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Studying the transmission dynamics of norovirus in a paediatric hospital

BSMT Autumn Microbiology Symposium, Merseyside Maritime Museum, Liverpool, Friday 19th October 2018

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Viral causes of diarrhoea

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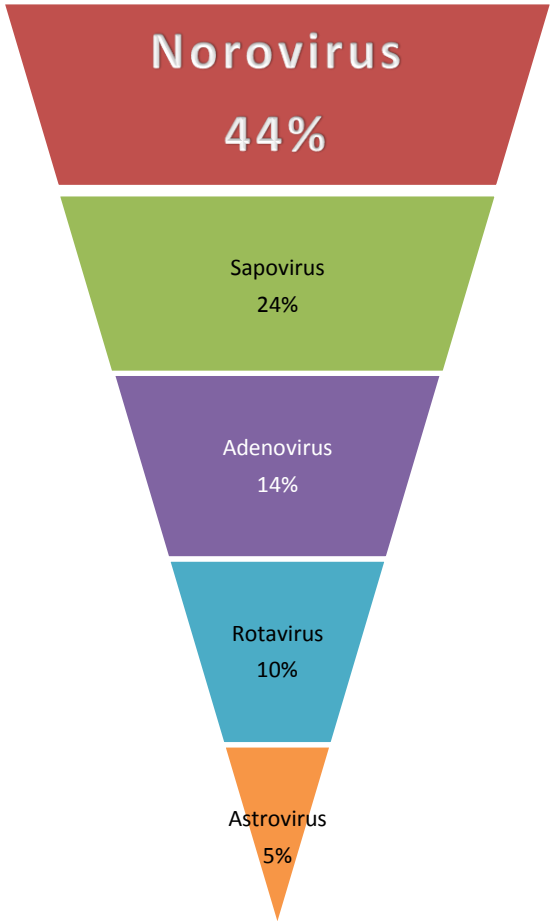
- Europe: 4 episodes of diarrhoea per child per year in under 5 year olds
- 147 community cases for every case reported in national surveillance
- 17 million community cases per year
- UK gastroenteritis: £115 million per year (63% norovirus)
- All faecal oral transmission (person-person)

Hospitalised Children (GOSH)



Norovirus

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	Immunocompetent	Immunocompromised
Prevalence	Leading worldwide cause of gastroenteritis	Not established (17-18%)[1, 2]
Seasonality	Winter peaks	Year-round[2, 5]
Clinical features	Acute onset, vomiting (projectile, <1 day), diarrhoea	Acute onset, vomiting (<2 days), diarrhoea
Duration	24 – 48 hours	Weeks to years (chronic)
Complications	Dehydration	Dehydration, malnutrition, dysfunction of intestinal barrier[3], dramatic weight loss[4], nutritional support
Prognosis	Excellent	Poor to excellent (deaths[3, 4]) Chronic infection common

[1]Schorn *et al* 2010, renal Tx; [2]Roddie *et al.* 2009 CID, HSCT; [3]Schwartz *et al.* 2011; [4]Roos-Weil *et al.* 2011, renal Tx; [5]Ludwig *et al.* 2008 cancer px

Norovirus genome & typing

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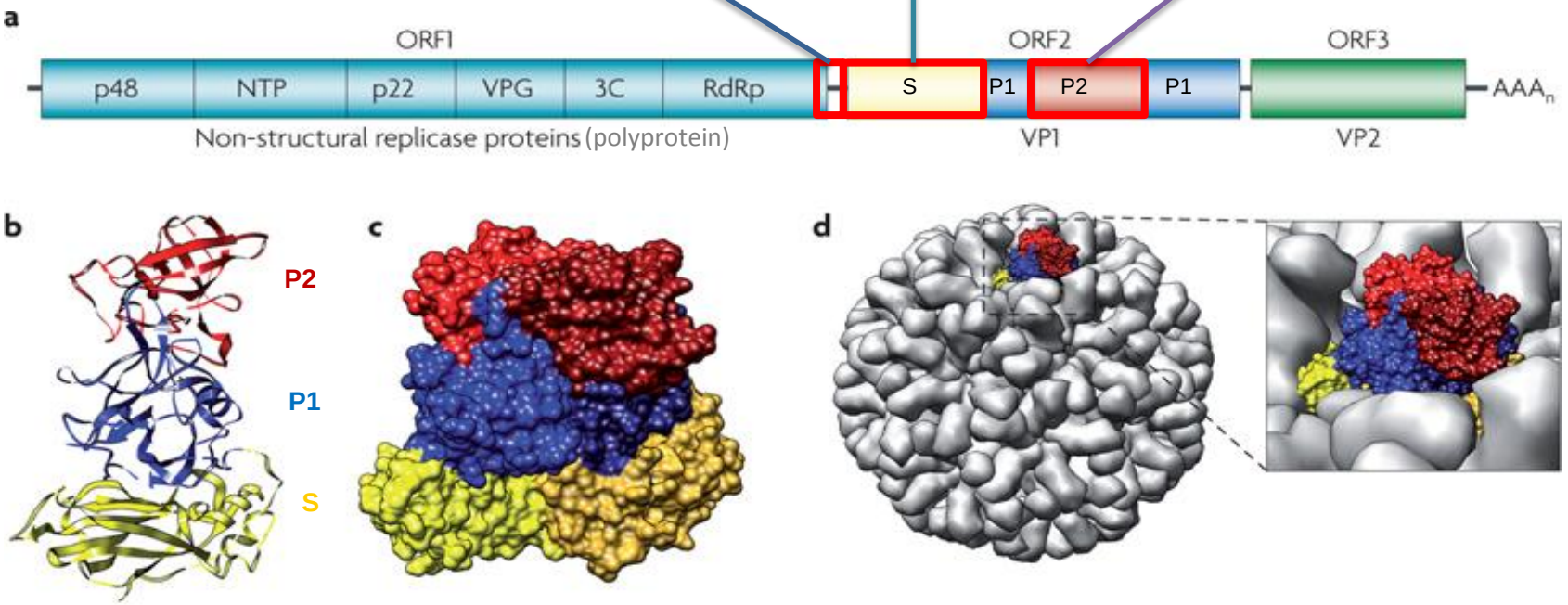
Genogroup (GII)

Genotype (GII.4)

Variant type (GII.4 Sydney 2012)



Nature Reviews | Microbiology

Norovirus genotypes identified in paediatric tertiary referral hospital (GOSH), July 2014 – February 2016 (n = 184)

