)	93% (85%, 99%)	92% (80, 98%)	87% (83%, 96%)
<b>EM</b>	18% (8%, 30%)	100% (100%, 100%)	100% (100%, 100%)	63% (55%, 70%)
<b>Composite Reference Standard, prevalence (95% CI) = 0.37 (0.26, 0.49)</b>				
<b>RT<sup>2</sup>-PCR</b>	100% (100%, 100%)	78% (66%, 88%)	82% (57%, 86%)	100% (100%, 100%)
<b>EIA</b>	97% (91%, 100%)	96% (90%, 100%)	96% (83%, 100%)	97% (94%, 100%)
<b>EM</b>	20% (9%, 33%)	100% (100%, 100%)	100% (100%, 100%)	68% (56%, 79%)

RT<sup>2</sup>-PCR, real-time reverse-transcriptase polymerase chain reaction; EIA, enzyme Immunoassay; EM, electron microscopy; 95% CI, 95% credible interval based on 100,000 bootstrap iterations.

# Current Algorithm of Testing Outbreak Specimens vs Clinical Specimens

- Outbreak specimens for viral gastroenteritis:
  - EM or PCR
    - If PCR negative, perform EM
- Routine:
  - EM testing

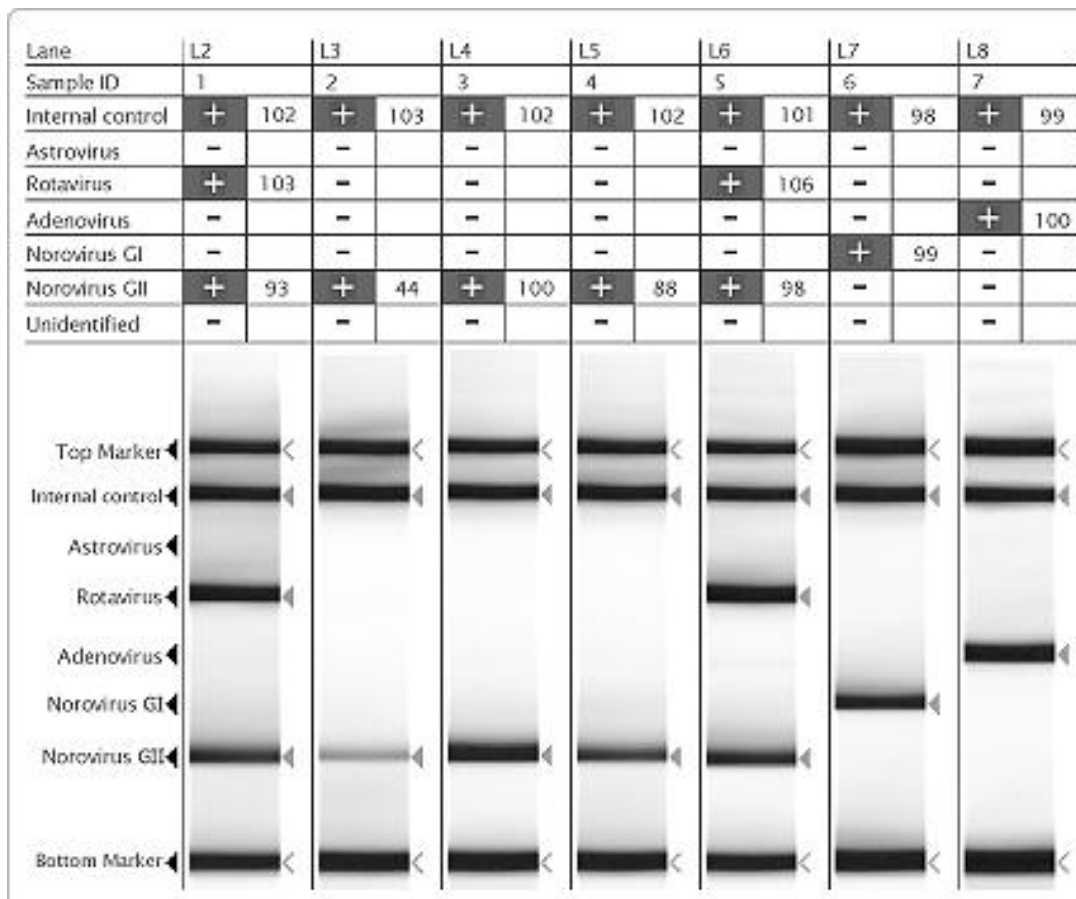
## Enteric viruses in Ontario Jan 09- Aug 10

	EM		EM Total	NORO_PCR		NORO_PCR Total	Grand Total
	Outbreak	Non-Outbreak		Outbreak	Non-Outbreak		
Adenovirus	13	138	151				151
Astrovirus	2	13	15				15
Calicivirus	4	4	8				8
Norovirus		5	5	1672	28	1700	1705
Norovirus-like	110	204	314				314
Picornavirus-like	1	4	5				5
Rotavirus	85	642	727				727
Negative	1506	5755	7261	691	44	735	7996
Grand Total	1721	6765	8486	2363	72	2435	10921