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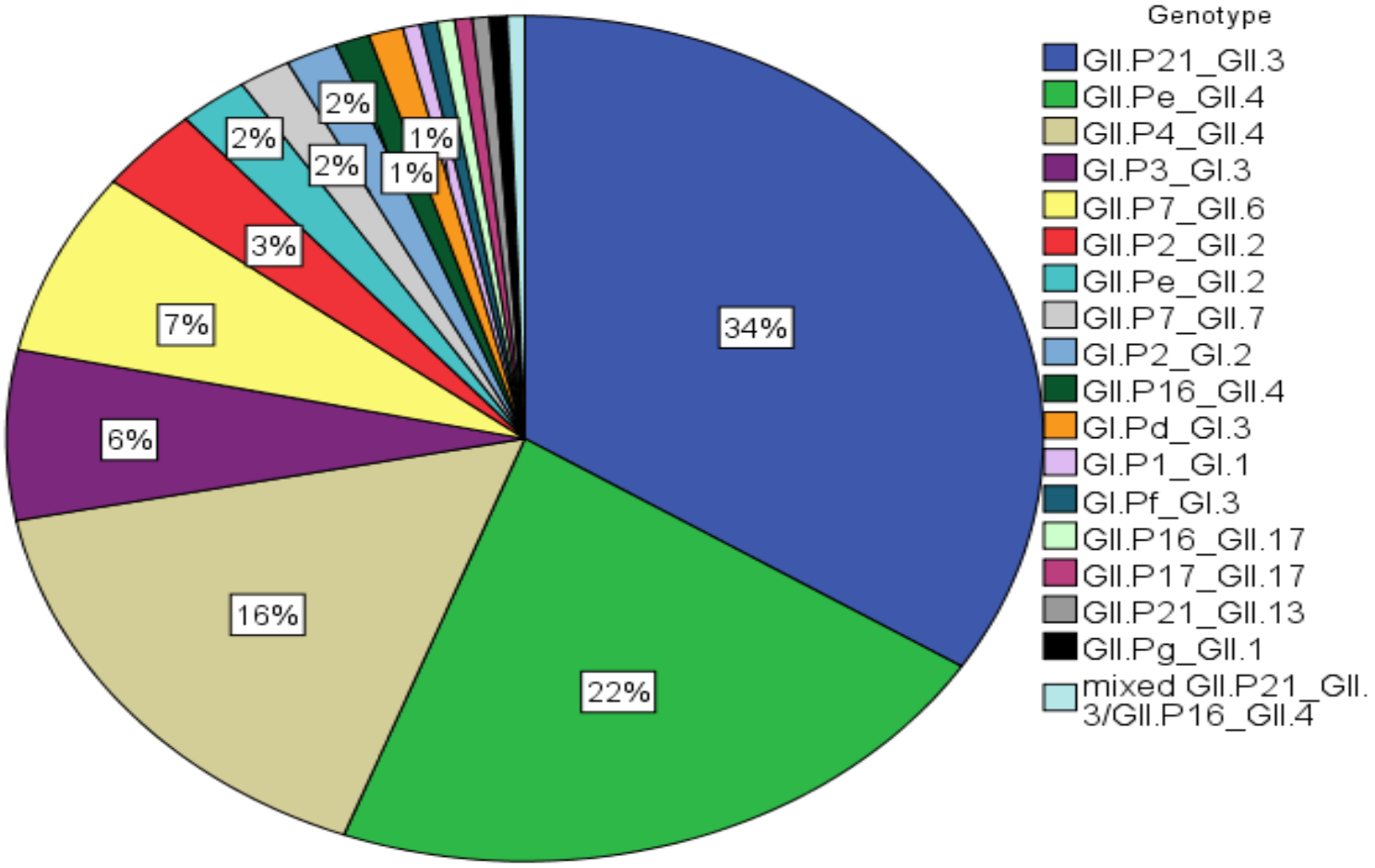

>90% of outbreaks worldwide = GII.4

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Norovirus capsid sequencing (1 kb)

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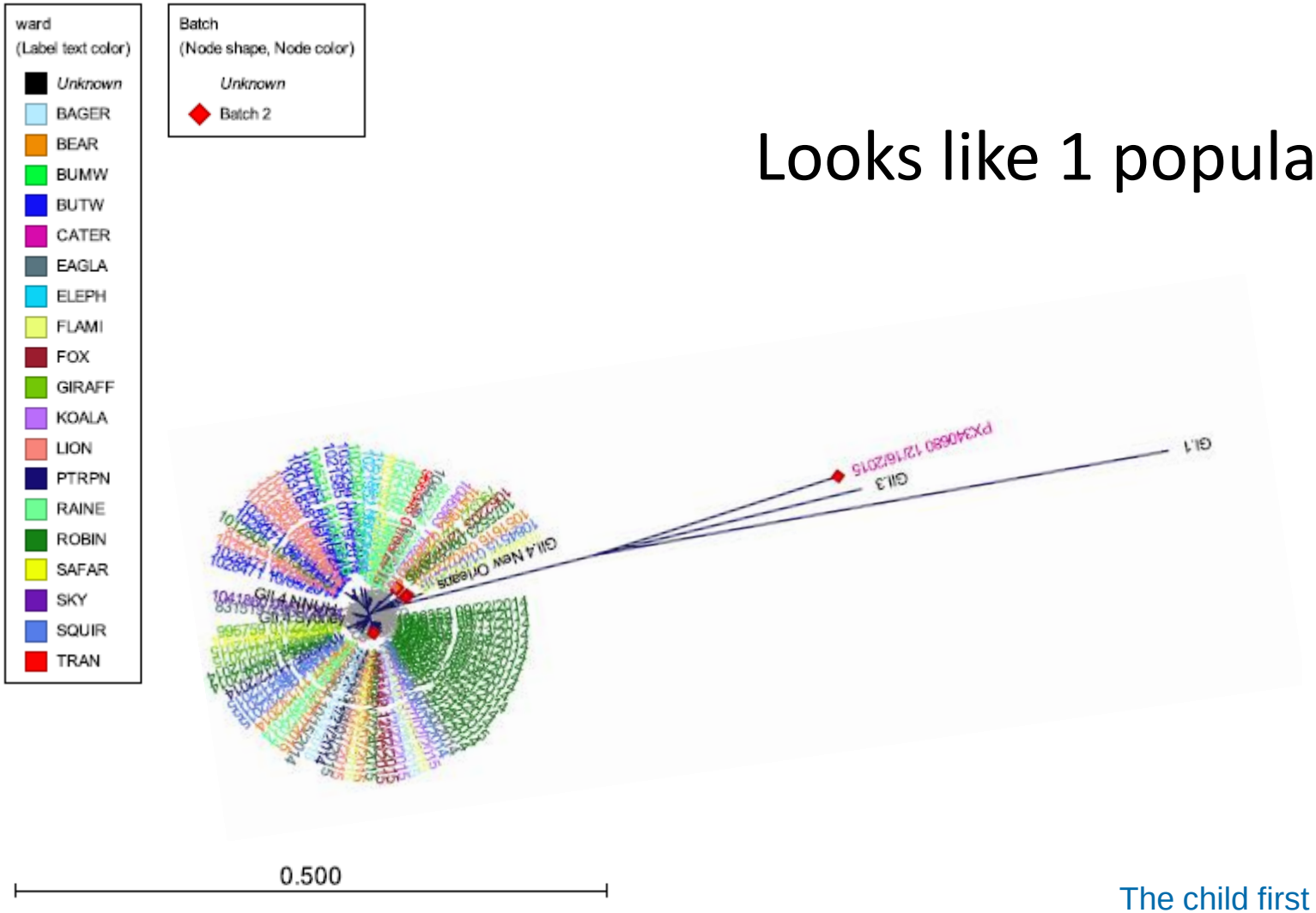

Looks like 1 population

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Clinical Infectious Diseases

MAJOR ARTICLE

Norovirus Transmission Dynamics in a Pediatric Hospital Using Full Genome Sequences

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Background. Norovirus is a leading cause of worldwide and nosocomial gastroenteritis. The study aim was to assess the utility of molecular epidemiology using full genome sequences compared to routine infection prevention and control (IPC) investigations.

Can WGS be used to better understand the sources of norovirus infection and transmission dynamics in a paediatric population with a high prevalence of immunocompromised patients.

Conclusions. We show there are frequent introductions of multiple norovirus strains with extensive onward nosocomial transmission of norovirus in a pediatric hospital with a high proportion of immunosuppressed patients nursed in isolation. Phylogenetic analysis using full genome sequences is more sensitive than classic IPC investigations for identifying linked cases and should be considered when investigating norovirus nosocomial transmission. Sampling of staff, visitors, and the environment may be required for complete understanding of infection sources and transmission routes in patients with nosocomial infections not linked to other patients and among patients with phylogenetically linked cases but no evidence of direct contact.

Keywords. norovirus; epidemiology; molecular epidemiology; sequencing; whole genome.

Study Cohort

- Paediatric tertiary referral hospital, 350 beds, 60% single isolation rooms, no A & E
- Residual specimen (where available) from the first positive sample from all norovirus positive patients between 1st July 2014 and 17th February 2016 (19 months) was submitted for whole genome sequencing
- A total of 205 norovirus PCR positive patients were identified during the study period, 189 of these were whole genome sequenced
- Median patient age was 2 years
- 59% of patients profoundly immunocompromised

Infection Prevention and Control Decision tree following detection of Norovirus infection

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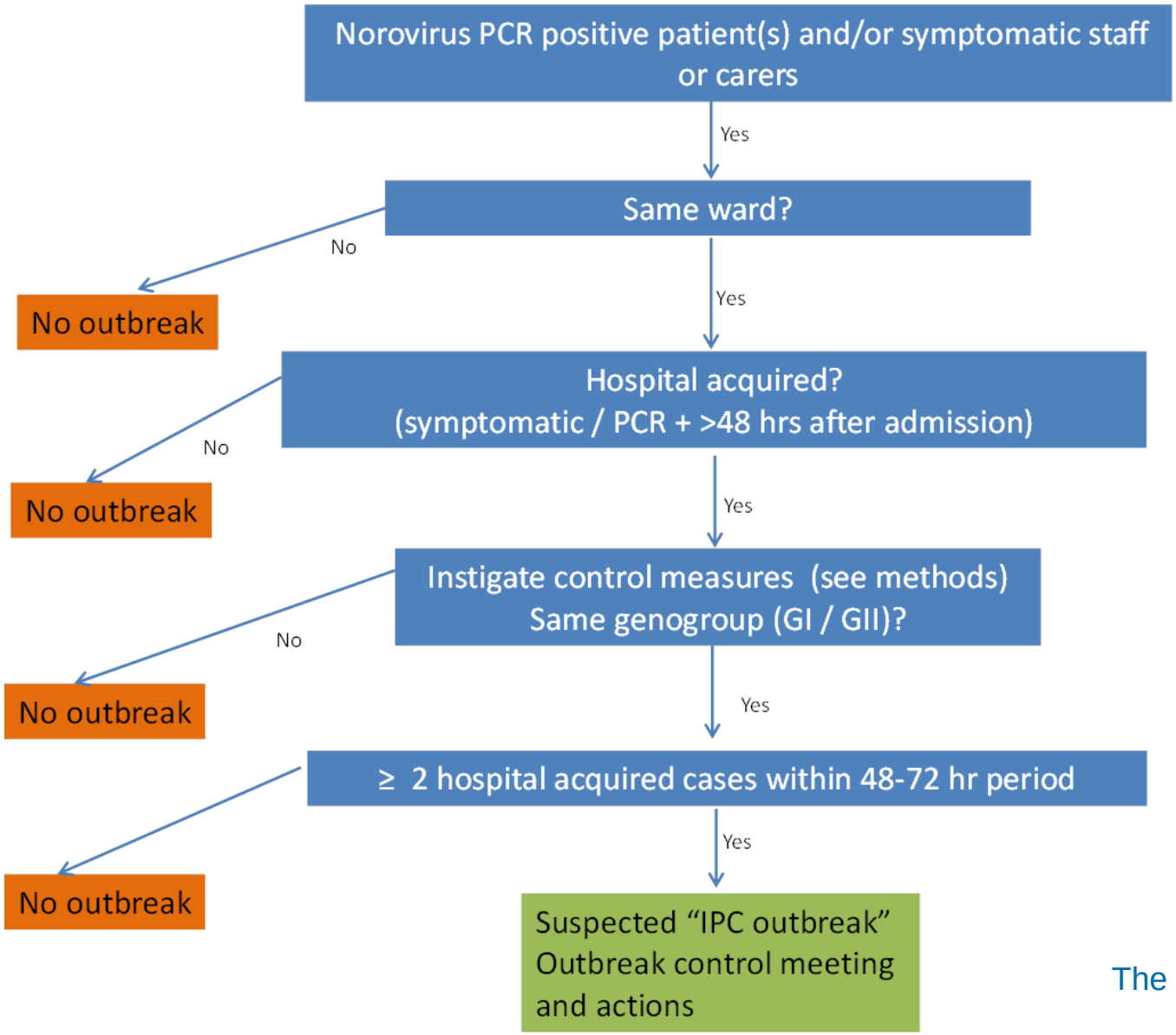

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Whole genome sequencing - SureSelect