## Typing method

- Sequence of VP1 region of the virus
- Useful to link outbreak specimens
- Role of typing in Norovirus outbreak?
- National surveillance Program (vironet)

# Evaluation of Multiplex Assay for Detection of Enteric Viruses



1	Rotavirus, Norovirus GII
2	Norovirus GII
3	Norovirus GII
4	Norovirus GII
5	Rotavirus, Norovirus GII
6	Norovirus GI
7	Enteric adenovirus 1~7: Clinical samples

Pathogen	No tested	Source
Adenovirus	29	Stool
Rotavirus	52	Stool
Norovirus GI	19	Stool
Norovirus GII	37	Stool
Astrovirus*	3	Stool
<i>S. paratyphi A</i>	2	Stool
<i>S. typhi</i>	1	Stool
<i>S. dysenteriae Type 2</i>	5	Stool
<i>E. coli - 0:157:H7</i>	1	Culture
<i>S. enteritidis</i>	1	Culture
<i>S. flexneri</i>	1	Culture
<i>S. sonnei</i>	1	Culture
<i>V. cholera</i>	1	Culture
<i>C. jejuni</i>	1	Culture
Influenza B	2	NP swabs
Influenza A/ H1N1	5	NP swabs
Influenza A/ H3N2	1	NP swabs
Picornavirus	2	NP swabs
<i>A. hydrophila</i>	1	Culture
<i>N. meningitidis</i>	1	Culture
<i>S. pyogenes</i>	2	Culture
<i>S. pneumoniae</i>	2	Culture
<i>S. epidermidis</i>	1	Culture
Negative Enteric Specimens	29	Stool

Various positive and negative stool samples and stool cultures were used to determine sensitivity and specificity

## For discordant analysis, real-time PCR was developed

Target	Primers and Probe Sequences	Amp Size (BP)	Position	PCR Method
<i>B-Fragilis</i>	F: GAG AGG AAG GTC CC R: CGC TAC TTG GCT GG P: FAM-CCA TTG ACC AAT ATT CCT CAC TGC TGC CT- <i>BHQ</i>	129	296-425	RT PCR
Norovirus G1	F:GCC ATG TTC CGI TGG ATG R:TCC TTA GAC GCC ATC ATC AT P:FAM- AGA TCG CGG TCT CCT GTC CA- <i>BHQ</i>	76	5282-5358	RT rt-PCR
Norovirus GII	F:CAA GAG TCA ATG TTT AGG TGG ATG AG R:TCG ACG CCA TCT TCA TTC ACA P:CY5- TGG GAG GGC GAT CGC AAT CT- <i>BHQ</i>	77	5003-5080	RT rt-PCR
Adenovirus	F: CAG GAC GCC TCG GRG TAY CTS AG R: GGA GCC ACV GTG GGR TT P: FAM-CCG GGT CTG GTG CAG TTT GCC CGC- <i>BHQ</i>	103	*	RT PCR
Rotavirus	F:CCA TCT WCA CRT RAC CCT CTA TGA G R:GGT CAC ATA ACG CCC CTA TAG C P: CY5-AGT TAA AAG CTA ACA CTG TCA AA- <i>BHQ</i>	86	963-1049	RT rt-PCR

## Summary of Results

Pathogen Tested	Source	Test Method 1: PHL	Test Method 2: Seeplex	Test Method 3: Real-Time rt-PCR	Co-infecting Pathogens	Comments
<b>Adenovirus</b>	Stool	29	28	29	1 norovirus GII	PHL test: EM
<b>Rotavirus</b>	Stool	52	51	51	5 norovirus GII	PHL test: EM
					1 adenovirus	
<b>Norovirus GI</b>	Stool	19	18	18		PHL test: real time rt-PCR
<b>Norovirus GII</b>	Stool	37	38	38	2 rotavirus	PHL test: real time rt-PCR
					3 adenovirus	
<b>Negative</b>	Stool	29	31	30		1 norovirus GII detected
<b>Enteric bacteria</b>	Stool	8	8	8		PHL test: bBacterial culture
<b>Other viruses</b>	NP Swab	10	10	10		PHL test: real time rt-PCR
<b>Other bacteria</b>	Culture	13	13	13		PHL test: bacterial culture
<b>Total</b>		<b>197</b>	<b>197</b>	<b>197</b>	<b>12</b>	

## Overall Sensitivity and Specificity

	<b>Sensitivity</b>	<b>specificity</b>
<b>Adenovirus</b>	97%	99.40%
<b>Rotavirus</b>	98%	99.30%
<b>Norovirus GI</b>	97%	99.40%
<b>Norovirus GII</b>	95%	99.40%

## Summary

- Majority of gastro outbreaks are caused by enteric viruses
- Norovirus is far more common in enteric outbreaks
- Molecular testing has greatly enhanced capability of detecting viruses in stool
- Typing Norovirus may have limited utility
- Further validation and verification is needed for multiplex testing