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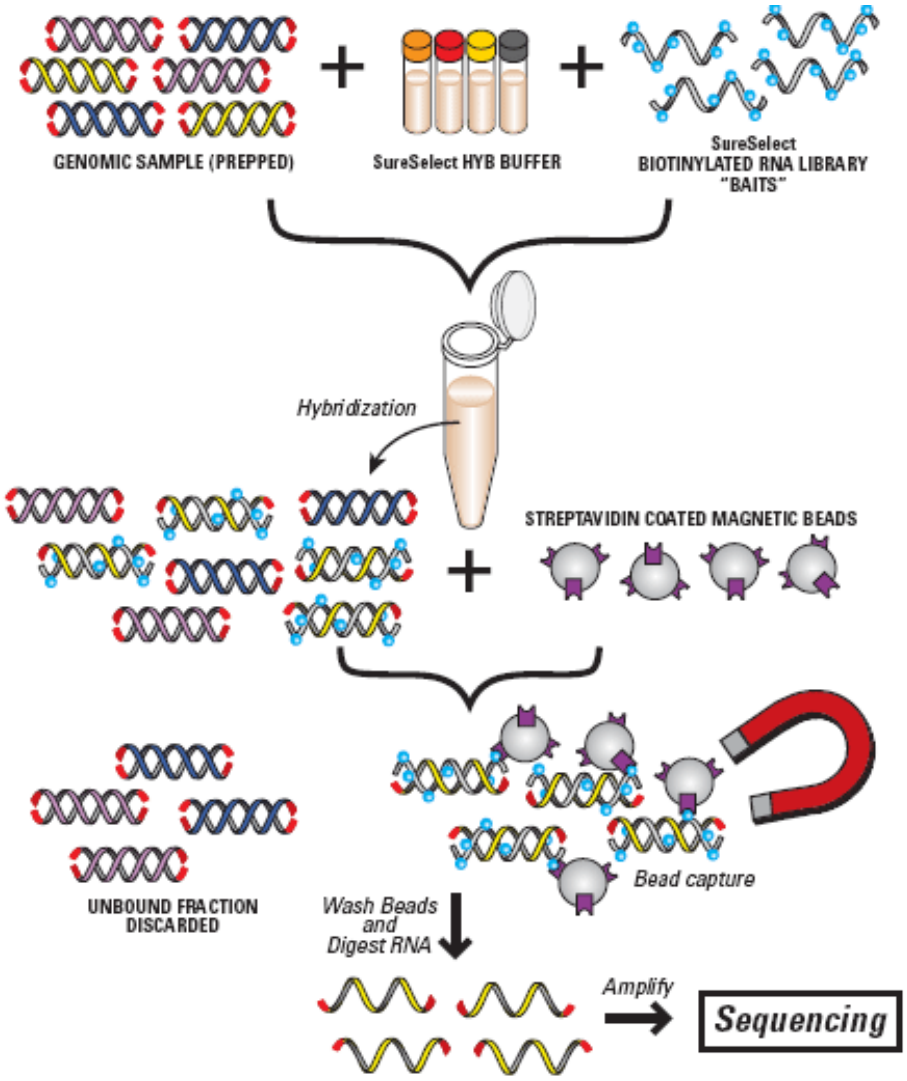
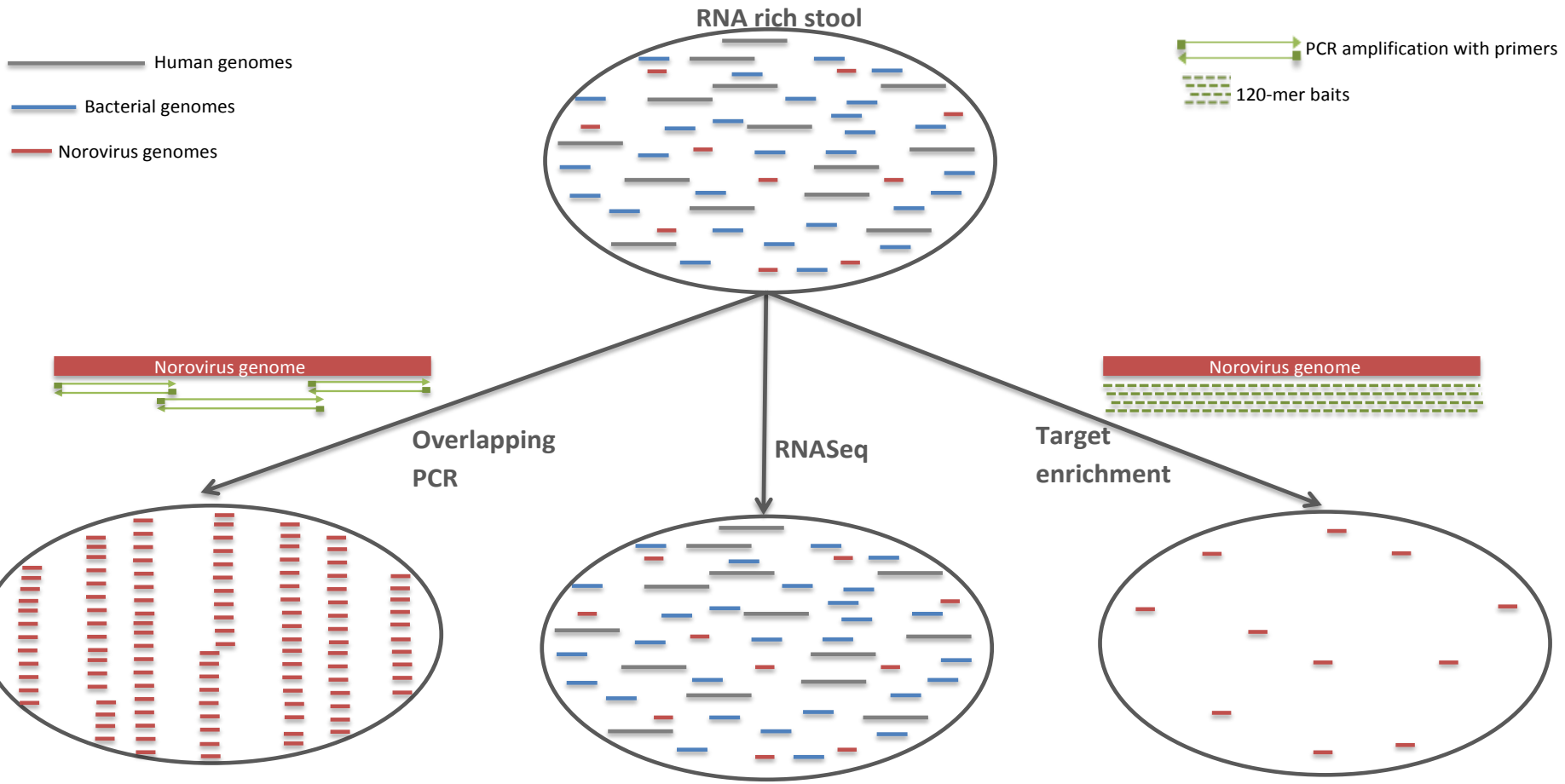


Hybridize sample DNA to 120bp biotin-labelled RNA fragments to cover entire genome

Magnetic beads bind biotin-labelled DNA-RNA fragments

Pull out target DNA using magnet

Sample is now concentrated (not pre-amplified) and ready for sequencing



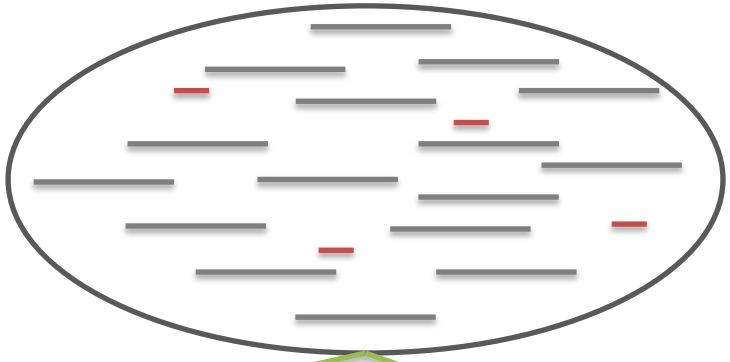
Pros	Cons
Generates high yields of target DNA	Sequence heterogeneity problematic for primer design therefore primers are often genotype specific. High failure rate, especially with non-target genotypes

Pros	Cons
Direct sequencing of total RNA; no need for primer design No prior knowledge of sequences required Not genotype specific	Majority of data is redundant with low proportion of reads generated corresponding to norovirus, resulting in low read depth and limited scope for variant analysis. Limited success with low titre samples

Pros	Cons
Bait design accounts for sequence heterogeneity Not genotype specific Good read depth, sufficient for variant analysis Successful with low titre samples	Bait design relies on availability of sequences; novel genotypes that are highly divergent from known genotypes may have limited success

Deep sequencing

Genomic material in a stool sample



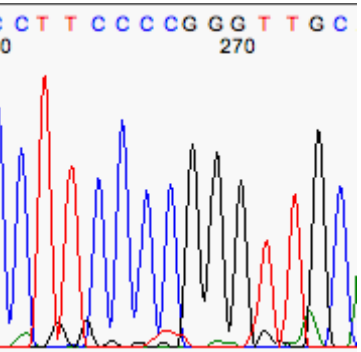
Human or bacterial genome

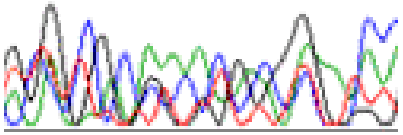
Viral genome

Sanger sequencing

One consensus sequence for the sample

Cannot separate mixed sequences

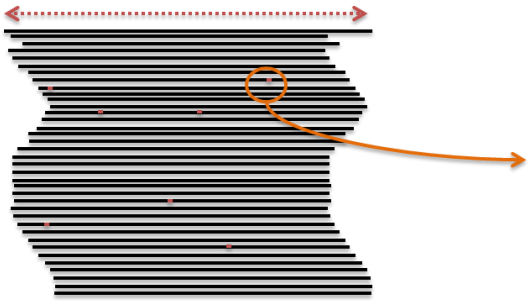




Deep sequencing

A sequence for every piece of genomic material in the sample

1 sequence = 1 read



ATACGCTAGCTAGCTAGCCCGTAGCTAATTCGATCGCCGTAGCTAATTCGA
ATACGCTAGCTAGCTAGCCCGTAGCTAATTTGATCGCCGTAGCTAATTCGA
ATACGCTAGCTAGCTAGCCCGTAGCTAATTCGATCGCCGTAGCTAATTCGA
ATACGCTAGCTAGCTAGCCCGTAGCTAATTCGATCGCCGTAACTAATTCGA
TACGCTAGCTAGCTAGCCCGTAGCTAATTCGATCGCCGTAGCTAATTCGA
TACGCTAGCAAGCTAGCCCGTAGCTAATTCGATCGCCGTAGCTAATTCGA
TACGCTAGCTAGCTAGCCCGTAGCTAATTCGATCGCCGTAGCTAATTCGA
CGCTAGCTAGCTAGCCCGTAGCTAATTTGATCGCCGTAGCTAATTCGA
CGCTAGCTAGCTAGCCCGTAGCTAATTCGATCGCCGTAGCTAATTCGA
CGCTAGCTAGCTAGCCCGTAGCTAATTCGATCGCCGTAACTAATTCGA
CGCTAGCTAGCTAGCCCGTAGCTAATTTGATCGCCGTAGCTAATTCGA

Norovirus full genome (7.5 kb)

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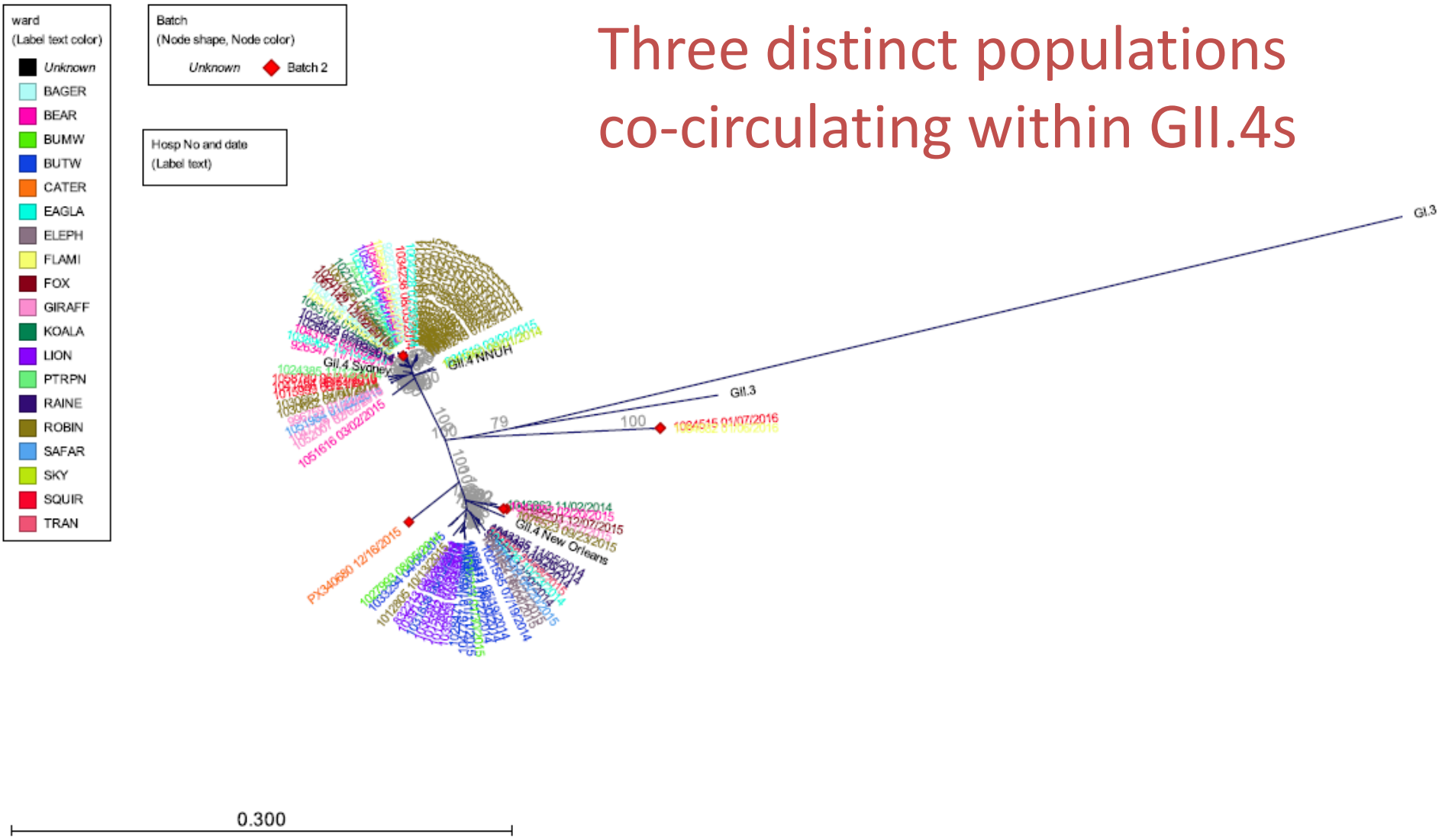
Three distinct populations co-circulating within GII.4s



Sequence clusters identified by maximum likelihood phylogeny using full genome sequences.

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Sequence cluster number	Genotype	Number of patients	Date range	Number of wards	Number of clinical specialties involved	Bootstrap support	Diversity within cluster (SNPs)	Identified by infection control (IPC) investigations	Supported by classical epidemiology*
4	GII.P7_GII.6	3	7 days	1	1	100	0	Yes	Yes
5	GII.P21_GII.3	17	3 months	6	3	100	0–22	Partially	Yes (16/17)
6	GII.P21_GII.3	2	3 days	1	1	82	14	No	Yes
7	GII.P21_GII.3	6	1 month	3	2	70	0–10	Partially	Yes
8	GII.P21_GII.3	2	2 months	1	1	100	11	No	Yes
9	GII.P21_GII.3	2	2 days	1	1	100	12	No	Yes
10	GII.P21_GII.3	9	17 months	2	1	100	19–149	Partially	Yes
23	GII.P21_GII.3	2	3 months	2	2	100	29	No	Yes
11	GII.Pe_GII.4	8**	2 months	2	2	100	1–24	Partially	Yes
12	GII.Pe_GII.4	2	6 days	1	1	100	3	No	Yes
13	GII.Pe_GII.4	2	3 days	1	1	100	0	No	Yes
14	GII.Pe_GII.4	4	11 days	2	1	100	1–4	Partially	Yes
15	GII.Pe_GII.4	3	3 days	1	1	100	1–3	Yes	Yes
16	GII.P4_GII.4	7	3 months	2	1	100	0–35	Partially	Yes
17	GII.P4_GII.4	2	25 days	2	1	100	14	No	Yes
18	GII.P4_GII.4	5	2.5 months	3	2	77	0–25	No	Yes
19	GII.P4_GII.4	2	19 days	1	1	100	6	No	Yes
1	GI.P3_GI.3	2	8 months	2	2	100	31	No	No
2	GII.P2_GII.2	2	2 months	2	2	100	7	No	No
3	GII.P7_GII.6	2	3 months	2	2	100	17	No	No
20	GII.Pe_GII.4	2	5.5 months	2	2	100	12	No	No
21	GII.P21_GII.3	3	4 months	3	1	95	17–28	No	No
22	GII.P21_GII.3	2	6 months	2	2	98	36	No	No
24	GII.P21_GII.3	2	3 months	2	1	100	18	No	No

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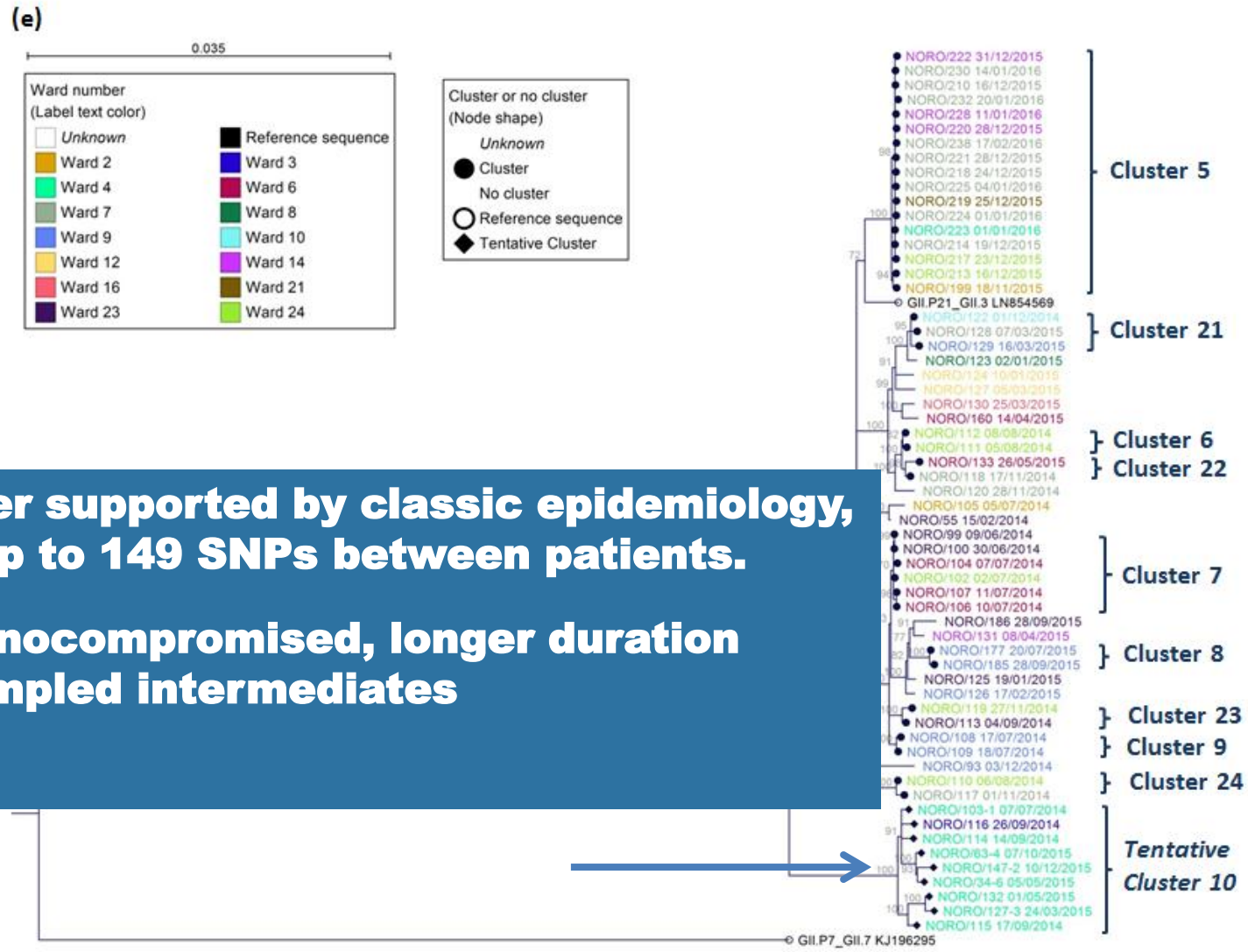
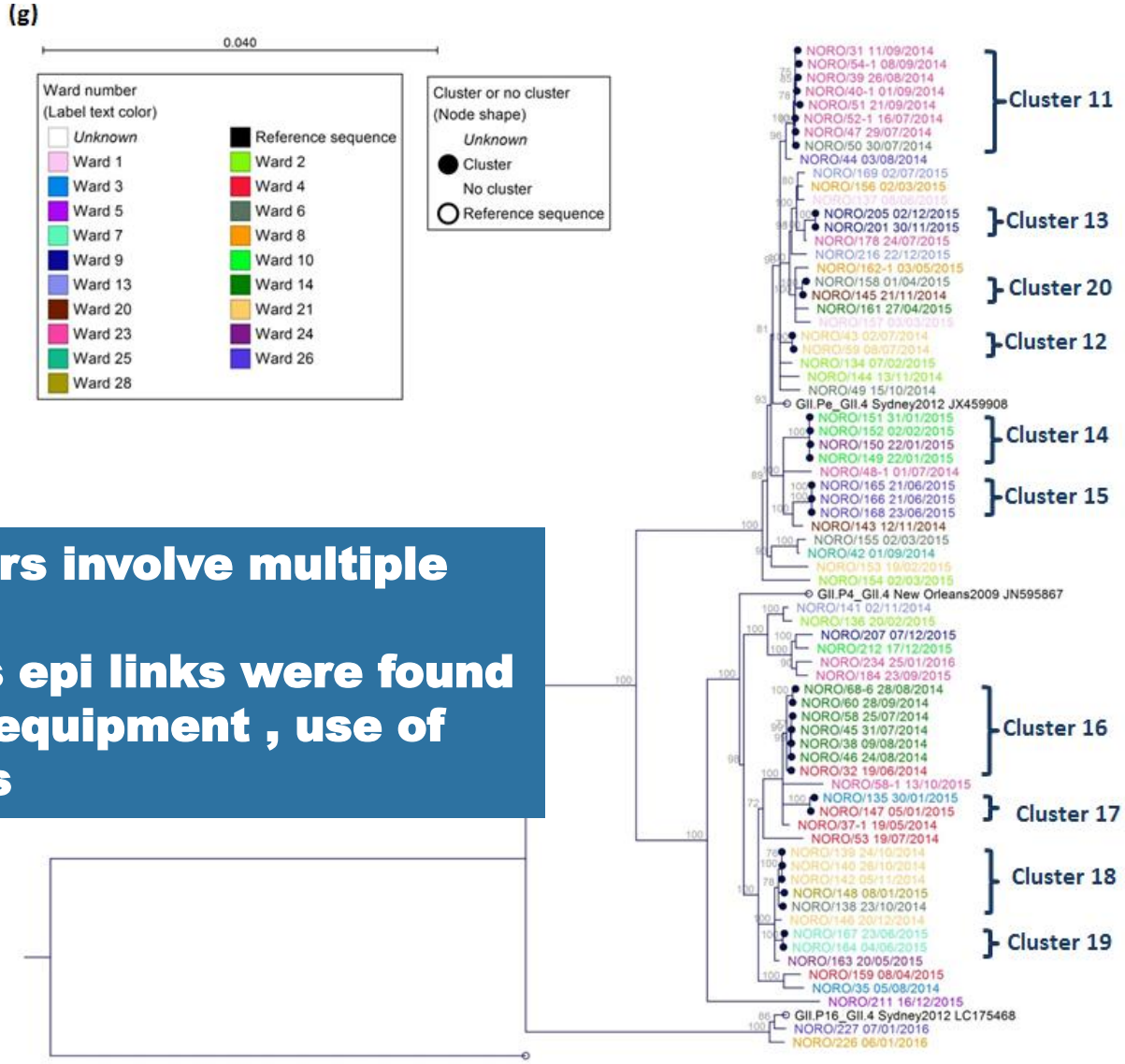
Maximum likelihood phylogeny of full genome sequences from norovirus episodes with GII.3 sequences



Cluster supported by classic epidemiology, but up to 149 SNPs between patients.

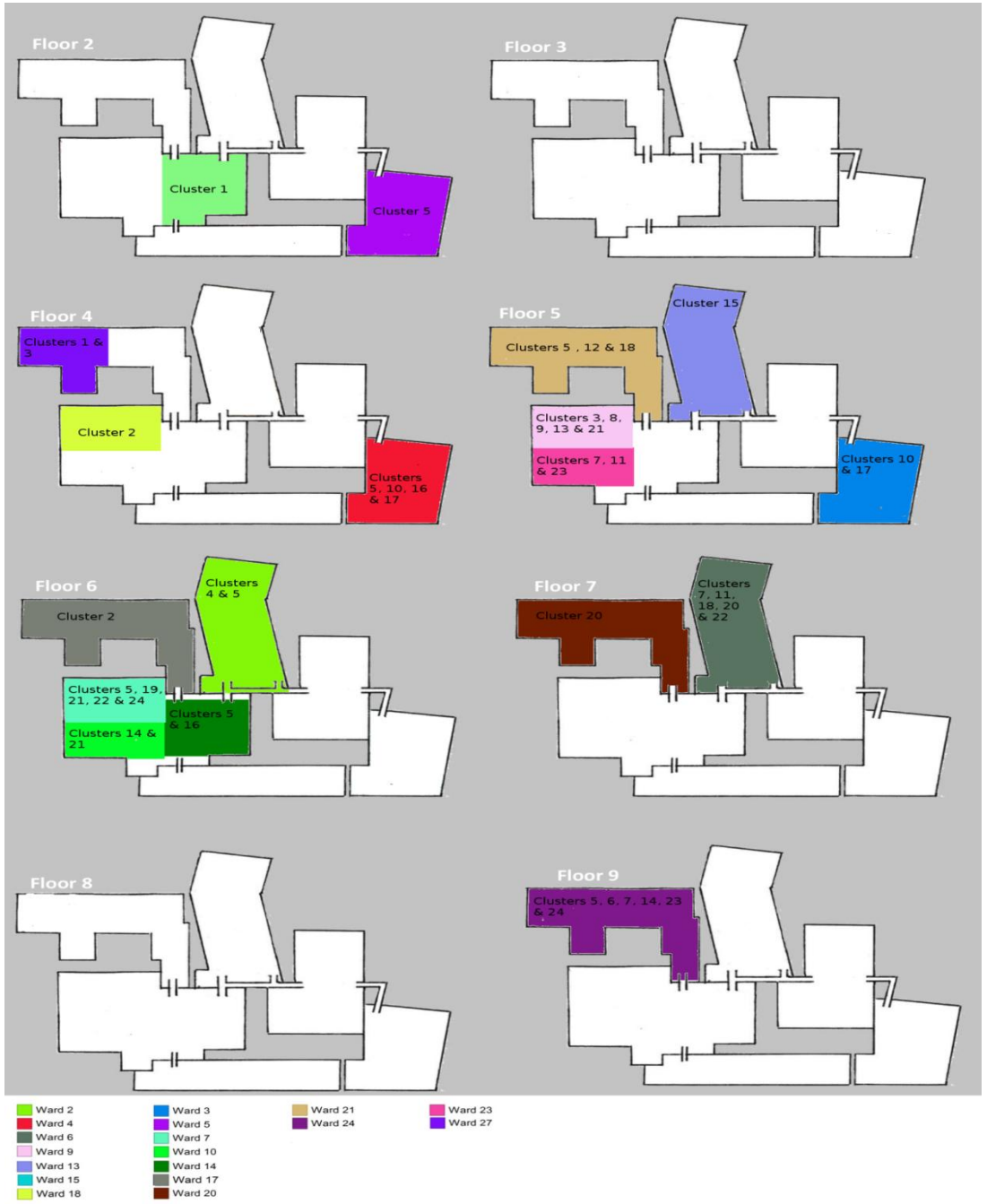
Immunocompromised, longer duration
Unsampled intermediates

Maximum likelihood phylogeny of full genome sequences from norovirus episodes with GII.4 sequences



Several clusters involve multiple wards
In many cases epi links were found (shared staff, equipment , use of common areas

Relationship between clusters and wards in the hospital



Decision Tree based on combining Norovirus Sequencing and Epidemiological Data

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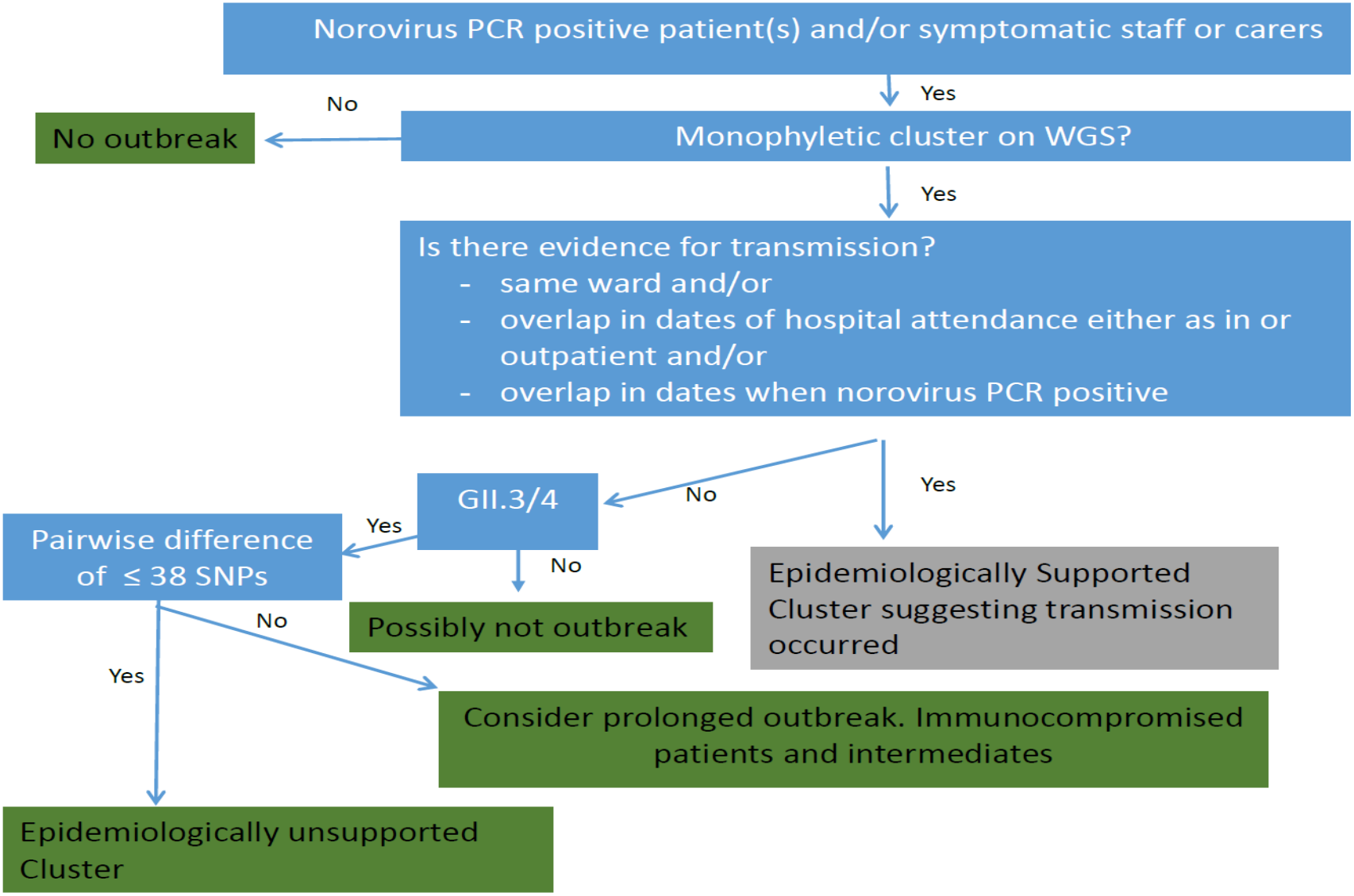

Conclusions

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- Routine IPC investigations alone only identified linked transmission in 44% of cases compared with IPC and WGS
- In this study 33% of new norovirus cases were acquired from another patient, despite isolation nursing and stringent IPC measures
- Source of infection for 43% of nosocomial infections remains unknown even with WGS, wider sampling of patients, staff, visitors and the environment needed
- With ever decreasing sequencing costs and technologies that allow rapid turnaround times the possibility that norovirus genome sequencing could be used routinely to control nosocomial infections is now a reality

Acknowledgements

- Julianne Brown
- Judy Breuer
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- UCL MRC CMMV.
- NIHR Great Ormond Street Hospital Biomedical Research Centre.